A Thesis on

A STUDY ON FLEXURAL BEHAVIOUR OF BIO-CONCRETE BEAMS

Submitted in Partial Fulfillment for the Award of DEGREE OF

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In

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Submitted by

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DECLARATION

We declare that the Project entitled **"A Study on Flexural behavior of Bio-Concrete Beams",** is the bonafide work carried out by me, under the guidance of **Mr. Mohd Bilal Khan, Assistant Professor, Department of Civil Engineering, Integral University, Lucknow.** Further we declare that this has not previously formed the basis of award of any degree, diploma, associate-ship or other similar degrees or diplomas, and has not been submitted anywhere else.

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CERTIFICATE

This is to certify that the entitled **“A Study on Flexural behavior of Bio-Concrete Beams”**, is being submitted by Hera Fatima (Roll No. 1801431005), in partial fulfillment of the requirement for the award of the **Master of Technology in Structural Engineering of Integral University, Lucknow** is a record of candidate’s own work carried out by him under our supervision and guidance.

The results presented in this thesis have not been submitted to any other university or institute for the award of any degree or diploma.

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ABSTRACT

Concrete is the most widely used construction material because of its high compressive strength, relatively low cost, etc. One adverse property of concrete is its sensitivity to crack formation as a consequence of its limited tensile strength. For that reason, concrete is mostly combined with steel reinforcement to carry the tensile loads. Although these rebar restrict the crack width, they Concrete is very sensitive to crack formation. As wide cracks endanger the durability, repair may be required. However, these repair works raise the life-cycle cost of concrete as they are labour intensive and because the structure becomes in disuse during repair. In the following years, several researchers started to investigate this topic. The "Bacterial Concrete" is a concrete which can be made by embedding bacteria in the concrete that can constantly precipitate calcite. Cracking in the surface layer of concrete mainly reduces its durability, since cracks are responsible for the passage of liquids and gasses that could potentially contain deleterious substances. When micro-cracks migrates towards the reinforcement, not only the concrete itself may be damaged, but also corrosion occurs in the reinforcement due to exposure to water and oxygen, and possibly CO_2 and chlorides too. Micro-cracks are therefore the main cause to structural failure. One way to circumvent costly manual maintenance and repair is to incorporate an autonomous self -healing mechanism in concrete. One such an alternative repair mechanism is currently being studied, i.e. a novel technique based on the application of bio-mineralization of bacteria in concrete. Synthetic polymers such as epoxy treatment etc. are currently being used for repair of concrete are harmful to the environment, hence the use of a biological repair technique in concrete is focused. In the present paper, an attempt is made to incorporate dormant but viable bacteria in the concrete matrix which will contribute to the strength and durability of the concrete. Water which enters the concrete will activate the dormant bacteria which in turn will give strength to the concrete through the process of metabolically mediated calcium carbonate precipitation. Concrete, due to its high internal pH, relative dryness and lack of nutrients needed for growth, is a rather hostile environment for common bacteria, but there are some extremophiles spore forming bacteria may be able to survive in this environment and increase the strength and durability of cement concrete. Overview of development of bioengineered concrete using bacterial strain Bacillus Cereus and its enhanced mechanical and durable characteristics will be briefly described in this paper.

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Worlds may fail us, when we think of all, that our parents have done for us.

Throughout life, they have always been a constant source of inspiration and whose administration helps us in our academic pursuits. Their sacrifices, prays and good wishes made us to reach at this stage.

Hera Fatima
M. Tech (Structural Engineering)

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CHAPTER 1: INTRODUCTION

Many concrete structures including infrastructure suffers from serious deterioration all over the world. Thus inspection and maintenance techniques for concrete structure have become the focus of increasing attention. A concrete mix composed only of hydrated cement and fine aggregate is defined as mortar. Usage of cement per year is million metric tons. Types of mortars are lime mortars, gypsum mortar and cement mortars. Natural processes like earthquakes and human activities create fractures and fissures in concrete structures and historical stone monuments due to their weak strength and durability. Even though the use of bacteria in cement mortar/concrete is an untraditional concept in current civil engineering, it is a new approach to an old idea that a microbial mineral deposit constantly occurs in natural environments.

The current demand for the concrete is massive. For centuries, one of the most common materials used in construction has been concrete. Formed from hardened cement, concrete has been used for everything from driveways to home foundations. However, as technology advances concrete has not remained the end-all tool for building. It has a variety of disadvantages that should be considered before use. One main disadvantage of concrete is that all structures made from it will crack at some point. Concrete can also crack as a result of shrinkage, which happens when it dries out. Earlier strength was the main criteria considered during construction. In recent years, it has been focused out that not only structural safety but also durability is significant when designing building or concrete structures. Concrete can bear up with compression load, but the material is weak in tension. Hence, steel reinforcement is provided and the steel bars take over the load when the concrete cracks in tension. However, the development of cracks in the concrete pose a problem. Due to reasons like freeze-thaw reactions, shrinkage, low tensile strength of concrete etc, cracks develop during the process of concrete hardening resulting in weakening of the buildings. Due to lack of permeability, water droplets seeping inside can damage the steel reinforcement present in the concrete member.

When this phenomenon occurs, the strength of the concrete decreases causing the decay of structure .Synthetic materials like epoxies are used to remediate, but costly, not compatible and need time to time maintenance. Using chemicals is also causing damage to the environment. Although concrete is the world’s most used building material, it has a serious flaw. It can easily crack under tension. The researchers said, if these cracks become too large, they will lead to corrosion of the steel reinforcement, which not only results in an unattractive appearance but also jeopardizes the structure’s mechanical qualities.

Crack prevention is often employed by steel reinforcement which is highly expensive and requires large amounts of steel which will never be acceptable. Another disadvantage is, in case of larger constructions such as big dams or projects, if there we observed any crack or corrosion, it becomes deadly difficult to go there and repair it. Although it is known that it is costly to inspect, monitor and repair cracks. Rapidly developing construction, particularly in developing countries, contributes to environmental pollution, high energy consumption and natural resources. These actions have a direct impact on the comfort and heath of building inhabitants. Already in the 1970s, research was commenced into the harmful effect of building materials on users' health. As a result of the research, ecological materials were introduced, e.g., silicate blocks, materials based on gypsum binders, paints, wood, etc. These materials are intended to promote human health .As a result of the research, ecological materials were introduced, e.g., silicate blocks, materials based on gypsum binders, paints, wood, etc. These materials are intended to promote human health. Additionally, they are supposed to be of only a minimal burden to the environment.

