

EXPERIMENTAL INVESTIGATION ON BACTERIAL CONCRETE

Submitted in partial fulfilment of the requirements for the
award of

Bachelor of Engineering Degree in Civil Engineering

By

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SCHOOL OF BUILDING AND ENVIRONMENT

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of DEEPAK PANDURANGAN (Reg. No. 37200018) and D.GEETHAN KISHORE KUMAR (Reg. No. 3720018) who carried out the project entitled **“EXPERIMENTAL INVESTIGATION ON BACTERIAL CONCRETE”** Under our supervision from December 2020 to March 2021.

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Submitted for Viva voce Examination held on _____

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DECLARATION

We, Deepak Pandurangan(37200018) and D.Geethan Kishore Kumar(37200703) hereby declare that the Project Report entitled “**EXPERIMENTAL INVESTIGATION ON BACTERIAL CONCRETE**”done by us under the guidance of Mrs.Nirmala.R is submitted in partial fulfillment of the requirements for the award of Bachelor of Engineering degree in Civil Engineering.

1.

2.

SIGNATURE OF THE CANDIDATES

DATE:

PLACE:

ACKNOWLEDGEMENT

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

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ABSTRACT

This research shows that using *Bacillus Subtilis* microorganisms to build a tough framework and put forward a concentrated effort mending concrete as a strategy for break control to improve administration existence in solid structures is successful. The Microbiologically Induced Calcite Precipitation (MICP) method is used in this article. A 24 ml liquid form of *Bacillus Subtilis* with a cell concentration of 10^5 cells/ml was used in a 1:1.3: 2.75:0.45 mixing proportion. The compressive and flexural strength of concrete mixes was tested using 150mm x 150mm x 150mm cubes for compressive strength and 150mm x 150mm x 70mm rectangular beams for flexural strength. The specimens used for recovery are purposefully broken. The research shows that there is a significant improvement in the consistency of bacteria-added cement or bacterial concrete as compared to traditional concrete, and that calcium carbonate precipitation is visible after 3-4 weeks in small scale splits.

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CHAPTER 1

INTRODUCTION

1.1 General

Concrete is a combination of natural cracks that is homogeneous. Water and other salts seep through the cracks, causing the concrete to deteriorate and its lifetime to be reduced. Steel corrosion can also be caused by water and salt seepage, which weakens concrete reinforcement. As a result, rehabilitating concrete is critical in order to extend the economic life of structures. Bacteria are used to produce an inherent biomaterial, a self-healing substance, to patch concrete cracks. Bacterial concrete is an excellent tool for crack repair because it induces calcium carbonate deposition as a result of microbial activity. When microbes are given the right environment and calcium source, they will thrive, calcium carbonates can precipitate in concrete due to a few strains of microbes. This precipitation potential has been evaluated in recent decades to justify improvements in concrete strength and durability properties.

In this analysis, *Bacillus subtilis* bacteria were used, and their suitability in concrete was tested. This paper shows how *Bacillus subtilis* bacteria affect concrete compressive power, workability, and self-healing cracks. The findings indicate that adding bacteria to concrete strengthens it. The nutrient source has a major impact on the workability of bacterial concrete. Concrete's workability can be improved by adding calcium lactate, for example. On the other side, bacterial cultures and spore powder have a slight impact on concrete workability. Cracks can be repaired for both cultured bacteria and spore powder bacteria.

Cracking is a natural occurrence in systems and one of their underlying flaws. Water and other salts seep through these cracks, causing deterioration and shortening the life of the structure. Any structure with cracks loses structural integrity and is therefore risky. The bacterial remediation technique may be used to restore historical buildings in order to maintain their architectural value. Since the mineral precipitation caused by microbial activities is pollution-free and normal, this technique is highly desirable.

Since bacteria's cell walls are anionic, metal accumulation (calcite) on their surfaces is significant: mineral crystals develop over time, gradually plugging pores and cracks in structures. Microbiologically Induced Crack Repair (MICR) is a novel technique for repairing cracks and fissures in calcium concrete. Calcite Or Carbonate (CaCO_3) Precipitation (MICP) is a technique that falls under the bio-mineralization branch of research. Since the Calcite precipitation caused by microbial activities is pollution-free and normal, MICP is highly desirable.

Broken concrete specimens can be reinforced and stiffened using this technique. Bacterial activities also trigger a chemistry change in the solution, leading to oversaturation and mineral precipitation. When these Bio mineralogy concepts are applied to concrete, a new material called Bacterial Concrete may arise.

1.2 Concept of Bio mineralization

Bio mineralization refers to the process of living beings arranging minerals, which is a natural wonder. Through an organically actuated mineralization process, bio mineralization can be mastered. Mineralization that is spontaneously prompted occurs in an open domain as a consequence of unregulated microbial metabolic movement. Bio minerals are framed in this procedure by the interaction of metabolic products formed by microorganisms with the surrounding environment.

1.3 Bio concrete mechanism

When the solid is mixed with microbes (*Bacillus subtilis*), the microscopic organisms go into a slow state, similar to seeds. To activate their capabilities, all microorganisms need to be exposed to the air. Any splits that may occur include the crucial presentation. Microbes close to the split begin accelerating calcite precious stones at the point where the splits frame breaks. When a solid structure is harmed and water starts to leak through the breaks that appear in the solid, microorganism spores grow on contact with the water and supplements.