Cracks in concrete are a common phenomenon due to the relatively low tensile strength. Durability of concrete is impaired by these cracks since they provide an easy path for the transportation of liquids and gasses that potentially contain harmful substances. If micro-cracks grow and reach the reinforcement, not only the concrete itself may be attacked, but also the reinforcement will be corroded. Current study has overview that biotechnology can really be a supportive device to reduce micro cracks in concrete structures by using bacillus species of bacterial in concrete. This latest category of concrete, that is set to fix itself, shows a powerful enormous improvement in community infrastructure's service-life, there by considerably reducing the maintenance costs and lowering CO2 emissions. Several investigations are made of bacterial concrete in last few years.

When this phenomenon occurs, the strength of the concrete decreases causing the decay of structure .Synthetic materials like epoxies are used to remediate, but costly, not compatible and need time to time maintenance. Using chemicals is also causing damage to the environment. The need for an environment friendly and effective alternate crack remediation technique leads to the development of using the bio mineralization technique in concrete. Here we are incorporating calcite precipitating bacteria to concrete in certain concentrations so that the bacteria will precipitate calcium carbonate when it comes in contact with water and this precipitate will heal the cracks.

Micro biologically Induced Calcite Precipitation (MICP) is the process behind bio mineralization. The basic principle in the process is that the microbial urease, hydrolyzes urea, to produce ammonia and carbon dioxide and the ammonia released in surroundings subsequently increases the pH, leading to accumulation of insoluble calcium carbonate. Thus, this self-healing system can help in achieving a massive cost reduction in terms of health monitoring, damage detection and maintenance of concrete structures which assures a safe service life of the structure.

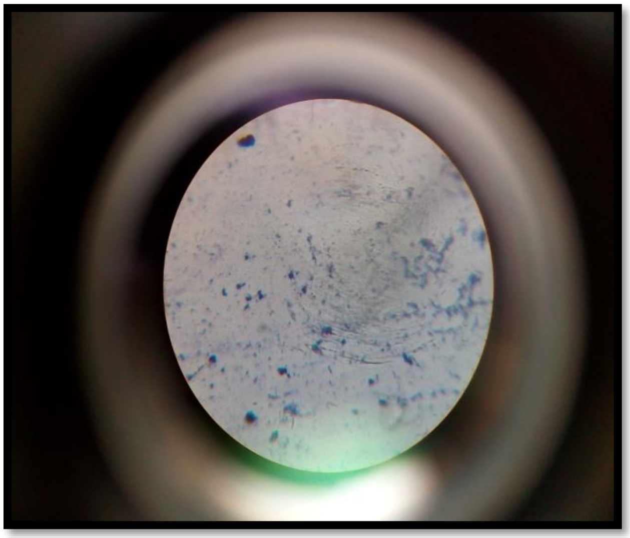


Fig 1.1- Microscopic View of Bacteria Strain

Principle of self-healing concrete

Self-healing concrete was invented by Henk Jonkers, a microbiologist and professor as Delft University of Technology in the Netherlands. Jonkers began developing self-healing concrete in 2006. After three years of experimenting, he found the perfect healing agent –bacillus. “You need bacteria that can survive the harsh environment of concrete,” Jonkers said in an interview with CNN. “It’s a rock like, stone-like material, very dry.”

Bacillus is a perfect match for the job. The bacteria will thrive in a high-alkaline conditions of concrete and produces spores than can live up to four years without any food or oxygen. Jonkers finalized his creation by adding calcium lactate to the limestone concrete mixture in order to feed the bacillus so that they can produce limestone to repair cracks in the concrete. “It is combining nature with construction materials,” Jonkers said. “Nature is supplying us a lot of functionality for free. In this case, limestone-producing bacteria.

Self-healing concrete could solve the problem of concrete structures deteriorating well before the end of their service life. Concrete is still one of the main materials used in the construction industry, from the foundation of buildings to the structure of bridges and underground parking lots. Traditional concrete has a flaw, it tends to crack when subjected to tension.

Concrete is one of the most used building materials. However, it is one of the major producers of carbon dioxide (CO_2) which is directly contributing to destroying our environment. Not to mention that enormous costs are being spent each year to maintain concrete constructions. Cracks of various form in all concrete constructions which need to be sealed manually shortening the life of a particular construction. On the other hand, self-healing concrete (SHC) is a revolutionary building material that has the solution to all these problems and is definitely the building material of the near future. Therefore, we need to understand its property and mechanism and foresee how it impacts the architectural designs of the time to come, which standers are needed to create useful and aesthetic buildings and constructions.

APPROACHES TO SELF HEALING

Several approaches towards self-healing of materials can be distinguished. These approaches can be subdivided in multiple categories.

a. Active versus passive

Dry (1994) proposed a distinction between active and passive self-healing materials. Active materials require a human intervention in order to fully complete the healing process, while passive materials can react to external stimuli without the need of human interaction. Although a stand-alone system might seem as the most desirable solution, the active materials allow for a larger degree of control and are thus likely to inspire greater confidence within the end user (de Rooij et al., 2013).

b. Autogenously versus autonomous

A second distinction between self-healing materials can be made based upon the autogenously or autonomous nature of the self-healing process. De Rooij et al. (2013) proposed the following definitions:

- Autogenic: the self-healing process is autogenic when the recovery process uses material components that could otherwise also be present when not specifically designed for self-healing (own generic materials).

- Autonomic: the self-healing process is autonomic when the recovery process uses material components that would otherwise not be found in the material (engineered additions).

An example of autogenous healing is the ability of cementitious material to repair cracks by further hydration of unhydrated cement particles and carbonation of dissolved calcium hydroxide. Autonomic systems, in which foreign materials are used in the 7 healing process, have received the bulk of the attention in research. Different autonomic self-healing approaches have already been investigated such as the implementation of glues and resins contained in capsules or brittle fibers (Dry, 1994; White et al., 2001), self-healing mechanisms activated by heating devices (Nishiwaki et al., 2006), minerals excreted by immobilized microorganisms (Ghosh et al., 2005), etc.

c. Intrinsic, vascular and capsule based self-healing

Blaiszik et al. (2010) state that self-healing materials can be classified broadly into three groups: capsule based, vascular and intrinsic. Each of these approaches differs by the mechanism used to accomplish healing in the damaged region. Figure 1-4 displays an example of three different systems in self-healing polymers using a two-component healing agent. The healing process commences when both components come in direct contact with each other.

Capsule based self-healing materials (Figure 5a) store the healing agents inside small capsules. When the material is damaged, the capsules in the damaged zone will rupture and release the healing agent. Once the capsules are broken, the healing agent is depleted entirely, meaning that the healing capability is a one-time event in that particular zone.

Vascular self-healing materials (Figure 5b) store the healing agent in a network of hollow tubes or capillaries. These channels can be interconnected one-dimensionally, two-dimensionally or three dimensionally. After damage has occurred, the healing agent 8 will be released from the capillary. However, the affected channel may be refilled, either from a neighboring capillary in an undamaged section or from an external reservoir. This allows that a particular zone can be healed multiple times. Intrinsic self-healing materials (Figure 5c) exhibit self-healing properties due to a latent material ability which is triggered by damage or an external stimulus such as heat, light or pressure. Multiple healing events on the same location are possible (Blaiszik et al., 2010).

CEREUS STRAIN CHARACTERISTICS
MTCC NO- 430

Name- Bacillus Organism- Bacteria Genus

Species Name- Cereus Strain designation- 11778

Temperature- 30^{o} C

Incubation- 24 hours Growth Media- 3

Growth condition- Aerobic

Sub culturing period- 30 day

Special features- Assay of Chlortetracycline, Oxytetracycline and tetracycline.

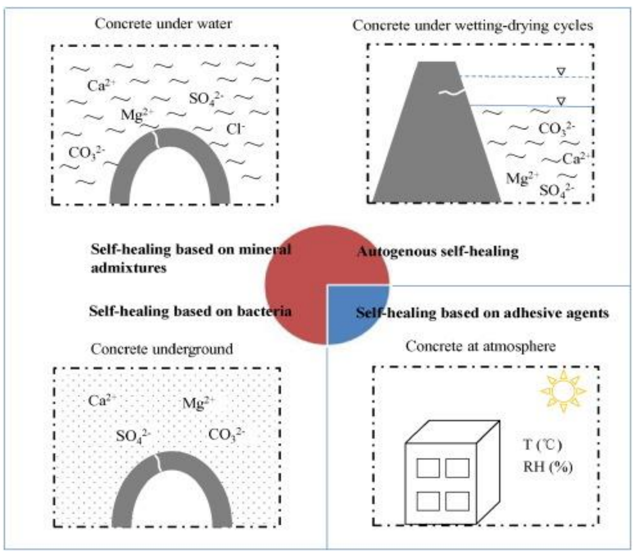
Fig 1.2- Rod like Structure of Bacillus ` Cereus

SELF HEALING IN CEMENTTIOUS MATERIALS

Mechanisms of self-healing in cementitious material, i.e. autogenous self-healing, self-healing based on mineral admixtures, self-healing based on bacteria and self-healing based on adhesive agents, are reviewed. Literature shows that all mechanisms of self-healing are effective, to some extent, under some particular conditions. It reveals that not any particular method of self-healing is the best, but one can be the most suitable for a particular situation.

For better application of self-healing concept in engineering practice, favorable situations for self-healing in cementitious materials are summarized. The required environmental conditions for each self-healing mechanism are analyzed. Additional costs for realizing self-healing in concrete structures are also discussed. Based on the aforementioned aspects of self-healing in cementitious materials, perspectives for further research on application of self-healing in engineering practice are proposed.

Cementitious materials are the most widely used building materials all over the word. However, deterioration is inevitable even since the very beginning of the service life, then maintenance and repair work, which are often labor- and capital-intensive, would be followed. Thus, self-healing of the affected cementitious materials is of great importance. Self-healing phenomenon in cementitious materials has been noticed and been studying for a long time.



The method of using microbes in bacterial concrete is known as microbial induced calcium carbonate precipitation (MICP) or bio- mineralization. Bio-mineralization is a biological precipitation in which organisms create a local micro environment by providing chemical precipitation of mineral phases extracellular. Some usually occurring metabolic processes including sulphate reduction, photosynthesis and urea hydrolysis end up in giving CACO3 as three byproduct. Various bacteria can precipitate calcium carbonate in both natural and laboratory conditions. Calcium carbonate precipitation is mainly governed by following factors.

- pH value
- calcium concentration
- DIC (dissolved inorganic carbon) concentration
- Nucleation sites

The main mechanism behind making a self-healing concrete is that the bacteria should be able to convert the soluble organic nutrients into insoluble inorganic calcite crystals which seals the cracks. The self-healing agent that is applied to the concrete consists of two components, bacteria which acts as a catalyst and calcium lactate i.e. the mineral precursor which is converted to calcium carbonate minerals9. The presence of CO2and 9 calcium hydroxide within the concretion of calcium carbonate in control concrete as shown in the reaction given below:

$$CO_2 + Ca(OH)_2 \rightarrow CaCO_3 + H_2O \quad (1)$$

The calcium carbonate is formed due to the presence of limited CO2 .Calcium hydroxide being soluble in nature dissolves in excess water and comes out from cracks as leaching. In self-healing concrete active metabolic conversion of calcium nutrients takes place due to the presence of bacteria.

$$Ca(C_3H_5O_2)_2 + 7O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O \quad (2)$$

There are two pathways of calcium precipitation done by microorganisms:

• It involves Sulphur cycle in which Sulphur reducing bacteria carry out Sulphur reduction in anoxic environment.

• It involves nitrogen cycle, explicitly the amino acid • oxidative deamination and urea or uric acid degradation using ureolytic bacteria in aerobic environment and in anaerobic conditions nitrate reductions. One of the most commonly used methods applied for MICCP is hydrolysis of urea through the urease enzyme in an environment in which calcium is in abundance. This method results in the hike in the dissolved carbon (inorganic) concentration and pH. Urease propels the hydrolysis of urea in bacterial environment to ammonia and CO2, resulting in pH and carbonate concentration increase. 1 mol of urea forms 1 mol of ammonia and 1 mol of carbonate by intracellular hydrolyzation, which in turn forms additional 1 mol of ammonia and carbonic acid spontaneously as follows:

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (3)$$

$$NH_2COOH + H_2O \rightarrow NH_3H_2CO_3 \quad (4)$$

A state of equilibrium in is attained to form bicarbonate in water, pH rises due to1 mol of ammonium and hydroxide ions.

$$H_2CO_3 \rightarrow 2H^+ + 2CO_3^{2-} \quad (5)$$
$$NH_3 + H_2O \rightarrow NH_4^- + OH^- \quad (6)$$

$$Ca^{2-} + CO_3^- \rightarrow CaCO_3 \quad (7)$$

Observations depict that in calcium precipitation a key role is being played by surfaces of bacteria due to the involvement of negatively charged ions and neutral pH, the metal ions with positive charge can combine with bacterial surfaces thereby encouraging heterogeneous nucleation. The possible biochemical reaction can be summed up as:

$$Ca2++ Cell \rightarrow Cell\text{-}Ca2+ \quad (8)$$

$$Cl- + HCO3 - + NH3 \rightarrow NH4Cl + CO3\ 2- \quad (9)$$

ROLE OF MICP IN STRENGTH AND DURABILITY OF CEMENT MORTAR/CONCRETE

MICP is a widespread biochemical process in soils, caves, freshwater, marine sediments, and hyper saline habitats. MICP is an outcome of metabolic interactions between diverse microbial communities with organic and/or inorganic compounds present in the environment. Some of the major metabolic processes involved in MICP at different levels are urea hydrolysis, denitrification, dissimilatory sulfate reduction, and photosynthesis. Currently, MICP directed by urea hydrolysis, denitrification, and dissimilatory sulfate reduction has been reported to aid in the development of bioconcrete and has demonstrated an improvement in the mechanical and durability properties of concrete.