The fluid calcium lactate is converted to insoluble limestone as the microorganisms absorb oxygen. The limestone adheres to the damaged area and repairs it. As steel corrodes, oxygen is a crucial ingredient, and once bacteria have absorbed it all, it expands the strength of steel-fortified solid inventions.

1.4 Activation of bacteria to remediate cracks by bacteria

Bacteria from a variety of natural environments have been confirmed to be capable of precipitating calcium carbonate in both natural and laboratory settings. Different types of bacteria, as well as abiotic factors (salinity and medium composition), appear to contribute to calcium carbonate precipitation in a number of ways in a variety of environments. CaCO_3 precipitation needs a large enough volume of calcium and carbonate ions so that the ion activity product (IAP) exceeds the solubility constant. Precipitation of calcium carbonate is a simple chemical process governed by four main factors:

- The calcium concentration.
- The concentration of dissolved inorganic carbon.
- The PH.
- The availability of nucleation sites.

1.5 Objective

- Optimization of bacteria density
- To determine the ideal bacteria dose for bacterial concrete.
- To study the crack healing process by bacteria on plain cement concrete.
- To suggest a better constructive material for sustainable building.

1.6 Scope

- To enhance the microbial activities for pollution free and natural environment.
- To introduce bio mineralisation in concrete enhancing the performance of concrete.
- To remediate cracks and fissures occurring on concrete.
- Develop efficient self-healing techniques that enable concrete to regain liquid tightness by suitable micro-organisms.
- To maintain a crack-removal method that is both environmentally sustainable and reliable.

CHAPTER 2

LITERATURE SURVEY

2.1 General

This is made and planned to by reviewing some of the literature. The importance of reviewing the literature is to analyse the research works related to the study.

2.2 Review of literature

Jonkers et al(2007), New type of self-healing concrete in which bacteria mediate the processing of minerals that quickly seal newly formed cracks, a process that also reduces the permeability of the concrete and thus improves its protection. Calcite-embedded steel reinforcement Endospores formed by *B.cohnii* appear as bright intracellular spheres in this culture. Since metabolic conversion of citrate resulted in increased alkalinity and decreased chelating ability, bacterial cultures did produce calcite. The addition of a large number of bacterial spores to the paste (6 108 cm³) resulted in a 10% reduction in compressive power, with values of 91, 92, and 93 percent after 3, 7, and 28 days (compared to controls).

Ramakrishnan et al (2005), It is discussed how microbiologically induced calcite (CaCo₃) precipitation can be used to patch concrete cracks and fissures. The technique of microbiologically induced calcite precipitation (MICP) is part of the biomineralization branch of research. Different bacteria concentrations were used in the study. Bacteria were found to boost the stiffness, compressive strength, modulus of rupture, and durability of concrete. The function of microbiologically induced mineral precipitation in improving the strength and durability of concrete was studied using a scanning electron microscope (SEM). Analysis of the precipitated crystal using an Energy Dispersive X-ray (EDX) spectrometer indicated abundance of calcium and the precipitation was confirmed to be calcite.

Yathishreddy et al(2020), Four distinct bacteria were compiled to study the mechanical properties of concrete. The addition of a large number of bacteria to the concrete mix. Polyurethane, Silica gel, Hydrogel, Diatomaceous earth, Melamine based capsules, Light weight aggregates, and Graphite Nanoparticles were among the materials used to add bacteria to the concrete, either directly or by encapsulation. Many scientists have used *Bacillus Subtilis* bacteria to build self-healing concrete. In the concrete industry, this bacterium-based self-healing concrete has a promising future.

Rathnayaka et al(2018), Alkalinity of bacterial concrete The addition of bacteria to the concrete significantly improves the overall consistency of the concrete compared to plain concrete. Bacteria fill in cracks in concrete by forming calcium carbonate crystals, which fill in and seal the cracks. According to the literature on self-healing concrete, bacterial concrete has a higher strength than standard concrete, rising by 13.75 percent in three days, 14.28 percent in seven days, and 18.35 percent in 28 days.

Shradha Jena et al(2020), The permeability and compressive strength of concrete are also impaired by the presence of bacterial specimens. The percentage of water and chloride permeability in concrete is significantly decreased when bacterial specimens are present due to their ability to block the cracks. Concrete is shielded from freeze-thaw damage by biological reactions involving bacteria, which enhances the concrete's properties. In comparison to control concrete, the volume of chloride that reaches the bacterial sample decreases as bacteria are used. The self-healing concrete stops new cracks from developing while also restoring those that already exist.

Chithambar Ganesh et al(2019), When *Bacillus sphaericus* and *Bacillus subtilis* are mixed with polypropylene fibre reinforced concrete, they have the ability to self-heal. The concrete was made with just the right amount of bacteria and polypropylene material. Artificial cracks in the concrete were made to conduct this investigation. The broken concrete was detected at regular intervals. The efficacy of self-healing was evaluated using a mechanical test over bacterial concrete with fibres (BCF). The findings of the mechanical analysis show that the BCF obtained

commendable results. SEM was used to evaluate the precipitation characteristics, and XRD diffractogram analysis was used to validate the precipitation in BCF. The results show that there is enough self-healing compound present in broken concrete. The efficacy of the formed BCF concrete in regaining strength shows a significant increase in strength after self-healing, so BCF self-healing is commendable.