Bio concrete is a promising sustainable technology which reduces negative environmental impact caused by CO_2 emissions from the construction sector, as well as in terms of economic benefits by way of promoting a self-healing process of concrete structures. Among the metabolic processes mentioned above, urea hydrolysis is the most applied in concrete repair mechanisms. MICP by urea hydrolysis is induced by a series of reactions driven by urease (Ur) and carbonic anhydrase (CA). Catalytic activity of these two enzymes depends on diverse parameters, which are currently being studied under laboratory conditions to better understand the biochemical mechanisms involved and their regulation in microorganisms. It is clearly evident that microbiological and molecular components are essential to improving the process and performance of bio-concrete.

COMPESSIVE STRENGTH

The process of microbial induced calcite precipitation (MICP) is widely used recently in construction engineering in improving compressive strength, durability and self-healing of building materials and culture heritages. However, most of researches to date have concentrated on prokaryotic systems despite of associated limitation of urease-positive bacteria in bio cementation.

The compressive strength is frequently used as a measure of the resistance because this strength is the most convenient to measure. The cement mortar/concrete properties of various ingredients of the mixture are usually measured in terms of their compressive strength. The compressive strength of concrete/mortar is usually determined by submitting a specimen of constant cross-section to a uniformly distributed increasing axial compression load in a suitable testing machine.

FLEXURAL STRENGTH

The durability of concrete/mortar is related to the characteristics of its pore structure. Furthermore, permeability of concrete/mortar is dependent on the porosity and the connectivity of the pores. The degradation mechanisms often depend on the way potentially aggressive substances can penetrate the cement-based material, possibly causing damage. The more open the pore structure and connectivity of the pores, the more vulnerable it is to degradation caused by penetrating substances.

One of the predominant causes of the corrosion of steel in cement-based structures is chloride attack. Chloride ions may be present in a cementitious material either as a result of aggressive ions ingress or incorporation of the aggressive ions during concrete/mortar preparation. Chloride ions may also penetrate from external sources such as seawater or deicing salts. In the marine environment, the in Chloride is bound if it has reacted with cement and is free if it is available in pore solution. Several chemical interactions between chloride and the cement constituents do affect the chloride penetration into the concrete/mortar bulk. The chemical reactions of chlorides with cement paste start with calcium hydroxide and calcium aluminate hydrate, depending on the cat ions in solution.

Chloride ingress in cement-based materials is mainly through capillary absorption, permeation, and diffusion. However, it may also occur through a multiple of the aforementioned mechanisms. Diffusion is the most prevalent process. The ingress of chlorides/sulphates due to the various transport mechanisms obeys different laws. Fick’s second law of diffusion is commonly applied to quantify the aggressive ion ingress due to the multiple transport phenomena.

Bio-cementation in OPC lowers chloride ingress and permeability into the cement matrix. This is due to the refinement of the pore structure. Chloride ions penetrate a pore system and form chloride salts which may crystallize within the pores inducing internal cracks. The cracks affect the mechanical and durability properties of concrete/mortar.

DURABILITY

The microbial concrete makes use of calcite precipitation by favorable bacteria. In this technique urolytic bacteria (microorganism) are used hence the concrete is called Bacterial or Microbial concrete. The “Microbial concrete” can be prepared by adding spore forming bacteria in the concrete that are able to continuously precipitate calcite, this process of production of calcite precipitation is called Microbiologically Induced Calcite Precipitation (MICP). Recently, it is found that microbial calcite precipitation resulting from metabolic activities of favorable microorganisms in concrete improved the overall properties of concrete. Bacterial Cultures improves the strength of cement sand mortar and crack repair on surfaces of concrete structures. The basic principle for this process is that the microbial urease hydrolyzes urea to produce ammonia and carbon dioxide and the ammonia released in surrounding subsequently increases pH, leading to accumulation of insoluble calcium carbonate. Calcium carbonate precipitation, a metabolic process which occurs in some bacteria, has been investigated and proven its wide range of scientific and technological implications. Calcite formation by Bacillus species is used in making microbial concrete, which can produce calcite precipitates on suitable media supplemented with a calcium source. Bacterial spores are specialized cells which can endure extreme mechanical and chemical stresses and spores of this specific genus are known to remain viable for up to 200 years. Spores are dormant but viable bacterial spores immobilized in the concrete matrix will become metabolically active when revived by water entering freshly into the concrete. Calcite precipitation is selective and its efficiency is affected by the porosity of the medium, the number of cells present and the total volume of nutrient added. The bacteria precipitate calcite in the presence of nutrients. The alkaline environment of concrete with pH around 12 is the major hindering factor for the growth of bacteria. However, some bacteria have the ability to produce endospores to endure an extreme environment, as observed by the studies. The technique is used to improve the compressive strength and reduce the permeability of concrete. There are various types of Bactria which are used for making microbial concrete and help to improve the concrete strength and durability. According to literature review, following are the some of the bacteria used in concrete.

One of the main characteristics influencing the durability of concrete is its impermeability to the ingress of water, oxygen, carbon dioxide, chloride, sulphate and other potentially deleterious substances. Most building materials, both natural and artificial such as bricks, cement mortar/concrete, contain a certain volume of empty space which is distributed within the solid mass in the form of pores, cavities, and cracks of various shapes and sizes. The total sum of these empty spaces is called porosity, a fundamental characteristic of building construction material that affects its physical properties. The knowledge of their pore structure is an important parameter for characterizing building materials in predicting their behavior under weathering conditions and for evaluating the degree of deterioration and establishing the effectiveness of conservation treatments of their surfaces.

The durability of mortar and concrete depends largely on the movement of water and gas enters and moves through it. The permeability is an indicator of concrete's ability to transport water more precisely with both mechanism that is controlling the uptake and transport of water and gaseous substances into cementitious material. Permeability is a measure of flow of water under pressure in a saturated porous medium while sorptivity is materials ability to absorb and transmit water through it by capillary suction.

To improve the durability of cement mortar/concrete structures, we must study the impermeability of water, chlorides for cement mortar/concrete and methods to improve the same. There are many alternatives available to improve impermeability to enhance the durability of building materials. Chemical admixtures such as plasticizers and water reducing agents help to improve the workability by reducing the inter-granular friction ultimately affecting the porosity and distribution of pores. If microbial deposition is able to seal the pores in the cover portion of the cement mortar/concrete in the top few millimeters, then the life of the reinforced concrete sections will improve substantially. Chloride ion penetration is the most frequently specified durability criterion/performance characteristic for durable performance of concrete structures. Resistance to chloride ion penetration can be considered to be the most positive, reliable and rational measure of the long-term durable service life of concrete structures in aggressive environments. Deterioration of cement mortar/concrete structures is directly proportional to the ingress of moisture and chlorides. Resistance to penetration of chloride ions is one of the most important measures in durability. The conductivity method is based on materials science and can therefore be used with confidence in durability prediction models.

BACTERIAL CALCITE TECHNOLOGY IN BIO-MINERALIZED MATERIAL

"Bio minerals are everywhere." If we take a look around, we see ourselves surrounded by bio minerals whether in the form of beautiful corals, ant hills, and caves, shells of mollusks, teeth, bones or rocks. Researchers around the globe are now focusing on harnessing the technical applications of these bio minerals in various fields.

Bio mineralization is a process by which living organisms produce minerals. These could be silicates in algae and diatoms, carbonates in invertebrates and calcium, phosphates and carbonates in vertebrates. The synthesis of minerals by prokaryotes is broadly classified into two classes: Biologically controlled mineralization (BCM) and biologically induced mineralization (BIM) (Lowenstam, 1981; Lowenstam and Weiner, 1989). Minerals are directly synthesized at a specific location within or on the cell and only under certain conditions in case of BCM but in case of biologically induced mineralization, the minerals are formed extracellular as a result of metabolic activity of the organism. The extracellular production of these bio minerals invited scientists worldwide for harnessing this capability of microbes for various bioengineering applications.

Minerals known to be formed via biologically induced mineralization through passive surface-mediated mineralization include Fe, Mn, and other metal oxides, e.g., ferrihydrite ($5Fe_2O_3 \cdot 9H_2O$), hematite (α-Fe_2O_3), and goethite (α-FeOOH); metal sulfates, phosphates, and carbonates; phosphorite; Fe and Fe-Al silicates; and metal sulfides. Of all the minerals that have been associated with bio-mineralization, carbonates are the most obvious. Microbially induced calcium carbonate precipitation (MICCP), most widely studied branch of biomineralization holds promise for variety of fields ranging from Biotechnology, Geotechnology, Paleobiology to Civil engineering.

APPLICATIONS OF MICROBIAL CEMENTATION

The microbial cementation could be used for the civil and environmental engineering applications like, to enhance stability of retaining walls, embankments and dams, reinforcing underground constructions, constructing a permeable reactive barrier in mining and environmental engineering, increasing the resistance to petroleum borehole degradation during drilling and extraction, controlling erosion in coastal areas and rivers, increasing the bearing capacity of piled or non-piled foundation and treating pavement surface.

OBJECTIVE

- To compare the flexural strength of normal concrete and bio concrete.
- To develop self- healing bio concrete using biological based techniques.

SIGNIFICANCE OF STUDY

Concrete is a building material that withstands environmental actions for centuries. Development of concrete technologies enables building up the lightweight, slender, and aesthetically attractive structures.

Concrete is the most widely used construction material which has high tendency to form cracks. These cracks leads to significant reduction in concrete service life and high replacement cost. These building structures, however, became vulnerable to cracking and detrimental effects of corrosion of steel reinforcement. In the last decade, self-healing technologies faced substantial interest of experts worldwide. These technologies are considered as a promising and sustainable solution increasing the durability of structural concrete.

Bio concrete can be used as the best alternative for constructions in extreme climates. As such extreme climate can deteriorate the concrete surfaces which may result in ultimately failure in concrete structure. Although it is not possible to prevent crack formation, various types of techniques are in place to heal the cracks. These treatment methods that are environmentally friendly and long-lasting and are in high demand. A microbial self-healing approach is distinguished by its potential for long-lasting, rapid and active crack repair, while also being environmentally friendly. This study provides an overview of the microbial approaches to produce calcium carbonate ($CaCO_3$). Prospective challenges in microbial crack treatment are discussed, and recommendations are also given for areas of future research.

CHAPTER 2: LITERATURE REVIEW

1).Hendrik et al. (1998). Generally, cement after responding with water during the primary reaction engenders calcium silicate hydrated (C-S-H) gel and calcium hydroxide in aqueous form. Existence of pozzolanic material inside the concrete triggers Secondary hydration and fabricates additional C-S-H gel that accelerates and augments the strength of final concrete. Though, the pozzolanic materials such as silica fume are exclusive and limited. Besides, the cement manufacture released about a tonne of greenhouse gases (GHG) for every tonne of cement

2). **V.Krishnan et al. (2001):** This paper has been informed that MICP it's the mechanism used for healing crack and fissure by using biotechnology and they use B. pasteruii. They observed that because of the production of calcite is in the natural way the MICP is cost effective. Durability, stiffness and strength of concrete is enhancing by the use of bacterial solution in concrete. They use XRD and SEM analysis to measure the amount of precipitation of calcite and pictured. Finally, they observed that the several chemical attacks and the shrinkage enhance because of introducing of bacteria in various medium of concrete.

3). Annie Peter.J et al, (2004): In order to understand the structural behavior of SCC & CVC in hardened state, reinforced concrete (RC)beams of size 150mm x 400mm x 3000mm with similar concrete strength and identical reinforcement were cast, tested and compared the structural behavior such as load-deflection characteristics, crack-widths, spacing of cracks, number of cracks, crack pattern, ultimate load- carrying capacity, moments-curvature relationship, longitudinal strain in both concrete and steel. Beyond the peak load stage, CVC beams showed no drop in load with increased deformation while SCC beams showed drop in load with increased deformation. Crack widths were within the limits specified by IS 456 at all load stages. The average crack widths of both the types of beams were comparable.

4). Lakshmanan.N, Devdas Manoharan (2004): Concrete is a absolutely essential component of construction materials used in infrastructure and most buildings. However, concrete is sometimes exposed to substances that can attack it and cause deterioration. The corrosion of the concrete is caused by the interaction between biological and chemical processs .Recent research has shown that specific species of bacteria can be useful to enhance the durability and strength of concrete structure. The microbial concrete presents a potentially enormous lengthening in service-life of infrastructure, substantially reduces the maintenances costs. This paper outlines the
Basic mechanism involved in enhancing the strength and durability of concrete.

5).Prasad et al. (2006): Cracking, strength loss and disintegration strikes concrete when endangered to acid attack, is one of the concrete durability aspects i.e. chemical attack. The acidic strike is subjective to the dissolution processes of the cement paste components. The acid attack menace can be diminished by hindering the 26 corridors present inside the concrete formation. Calcium Carbonate, from microorganism as grout material can shrink the pores and mend the concrete durability.

6). Willem De Muynck et al, (2008): Shortcomings of conventional surface treatments have drawn the attention to alternative techniques for the improvement of the durability of concrete. This paper report the effects of bacterial carbonate precipitation on the durability of mortar specimens with different porosity.. An increased resistance towards freezing and thawing was also noticed. The results obtained with their bio deposition treatment were similar as those obtained with conventional surface treatments.