Lagazo et al(2019), To plug holes, *Bacillus Subtilis* has been used to precipitate calcium carbonate. MICP, or Microbiologically Induced Calcite Precipitation, When bacteria are mixed with nutrient broth to create conditions where they can close cracks and pores, is a urease enzyme phenomenon that can be effectively used. Calcite deposits have been discovered to be effective at sealing cracks and solidifying the surface finish of concrete. This process, which can be referred to as an organic remediation for concrete healing, can be used in a number of industrial applications. Calcite precipitation may aid in the exclusion of moisture. When the concrete or the crack comes into contact with moisture, the bacteria will activate and the crack will heal.

Abhishek Pandit et al(2018), Bacteria patch concrete cracks by forming calcium carbonate crystals, which seal micro cracks while increasing the material's strength and durability. Bacterial concrete has been researched extensively, and it has been discovered that it raises the strength of traditional concrete by 15% in 7 days and 18-20% in 28 days. By narrowing the gaps, microbiology-induced calcium carbonate precipitation fills the voids and decreases water permeability. Corrosion resistance, drying shrinkage resistance, acid attack resistance, and sulphate attack resistance all increase with the addition of bacteria. Bacterial concrete with admixtures such as silica fume was also discovered. Fly ash and natural or synthetic fibres (polypropylene, steel, etc.) also improve strength and durability. Although the cost of bacterial concrete tends to be higher at first sight, it is more cost efficient when bought in bulk and offset by lower repair costs.

Seshagiri Rao et al(2017), The use of bio-mineralization of bacteria in concrete was tested. Micro cracks penetrate the reinforcement and are responsible for the transport of gases and harmful chemicals, causing concrete to deteriorate

and start to corrode. The use of calcite mineral precipitating bacteria for concrete repair and plugging of pores and cracks in concrete has been studied. Synthetic polymers used in concrete repair, such as epoxy, are detrimental to the environment; therefore, the use of a biological repair technique in concrete is prioritised. *Bacillus subtilis* JC3, an alkaliphilic aerobic microorganism, was induced into cement mortar samples at different cell concentrations. The use of bio-mineralization of bacteria in concrete was tested. Micro cracks penetrate the reinforcement and are responsible for the transport of gases and harmful chemicals, causing concrete to deteriorate and start to corrode. The use of calcite mineral precipitating bacteria for concrete repair and plugging of pores and cracks in concrete has been studied. Synthetic polymers used in concrete repair, such as epoxy, are detrimental to the environment; therefore, the use of a biological repair technique in concrete is prioritised. *Bacillus subtilis* JC3, an alkaliphilic aerobic microorganism, was induced into cement mortar samples at different cell concentrations.

Farzaneh Nosouhian et al (2015), Researchers studied concrete toughness improvement in a sulphate setting using two different bacterial strains in varying concentrations of bacteria. After seven groups of 70-mm concrete prisms were made using two different bacterial strains and mixing water, the effects of sulphate solution exposure on durability properties of tested specimens, including variations in density, volume, water absorption, and compressive power, were measured. Concrete discs with a diameter of 100 mm and a thickness of 50 mm were made from the same batches to investigate the chloride permeability of bacterial concrete using RCPT. The average compressive strength of bacteria-infested concrete increased by 16.2 and 20.8 percent after 28 and 270 days, respectively. Concrete chloride penetration, mass, volume variation, and water absorption were found to be reduced by *S. pasteurii* and *B. subtilis*, resulting in an increase in bacterial concrete compressive strength. Calcium precipitating bacteria tend to strengthen concrete resilience by producing a denser and thus less permeable microstructure, resulting in increased durability.

Naveenand et al(2012), A paper on the effect of calcite-hardening microbes on the self-mending portion of cement was distributed. The researchers used a natural treatment that included a *B.sphaericus* culture concentrated in a gel grid with a calcium source added. They used silica gel to protect the microscopic organisms from the solid's pH, which they discovered was still viable as the CaCO_3 gems accelerated within the grid. As a result of the break patch, the water became brittle. Precious stone precipitation increased the material's power.

Day et al(2017), The findings of a novel approach to solid break remediation using microbiologically prompted calcite are depicted in this paper. *Bacillus pasteurii*, a popular soil bacterium, The calcite precipitation process was started using this method. The microbial urease hydrolyzes urea to create smelling salts and carbon dioxide, and the alkali emitted into the atmosphere increases pH, allowing insoluble calcite to accumulate, are the basic requirements for this application. To protect the microorganisms from the high pH of cement, they were immobilised in polyurethane polymer, lime, silica smoke, and fly fiery debris, and then connected in solid split remediation.

CHAPTER 3

MATERIALS AND METHODS

3.1 Cement

Binder is a building material that sets, hardens, and adheres to other materials to hold them together. Cement is typically used to bind sand and gravel (aggregate), rather than being used alone. Concrete is made from sand and gravel, while masonry mortar is made from cement mixed with fine aggregate. Concrete is the world's most widely used material, accounting for more than a fifth of all building projects.

Table 3.1 Chemical composition of cement

Components	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	SO ₃
PPC %	21.28	5.60	2.85	64.18	1.66	2.57

Table 3.2 Properties of cement

Particulars (IS 456:2000)	Range
Specific Gravity	3.2
Consistency	37%
Fineness modulus	10% should be retained
Initial setting time	30 mins
Final setting time	600 mins

3.2 Aggregate

Aggregate is a building and construction material that is combined with cement, lime, gypsum, or another adhesive to create concrete or mortar. The aggregate guarantees consistency and volume in the finished product, as well as resistance to wear and erosion and other physical properties. Fine aggregate is used to make thin concrete slabs or other smooth-surfaced structural members, while coarse aggregate is used to make thick concrete slabs or other rough-surfaced structural members.

3.2.1 Classification of the Aggregates

The coarse Aggregates are mainly classified are as follows

- According to the source or the nature of the formation of the aggregate.
- According to the size of the aggregate
- According to the shape of the aggregate

3.2.2 Fine aggregate

Fine aggregates are any natural sand particles extracted from the ground through the mining process. Fine aggregates are made up of natural sand or crushed stone particles with a diameter of 14 inches or less. Because of the scale, or grading, of this particular aggregate, it is sometimes referred to as 1/4" minus.

3.2.2.1 Sand

Fine aggregate, which is made up of natural sand or crushed stone, is an important component of concrete. The hardened properties of concrete are highly affected by the fine aggregate density and strength. Fine aggregates are the structural filler in concrete mix formulations, accounting for the bulk of the volume. The production can be significantly affected by the composition, form, scale, and other properties of fine aggregate.



Fig3.1 River sand

Table3.3 properties of sand

Particulars (IS383)	Range
Specific gravity	2.65
Bulk density	1.71 kg/m ³
Fineness modulus	5.24

3.2.2.2 *Msand*

River sand extraction is detrimental to the ecosystem because it lowers groundwater levels and dries up river water. Excess dragging of sand from the river, the water table level decreases, and tree roots may be unable to receive water in the absence of sand. In the absence of sand, river water evaporates due to direct sunlight. It is suggested that crushed sand be used. It is a cost-effective and environmentally friendly alternative to natural sand. Except for plaster and waterproofing work, crushed sand can be used in construction for anything except bedding under the stone. For better results, 50 percent m sand and 50 percent river sand should be used for hardscape work.



Fig3.2 Manufactured sand(msand)

Table 3.4 Properties of Msand

Particulars (IS383)	Range
Specific Gravity	2.5-2.9
Bulk density	1.75 kg/m ³
Fineness modulus	4.66
Grading	Zone I, Zone II, Zone III, Zone IV.
Water absorption	The ability to old surface moisture of Crushed sand is up to 10%.

3.2.3 Coarse aggregate

Coarse aggregates are irregularly broken stone or naturally rounded gravel that are used to make concrete. Coarse aggregates are materials that are too wide to pass through a 4.75 mm sieve. Blasting in stone quarries, or breaking them by hand or with crushers, are the most common methods for extracting coarse aggregates. Machine-crushed aggregates are made up of stones of different sizes, while hand-broken aggregates are made up of just one size stone. To make graded aggregates for high-class concrete, they're mixed together again in various proportions. With a maximum size of 20 mm and a minimum size of 10 mm, coarse aggregates must be well graded and uniform in scale.

Table3.5 Properties of coarse aggregate

Specific gravity	2.78
Water absorption	1.0%
Impact value	5.7%
Crushing value	18.72%
Bulk density	1935.3kg/m ³



Fig3.3 Coarse aggregate

3.3 Water

Water is the least costly but most essential component of concrete. Water used to mix concrete should be sterile and clear of harmful impurities such as tar, alkali, acid, and other chemicals. For mixing, curing and other constructional works portable water is used.

3.4 Bacteria

Bacteria are single-celled organisms with a limited footprint. Bacteria can be found almost anywhere on the earth and are essential to its ecosystems. Some animals are able to survive in extremes of temperature and pressure. Bacteria

abound in the human body, which is estimated to contain more bacterial cells than human cells.

3.4.1 Selection of bacteria

The most suitable bacteria for self-healing concrete are chosen primarily for their carbonate yield. Bacteria are often isolated from carbonate-producing habitats including calcareous blocks, calcareous sludge, and calcareous soil. The best results were obtained with *B. Subtilis* (via oxidative deamination of amino acids), which produced 0.6 g CaCO_3 per gramme of organic matter. This organism was then used in the field to consolidate limestone monuments. *B. cohnii* cells in suspension (108 cells/mL) When 7.5 g/L acetate was degraded, it produced about 25 g/L CaCO_3 (1.7 g/(L.d)), with the first two days producing the most. Several ureolytic bacteria strains Even at lower temperatures of 10°C, the mesophilic *Bacillus sphaericus* produced more carbonate in the shortest amount of time than other cold-tolerant strains like *Sporosarcinapsychrophila*. MICP was investigated in a low-nutrient environment using denitrification. The ability of two newly isolated resilient strains to precipitate CaCO_3 , *Diaphorobacternitroreducens* and *Pseudomonas aeruginosa*, were optimised in this analysis, as well as the repetitive CaCO_3 precipitation of a single inoculum. CaCO_3 precipitation yields ranged between 14.1 and 18.9 grammes. However, a direct comparison is difficult to make because there are numerous variables regulating the CaCO_3 precipitation yield that must be optimised and that interfere with one another, such as bacterial cell concentration, urea concentration, organic acids or nitrate concentration, and calcium concentration.