7). H.M Jokers and Erik(2009): Concrete structures usually show some self-healing capacity, i.e. the ability to heal or seal freshly formed micro-cracks. This property is mainly due to the presence of non-hydrated excess cement particles in the materials matrix, which undergo delayed On secondary hydration upon reaction with ingress water. In this research project we develop a new type of self-healing concrete in which bacteria mediate the production of minerals which rapidly seal freshly formed cracks, a process that concomitantly decreases concrete permeability, and thus better protects embedded steel reinforcement from corrosion. Further development of this bio-concrete with significantly increased self-healing capacities would represent a new type of durable and sustainable concrete with a wide range of potential applications.

8). Jonkers et al. (2010); Van Tittelboom et al. (2010), Wiktor and Jonkers(2011); Wang et al. (2012).Consequently, a more natural way to instigate self-healing mechanism is to use bio-materials, Such as microorganisms which work on the basis of biomineralization. Since, most compatible substance with concrete compositions is calcium carbonate; the techniques of conventional treatment of crack can be replaced by microbial metabolic pathways.

9). Dhami et al (2012): Epoxy treatments are currently used but they are harmful for environment and health as lethal fumes emanated may cause skin and breathing issues. These treatments usually encompass chemical constituents such as epoxy resins, chlorine infused rubbers, waxes, polyurethane, acrylics and siloxane. While passive treatments are appropriate for numerous standing concrete constructions, they have countless restrictions which deter their practice. Some of the shortcomings in the use of chemical treatments have inferior weather endurance, moisture vulnerability, low heat confrontation, un-sustainability, poor adhering with concrete, exposure to decomposition and de-lamination with time and diverse thermal expansion coefficients between concrete and chemical treatments.

10). Vekariya M. et-al(2013): This have defined the bacterial concrete, its classification and types of bacteria, chemical process to fix the crack by bacteria, advantages and disadvantages and possibilities of application of MICP and advantages by switching it over epoxy resins.

11). Wang et al. (2014): It was established that to fill the cracks and porosities the precipitation of calcium carbonate through ureolytic pathway was only required. Their analysis demonstrated that the bio-treated mortar soaked up six times less water than crude mortar. The principle of the analysis executed by was to find out the effect of microbial agent on the crack healing capacity and water permeability. It was established that the crack healing capability amplified from 20–48 % to 50–80 % in the company of the microbial agent. A tenfold reduction in water permeability was observed by the precipitation of calcium carbonate through ureolytic pathway. To establish the competence of the bio self-healing methodology distribution and the amount of calcium carbonate precipitate across the concrete formation are the core prerequisite.

12). Prof. M. Manjunath, A. A. Kalaje, Santosh A. Kadapure, (2014): This paper was presented the observation they did the tests on the mechanical properties of concrete, chloride permeability and water absorption and also fly ash replacing cement by 10% and 20% with bacterial solutions of 103,105,107 using B. sphaericus at age of 28 days. Generally, they concluded that mechanical properties are improved by the presence of bacteria and decrease water absorption and permeability. The better results gains at bacterial solution of 105cells/ml.

13). N. Kannan, Ravindranatha, Likhit M. L(2014): This paper has presented a comparison of bio concrete and normal concrete regarding to flexural and compressive strength tests using beams and cubes moulds with B. pasteurii. The concrete cubes and beams were prepared adding a calculated quantity of bacterial solution and they were tested for 7days,14 days and 28 day compressive and flexural strengths. Finally found that there was a high rise of remedial of cracks and strength exposed to loading the concrete sample. The properties of concrete effectively improving due to bacterial species by attaining a very high early strength rise and also found that the structure produced by the bio concrete is resistive to seepage and more compact due to the bacterial produce calcium carbonate in the concrete.

14). R. Sri Bhavana, P. Polu Raju, S S Asadi (2014): In this study has been presented; a biological repair technique was used in which bacteria of 105cells/ml were mixed with concrete to seal the micro-cracks. The tests they did on this experiment are like Flexural, split tensile and compressive strength by B. subtilis type of bacteria for 3, 7 and 28 days. In addition to above technique fly ash was partially added in the place of cement. The cement is replacing by 10% and 30% fly ash in concrete mix tests were did generally found that cement when replacing by 10% fly ash attained maximum strength with and without bacteria than normal concrete.

15). S. Krishnapriyaa, D. Babub, G. Arulrajc(2015): This have been published a paper and found that significant rise in strength and cracks healing in concrete sample cast B. megaterium B. licheniformis and bacillus megaterium MTCC 1684. They observed that because of presence of bacterial in the concrete strength and other properties of concrete rise and also production of calcite in concrete healing a crack.

16). N. Amudhavalli, K. Keerthana and A. Ranjani (2015): This paper has presented the overview of bacterial concrete, bacteria the state of art results in all projects show that material designed as self-healing agents. Some of the bacteria is drawbacks not directly functional in Construction structure like houses and offices because of health concerns this bacteria like B. Pasteuri, B. megaterium, B. subtilis. Lastly, they achieve that bacterium that have used in concrete in better way because of their advantages than other bacteria that are B.Sphaericus And Eschericheria Coli.

17). Hanumanthrao M. and Vishwanad G(2015): Self-healing agents such as epoxy resins, bacteria, fiber are used to heal cracks in concrete.Among these, bacteria used in concrete are effective. When the bacteria is mixed with concrete the calcium carbonate precipitates forms and these precipitates filling the cracks and makes the crack free concrete and has been studied significantly.

18). Thakur A. et-al(2016): They have studied different bacteria's, their isolation process, different approaches for addition of bacteria in concrete,their effects on compressive strength and water absorption properties of concrete and also the SEM and XRD analysis of concrete containing bacteria. Durability can be enhanced by preventing further ingress of water and other substances. Self-healing agents such as epoxy resins, bacteria, fiber are used to heal cracks in concrete. Among these, bacteria used in concrete are effective.

19). B. Chithra P Bai and V. Shibi(2016): This paper was presented, bacterial concrete with various bacterial solution of 103ml105ml and 107ml and they have been used B. Subtilis bacteria species in this experiment and also bio concrete is formed by 10%,20%, and 30% fly ash replacing cement by its mass. The Ultrasonic Pulse Velocity, split tensile, Flexural and compressive strength tests have been doing after 28 and 56 days for M30 grade .All mechanical properties of bio concrete enhancing at 10% fly ash replacing cement and by 105ml

Bacterial solution achieved maximum values for all test they conducted. Finally, The precipitation of CaCO3 due to bacteria in the concrete by bio technology concept that improves mechanical properties of fly ash concrete.