3.4.2 *Bacillus subtilis*

Bacillus subtilis is a Gram-positive, catalase-positive bacterium that can be found in soil, ruminant intestines, and human intestines. *B. subtilis* is a rod-shaped *Bacillus* species that produces a tough, protective endospore that helps it to survive in harsh conditions. Despite proof that it is a facultative anaerobe, *B. subtilis* has been classified as an obligate aerobe. The model organism *B. subtilis* is used to

research bacterial chromosome replication and cell differentiation. The Gram-positive bacterium has received the most attention. *B. subtilis* has been studied extensively for cytoplasmic pH homeostasis, which preserves cytoplasmic pH between 7.3 and 7.6 during vegetative growth over a pH range of 6.0 to 9.0 in the environment.

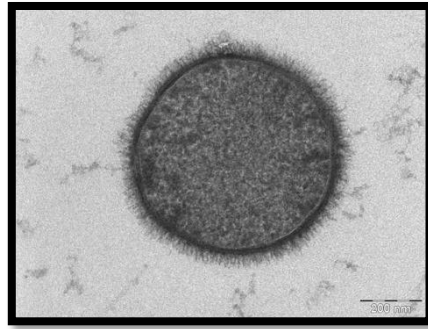


Fig3.4TEM micrograph of *B.subtilis* cell in cross-section

3.4.2.1 Characterization of *bacillus subtilis*

Bacillus Subtilis is a bacteria that is also known as hay bacillus or grass bacillus. *Bacillus* is a Gram-positive bacterium. *Bacillus B subtilis* is a rod-shaped bacteria that can form a solid, protective endospore, allowing it to survive in harsh environments. *Bacillus Subtilis* cells are rod-shaped gram-positive bacteria living in soil and vegetation.

Bacillus Subtilis thrives in a mesophilic setting. The ideal temperature range is between 25 and 35 degrees Celsius. In this setting, stress and hunger are normal. *Bacillus Subtilis* has developed a range of adaptations to help it survive in such harsh environments. The formation of stress-tolerant endospores is one such technique. *Bacillus Subtilis* bacteria are strictly aerobic bacteria, which means they require oxygen to grow and cannot ferment. They are not pathogenic in any way. They can contaminate food, but only a small percentage of the time do they cause food poisoning. *Bacillus Subtilis* is currently the topic of many scientific studies.

3.5 Mix design

The process of selecting suitable elements of cement, such as bond, totals, and water, and determining their relative proportions with the aim of producing cement with the least amount of consistency, workability, and durability for the least amount of money, is known as blend configuration. The above definitions provide an explanation for outlining as a two-overlay. The main goal is to meet the minimum quality and solidity requirements. The cement evaluations used in this analysis were standard grade concrete and conventional examination concrete.

Table3.6Mix design for plain concrete(IS 456:2000)

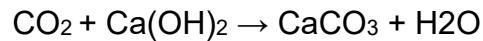
Material	1:1.55:2.45:0.44
Cement(kg/m)	435.45
Sand(kg/m)	676
Aggregate(kg/m)	1067
Water(L)	191.5

Table3.7Mix design for bacterial concrete(IS10262:2009)

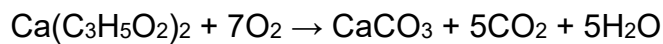
Material	1:1.3:2.75:0.45
Cement (kg/m)	403.2
Sand (kg/m)	642
Aggregate(kg/m)	1052
Water(L)	201.6

The table 3.5, explains the mix proportion for bacterial concrete with different ratio 1:1.3:2.75. So the concrete bacterial mix ration contains 1 part of cement, 1.3 part of sand which fine aggregate and 2.75 part of aggregate . After reviewing the above-mentioned literature, preparation of bacterial cement with the replacement of water with bacterial water . Table3.6 explains the mix ration for M25 concrete which has 1:1.9:3.1 ratio . Calcium Carbonate can form on the surface of

control concrete as a result of CO₂ in the concrete matrix reacting with Calcium Hydroxide in the concrete matrix, as shown below:



Since Ca (OH)₂ is a soluble mineral, it dissolves in water and then diffuses out of the crack as leaching. Due to the active metabolic conversion of Calcium nutrients by the bacteria present in concrete, the self-healing process in bacteria integrated concrete is much more efficient:



Microbial metabolic processes and autogeneous healing contain calcium carbonate both directly and indirectly. A bacteria-based crack sealing mechanism is formed as a result of this process.

3.6 Factors affecting bacterial growth

Physical factors:

- Temperature affects bacteria development. Over a temperature range of 300oC, most bacteria can grow.
- pH- Alkliphiles thrive in high pH (Bacillus Subtilus).
- Moisture – For transport, single cells require nutrients to be dissolved in a solvent, which is influenced by the amount of oxygen present in the atmosphere.
- Hydrostatic pressure and chromatic pressure are two different types of pressure.

3.7 Experimental methods

Experimental methods explain about the methods used in the preparation of Bacterial concrete and also the types of test to be conducted for the prepared specimen according to the standard procedure.



Fig.3.5 Bacillus subtilis



Fig.3.6 Addition of bacteria to water



Fig.3.7 Adding Bacteria water



Fig3.8Bacterial concrete



Fig3.9Casting



Fig3.10Compaction

3.7.1 Preparation bacterial solution

The solution is prepared with 1000ml of water mixed with 24ml of bacteria broth into it. Distribute the solution into 4 different containers equally (250ml) for easy growth of bacteria in small quantities. Then the germination of bacteria occurs within the container. Transfer the bacterial solution to single container after 12 hours of growth in four different containers and it is stored in the growth. The temperature is held at 37 degrees Fahrenheit.

3.7.2 Bacterial concrete

Once the solution is ready, the bacterial solution is added during the mixing of concrete. Instead of using water for mixing of concrete, the proportion of bacteria is fixed and introduced as per the portion of water in concrete. The proportions of the bacteria are 10^5 cells and 10^6 cells i.e 24ml in 1litre and 240ml in 1litre

3.7.3 Casting of self-healing concrete

The casting of convention and bacteria concrete for flexural strength test and compression strength test are described as follows

3.7.3.1 *Casting of cubes*

The concrete cube specimen are casted by the consideration of following steps

- Cube Mould - The cube moulds of required size 150 x 150 x 150 mm for nominal size aggregate not exceeding 38 mm. cube moulds shall be provided with a base plate and they shall be as per IS:10086-1982.
- Tamping rod - As per IS10086-1982, 16 mm diameter and 600 mm long with rounded working end and shall be made of mild steel.
- Casting process - Remove the cube mould plates, thoroughly clean them, and completely tighten all of the bolts. After that, apply a thin layer of oil to each of the mould's faces. It's vital that the cube's side faces are parallel. The cubes should be cast as soon as possible after taking concrete samples and combining them. The concrete sample should be poured into the cube moulds in 5 cm thick layers. To ensure a symmetrical distribution of concrete inside the mould, each scoopful of concrete should be pushed around the top edge of the mould as it slides out. Both sheets need hand or vibration compacting.

3.7.3.2 *Casting of Beams*

According to standards Concrete beam are casted to evaluate the flexural strength of the concrete. The casting and testing procedure to be followed as Indian standard code IS 516:1959.

- Cylinder Mould - The mould standard size will be 150 x 150 x 700 made out of metal. The tamping bar must be a steel bar that weighs 2 kg, is 40 cm long, and has a ramming face that is 25 mm square. The test specimen must be rendered as soon as possible after mixing and in such a way that the concrete is completely compacted with no segregation or unnecessary laitance.
- Casting Process - The compacted material must be distributed uniformly across the mould section. Rodding the bottom layer all the way to the bottom is needed. For each upper layer, the rod can penetrate about 25 mm into the underlying layer. If the rod creates

voids in the mould, tap the sides of the mould to fill the voids before adding the next layer of material.

3.7.4 Demoulding

Between 16 and 24 hours after being made, test concrete test specimens should be demoulded. If the concrete has not reached sufficient strength to enable demoulding without damaging the specimens after this time, the demoulding should be postponed for another 24 hours. Remove the concrete specimens from the mould by dismantling it entirely. Take care not to harm the specimens because any cracking would reduce any of the strength parameters. After demoulding, use a waterproof crayon or ink to write a legible identification on the top or bottom of each test concrete specimen. After demoulding, the mould must be thoroughly washed. Ensure that no grease or dirt collects between the flanges' faces; otherwise, the two halves would not fit together properly, causing leakage through the joint and possibly an irregular form.



Fig3.11Demoulded block

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Compression test

The compression test of plain concrete cube and bacteria concrete cube could do with the help of the Universal testing machine with the capacity of 400 KN. The compressive strength of the concrete blocks was measured on 150 mm x 150 mm x 150 mm. All cubes were casted and kept in curing tank until the testing dates. The first compression test for plain concrete block and bacteria concrete should be done at 7 days. The strength of self-healing concrete is higher than the plain concrete.



Fig4.1 Compression testing machine under load

Table 4.1 Compression test value

Days	Plain concrete (N/mm ²)	Bacterial concrete (N/mm ²)	Increase in strength(%)
7	20.57	25.34	23.71
14	25.93	30.51	17.65
28	37.90	44.37	16.42

The bar graph 4.2, is the compression test value obtained from observed result.