20). S. Sanjay, S. Neha, and R. Jasvir (2016): This paper was presented the experimental investigation on bacterial concrete to increase the strength of bio concrete and to inform the process involved in the bacterial concrete. To know the calcite crystals formed in bacterial

Concrete analysis of microstructure has been done that is used for the potential to recovery the cracks in bacterial concrete and also to inform the biological reaction in concrete. As a result, has been got because of good adaptability of nutrient broth medium of bio concrete at 28 days attained better strength when compared to urea medium.

21). K. Chintalapudi, R Mohan Rao.P (2016): This paper has been presented the bio technology that gains the satisfactory outcomes in remedying the micro cracks in concrete and informed that micro- cracks sealed by process of hydration in continuous situation. For better

Outcomes in strength and durability the improvement of pore structure and optimum bacterial solutions were did. They concluded that by the introducing of bacteria in concrete achieved reduction of permeability, keeping pH under favorable situations rise durability and compressive strength and also the potential to seal and heal the micro-cracks in concrete was found.. The bacterial solutions are the ability used as admixtures in concrete helps in enhancing the mechanical performance of concrete.

22). Kunal. R. Patil, B. Waghere, B. K. Ahire, et al (2016): This research has been informed that an experiment on bio concrete with the several type of bacteria B. pasteruii and bacillus sharicus to enhancing durability and strength of concrete with the mechanism of MICP at

age of 7,28 days. They found that when bacteria are added to the concrete its gives less compressive strength than nutrient broth solution by bacillus sphaericus and B. pasteruii.

23). E. Madhavi et al. (2016): It has published a paper on utilized that fly ash and GGBS materials and to decrease this kind of thermal power waste in the environment, the Ground Granulated Blast

Slag and fly ash replacing cement contain bacteria of 106 bacillus pasteurii in M40 mix. The fly ash and Ground Granulated Blast Slag used in the amounts of 10% of cement. From this research, the results are much better as compare to that of the convention concrete.

24). Seifan et al. (2016) : Concrete is an imperative and used to a great extent used building material for construction purposes. Even after using so many safety measures in design mix and using materials of good strength, concrete builds up cracks in it. Concrete cracks as it shrinks, it is an inherent property. This causes durability, appearance and strength damage to concrete. The ingress of water through these cracks and exposure to acidic corrosive conditions such as acid rain, sewer water etc seeps through; which is detrimental for concrete. Thereby it can be said that primary cause of structure failure is crack formation.

25). K. Pappupreethi, A. Rajisha and P. Magudeaswaran (2017): This paper has been presented that usingbacterial in concrete to enhancing the properties of bacterial concrete when it compared to normal concrete such as compressive and flexural strength and within the same
Time to decrease the water absorption, permeability and reinforcement corrosion. This paper enhanced the knowledge about bacterial concrete by defining the type, merit, and demerit and how it's used as repair material and also used different admixtures such fly ash, silica fume in bacterial concrete due to this condition bio concrete achieved improved durability and strength.

26). H. Ling, C. Qian(2017): This paper has been presented the effects of bacterial on self- healing cracks by chloride tests. Besides the method of electro-migration was used to accelerate the transmission of chloride.They observed that bacteria can heal the crack by itself that really delay the chloride transmission in crack and take defensive effects for RC. This study also shows good application values of microbial self healing technique used in the practical construction and provides a new approach to reinforce the durability of structure.

MICROBIOLOGICALLY INDUCED CALCITE PRECIPITATON

Microbiologically induced calcium carbonate precipitation (MICP) is a bio-geochemical process that induces calcium carbonate precipitation within the soil matrix. Biomineralization in the form of calcium carbonate precipitation can be traced back to the Precambrian period. Calcium carbonate can be precipitated in three polymorphic forms, which in the order of their usual stabilities are calcite, aragonite and vaterite. The main groups of microorganisms that can induce the carbonate precipitation are photosynthetic microorganisms such as cyanobacteria and microalgae; sulfate-reducing bacteria; and some species of microorganisms involved in nitrogen cycle.[4] Several mechanisms have been identified by which bacteria can induce the calcium carbonate precipitation, including urea hydrolysis, denitrification, sulphate production, and iron reduction. Two different pathways, or autotrophic and heterotrophic pathways, through which calcium carbonate is produced have been identified. There are three autotrophic pathways, which all result in depletion of carbon dioxide and favouring calcium carbonate precipitation. In heterotrophic pathway, two metabolic cycles can be involved:

the nitrogen cycle and the sulfur cycle. Several applications of this process have been proposed, such as remediation of cracks and corrosion prevention in concrete.

CONCEPT OF MICP

The major perception following the precipitation of CaCO3 by means of biogenic methods is MICP (Microbiologically Induced Calcite Precipitation). Bio mineralization can be interpreted as the chemical modification of surroundings and atmosphere owing to microbial commotion that consequently leads minerals to precipitate (Stocks-Fischer et al. 1999). Microbially induced calcite precipitation (MICP) insinuate to the development of calcium carbonate originating from a super-saturated suspension attributable to the existence of their microbial cells in addition to biochemical actions (Bosak 2011). The aptitude of urease to stimulate carbonate precipitation in microorganisms has formerly been debated by quite a lot by researchers (Hammes et al. 2003a; Burbank et al. 2012; Li et al. 2013; Stabnikov et al. 2013). Urease activity occurs in a plentiful stretch of microorganisms, but a quantity of strains fabricate predominantly immense amount of urease.(table 2.1)

Table 2.1. Urease production of various organisms

BACTERIA	UREASE ACTIVITY	CALCITE PRECIPITATION
B. spaericus CR2	432 U/ml	2.32 mg/cell mass (mg)
L. sphaericus CH5	-	980 mg/100 ml
S. pasteurii	550 U/ml	-
B. pasteurii NCIM	2477 18 U/ml	-
K. flavaCR1	472 U/ml	-
B. megateriumSS3	690 U/ml	187 mg/100 ml
B. thuringiensis	620 U/ml	167 mg/100 ml

Urease have an effect on the chemical progression linked with the generation of biominerals in accordance with dissimilar constraints (Hammes and Verstraete 2002) for instance pH, dissolved inorganic carbon (DIC) concentrations, Calcium ion concentrations along with the accessibility of nucleation sites. In saturation state, Calcium concentration, pH and DIC manipulate the carbonate ions concentration (CO3 2−), whereas the last constraint (i.e., accessibility of nucleation sites) is crucial for steady and incessant calcium carbonate development (Phillips et al. 2013). In the biomineralization process, nucleation sites are served by means of bacteria.