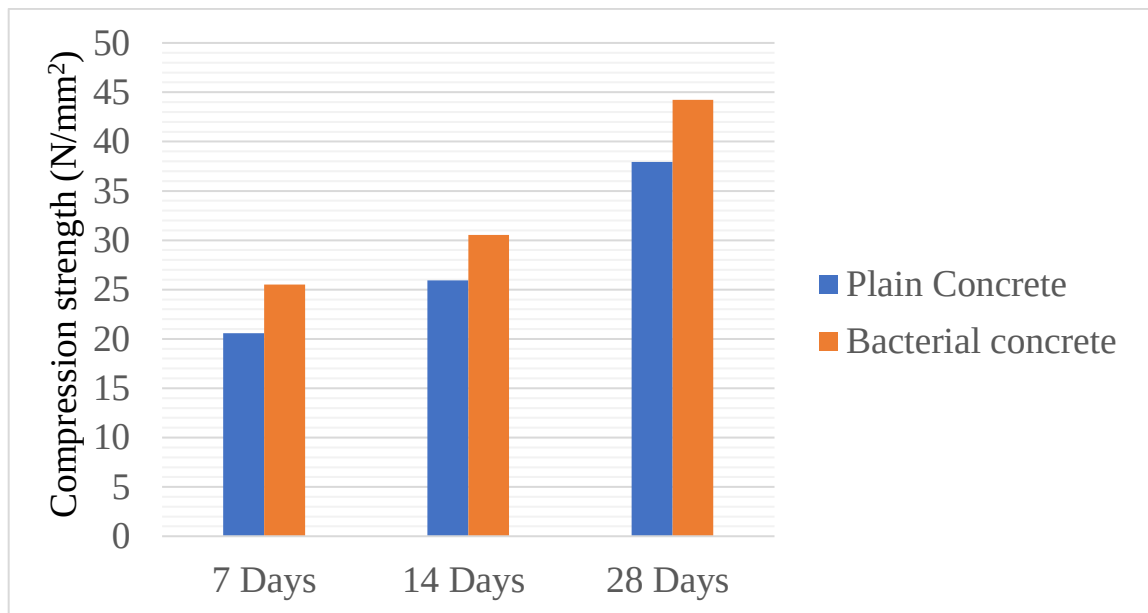


Fig.4.2 Compression strength

Therefore, in this study that bacterial concrete gives a positive result when evaluating compressive power. It also aids in the healing of cracks and the corrosion of steel. Consequently, concrete's permeability is also decreased.

$$\text{Compressive strength of concrete} = \frac{\text{Max load carried by specimen}}{\text{Top surface area of specimen}}$$

$$\begin{aligned} \text{The top surface are of specimen} &= 150 \times 150 \\ &= 22500 \text{ mm}^2 \end{aligned}$$

The compression load for 7th day specimen is 463KN

$$\text{Compressive strength of concrete} = 463000/22500=20.57 \text{ N/mm}^2$$

4.2 Flexural test

The flexural strength of concrete is a strong predictor of its tensile strength. At the point of failure in bending, it is the maximum stress on the strain face of an unreinforced concrete beam or slab. It's calculated by pouring three times the depth span capacity of 150 x 150 mm concrete beams. Flexural strength is calculated

using ASTM C293 standard test methods and expressed as Modulus of Rupture (MR) in MPa (center-point loading). Since the size of the specimen and the form of loading affect the flexural power measured, comparisons and specifications should be made for the same beam size and loading configuration.



Fig.4.3 Flexural strength test Machine

Table4.2 Flexural strength value

Days	Flexural strength of normal concrete (Mpa)	Flexural strength of bacteria concrete (Mpa)	Increased strength
14	2.72	3.10	18.09
28	3.24	3.97	26.48

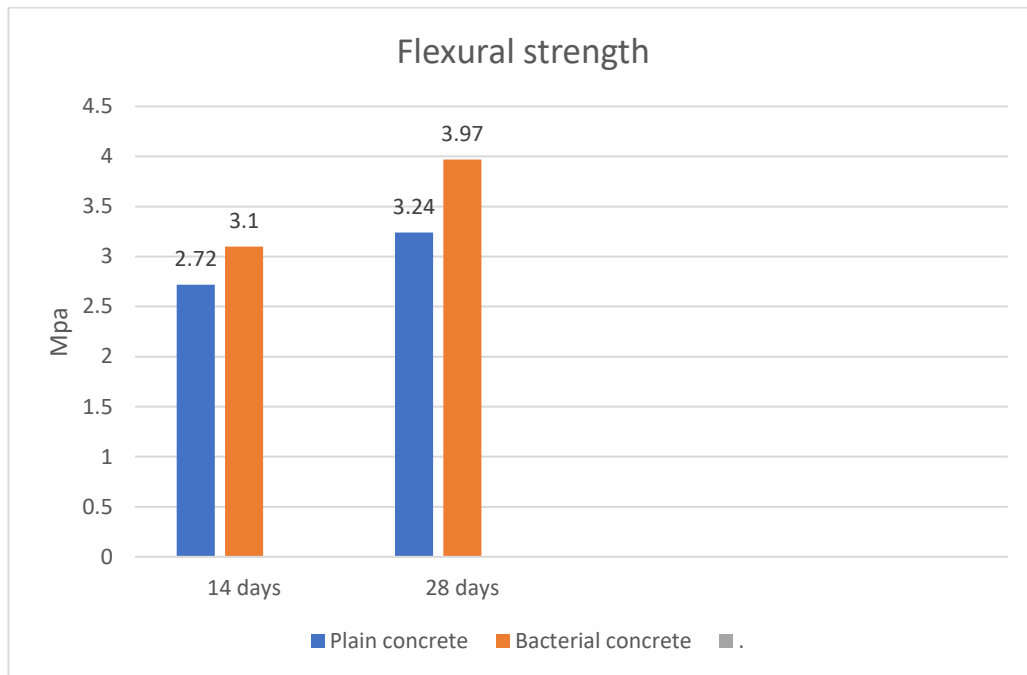


Fig4.4 Flexural strength

The above table 4.5 defines the flexural strength qualities determined in both natural and bacterial concrete. The Modulus of Rupture (MR) in psi was used to determine the flexural power (MPa). It assesses the concrete or slab's tensile strength in order to prevent bending failure. The flexural strength of normal concrete increases by 2.71 percent as compared to bacterial concrete. As a result, if we use self-healing concrete, we will minimise bending failure while increasing tensile strength.

CHAPTER V

SUMMARY AND CONCLUSIONS

5.1 Summary

The aim of this project is to describe self-healing concrete using the *Bacillus subtilis* bacteria. Many other bacteria have been identified in the literature as having the ability to enhance the strength and other chemical properties of concrete. Many literatures were studied related to our project to gain knowledge and to develop our creative and innovative skills. The properties of materials such as sand, Msand, aggregates, and cement were tested and compared with IS considerations which should be satisfied. Then the bacterial concrete 1:1.3:2.75 were allowed to prepared. The hardened specimen was tested at the required testing date. Till the test date the specimen was kept in curing tank, where we observe the change of water colour in bacterial concrete tank which shows the activation of bacteria

As compared to traditional concrete, the compressive strength of bacterial concrete gradually increases. The open porosity and apparent density of concrete mix ratio were considered to be different for conventional concrete to bacterial concrete to study the variance of compressive strength.

The presence of ample organic matter derived from microorganism biomass in the matrix also led to the overall increase in strength. This biomass is generated by the death of cells or the transformation of bacteria into endospores, which function as organic fibres.

The presence of a calcite layer on the surface limited the water absorption in the mortar samples. *Bacillus subtilis* deposition resulted in a decline in water permeability in concrete that had cracks fixed. The porosity was decreased while the dynamic modulus strength was increased. The permeability of both gas and chlorine was reduced. The 400 m crack surface width was fully filled after 44 days. *Bacillus Subtilis*, as a result, may be a promising concrete admixture for enhancing

the hardness and mechanical properties of concrete. The percentage difference in flexural strength between natural and bacterial concrete.

5.2 Conclusions

Self-healing concrete has the potential to address the issue of concrete buildings rotting long before their service lives are over. From building foundations to bridge structures and underground parking lots, concrete is one of the most commonly used materials in the construction industry.. When exposed to tension, traditional concrete has a flaw: it cracks.

Owing to the poor tensile strength of concrete, cracks are a normal occurrence. By providing a convenient route for the transportation of liquids and gases that may contain toxic chemicals, these cracks minimise the toughness of concrete. Concrete is a multiscale material with a heterogeneous internal material structure that has a significant impact on its engineering behaviour. A multiscale approach is needed to evaluate the significance of a bacteria in relation to concrete cracks.

In Bio Geo Civil Engineering, microbial concrete is becoming more natural. Microbial concrete has been found to be useful in a variety of applications, including increasing the hardness of cementitious materials and improving sand properties, as well as preserving limestone monuments, sealing concrete cracks, and making highly durable bricks. A bacterial cement building's longevity is determined by the environment in which it is built. A movement of water and chloride is present. The high costs are difficult for investors to justify. The high costs are difficult for investors to justify. Investors and designers are unaware of bacterial concrete's ability to self-repair, allowing the structure to last longer and cost less. Only the high production costs and, as a result, the initially high commodity costs are apparent to them. Another problem is that most contractors only have a ten-year house warranty, which does not cover cracks. The advantages of such concrete could not become evident for several years, if ever. As a result, contractors are unlikely to invest in this technology. There are moments, however, when the benefits of self-healing

concrete outweigh any cost considerations. There are examples of problems where the preservation of precious items is more important than the preservation of money.

Concrete self-healing technology is one of the possible solutions for eliminating carbon dioxide emissions from cement production while also avoiding unnecessary concrete structure repair and maintenance. Concrete as a construction material would be in higher demand as the need for infrastructure expansion grows. Concrete manufacturing has a high carbon footprint and requires a lot of resources. Due to limited capital and environmental considerations, concrete material cannot be sustained in the long run. As a result, a sustainable concrete material must be developed to meet demand while still preserving the environment. The properties of this concrete will be better understood in the coming years as a result of a greater number of full-scale experiments, and the manufacturing methods will be less expensive. It has the potential to be a long-term solution to the concrete industry's current problems. Both the manufacturing industry and the general public are looking for products that use less energy and emit less carbon dioxide from the moment they are produced until they naturally decompose. It's also anticipated that such materials and structures would last a long time, at least 50 years (according to the standard), and will be simple to repair.

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