IMPORTANCE OF UREA

This research represents an experimental study on influence of urea on concrete through various tests on urea, cement, concrete and water. Test of finesses modulus, slump test, carbonation test, pH test, urea ingression test and increase in strength with urea percentage .This study deals to overcome three major problems in the concrete namely heat of hydration, permeability, and corrosion of steel bar embedded in concrete. Urea can generally reduce the temperature of concrete both at casting phase and during the procedure of hydration. Urea does not opposite effect the durability of reinforced concrete, except where there is an accumulation of urea crystal growth.

Addition of urea is extremely recommended. Bacteria are acknowledged to hydrolyze urea by urease to:(1) increase the general pH,

(2)exploit it as a nitrogen source, and

(3) consume it as a reserve of energy.

B. pasteurii is identified to yield a huge quantity of urease in soil atmospheres (Ciurli et al., 1996). The solubility of calcite is a utility of pH and achieved by aqueous medium's ionic strength (Stumm., 1981). Achal V et al (2009) supplemented urea and calcium chloride in the media that encourages microbial sporulation. Attributable to hydrolysis of urea, the pH of the medium was expressively amplified, and the isolates were capable to endure in this setting.

MECHANISM

1mol of carbamate and 1 mol of ammonia is result of hydrolysis of 1mol urea (Eq. 1). (1)

$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3$ (1)

This later hydrolyzes into carbonic acid and 1mol ammonia (eq. 2).

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

Consequently, these products react with water to produces 2 mol of hydroxide ions, bicarbonate and 2 mol of ammonium (Eq. 3 & 4).

$H_2CO_3 + H_2O \rightleftharpoons HCO_3^- + H^+$ (3)

$2NH_3 + 2H_2O \rightleftharpoons 2NH_4^+ + 2OH^-$ (4)

Hydroxide ions are responsible for increasing pH. Carbonate ions develops due to changed equilibrium of bicarbonate (Eq.5).

$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \rightleftharpoons CO_3^{2-} + 2NH_4^+ + 2H_2O$ (5)

$CaCO_3$ precipitation takes place when Carbonate ions come across soluble calcium ions (Eq.6).

$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3$ (precipitation) (6)

The precise reaction for system is indicated in Eq. 7-

$CO(NH_2)_2 + 2H_2O + Ca^{2+} \rightarrow 2NH_4^+ + CaCO_3 \downarrow$ (7)

Calcium carbonate precipitation in aqueous environment represents the following equation (Stumm & Morgann, 1981)-

$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \downarrow$ (8)

$CaCO_3$ thus formed is influenced by pH and ionic capability in the aqueous medium. When a medium a provided with the medium that favors microbial growth e.g. Urea$CaCl_2$ medium, the ions NH_4^+, Cl^-, Na^+, OH^- and H^+ controls precipitation at varied pHs (Siddique R and Chahal N, 2011).

Reactions taking place at cell surface are illustrated below (Stocks-Fischer et al., 1999):-

Ca2+ + CellÍ Cell − Ca2+ (9)

Cell − Ca2+ + CO3 2−Í CaCO3 − Cell Ļ (10)

BACTERIAL CELL CONCENTRATION.

Augmentation in the urease concentration intended for urea hydrolysis accounts for high concentrations of bacterial cells (from 106 to 108 cells) thereby escalating the quantity of calcite precipitation by MICP (Okwadha and Li 2010). As a result, urea hydrolysis (Ng et al. 2012) has an unwavering association with bacterial cell concentrations. Compressive strength test conducted by Shaikh et al., (2017) concluded that optimum level of concentration was found to be 105 cells/ml. If the concentration is increased further; strength of concrete tends to decrease.

THE pH EFFECT

Third important factor is ph which influences calcite precipitation. Urea hydrolysis will occur only when urease enzyme will attain specific pH values which would result in Calcite precipitation, which is subjective to pH. Many researchers have proclaimed that the optimum pH in favor of urease is 8.0, beyond which the activity of enzyme dwindles (Stocks-Fischeret al. 1999; Gorospe et al. 2013). An increased pH is imperative for ammonia creation via urea hydrolysis. Cell respiration allows Aerobic bacteria release CO2, which is complemented by an boost in pH owing to ammonia creation (Ng et al. 2012) .The carbonate is liable to liquefy than to precipitate if the pH levels reduce (Loewenthal and Marais1978).Most calcite precipitation occurs under alkaline conditions frompH 8.7 to 9.5 (Stocks-Fischer et al. 1999), but Mobley et al. (1995) found that the acid urease and optimum pH were nearly neutral. Stabnikov et al. (2013) recently investigated whether halophilic and alkaliphilic urease producing bacteria are active at high concentrations of inorganic salt and pH above 8.5 and the conditions suitable for manufacturing biocement.

TEMPERATURE

Temperature plays an important role in catalysis process of urea by means of urease. The most favorable temperature stretches from 20 to 37 °C (Okwadha and Li 2010). Mitchel and Ferris (2005) reported that the urease activity increased by about 5 times and 10 times when the temperature increased from 15 to 20 °C and 10 to 20 °C, respectively. Ferris et al. (2003) investigated the kinetic rate of urease and the temperature dependence of ureolytic CaCO3 precipitation by B. pasteurii at 10 and 20 °C in artificial ground water. Urease is totally stable &steady at 35 °C (Dhami et al. 2014), however as soon as the temperature is escalated and reaches beyond 55 °C there is 47% decline in enzyme activity.

Ca+ CONCENTRATIONS

The surface of microbes' cell is negatively charged. They work as foragers of cations specially calcium ion. The microbes acts as 'nucleation sites' for cations and bind themselves to their cell periphery (Stocks-Fischer et al. 1999; Ramachandran et al.2001). This manifests the need of an ideal calcium supplement with specific concentration for CaCO3 precipitation. If the concentration of urea and CaCl2 surpasses 0.5M, the decrease of efficiency of calcite precipitation takes place (Okwadha and Li 2010). De Muynck et al. (2010) stated paramount concentrations of urea and CaCl2 are 0.5 and 0.25 M for calcite precipitation respectively. Okwadha and Li (2010) reported that Ca^{2+} concentration decides the amount of $CaCO_3$ precipitation than urea concentrations.

EVALUATION OF SELF–HEALING CONCRETE

Each specimen was cast in a steel prismatic mold after mixing. Then, 24 hours later, specimens were demolded and cured in fresh water with a temperature of (20 ± 3)°C. Crack inducements (1st loading) were conducted at 7, 28, 49, and 91 days from the casting. The 2nd loading was conducted just after crack inducement, and it was the control value before the self-healing. The same 21 days of the healing period were applied to specimens after the 2nd loading. The self-healing environment was different for each case, such as in water and air. The water environment means healing in fresh water with a temperature of (20 ± 3)°C, which is the same condition as the curing after casting. The air environment means healing in the curing room with a temperature of (20 ± 3)°C and relative humidity of 60%. The 3rd loading was conducted after the healing period, and it was the evaluation value of the self-healing performance. Table 5 shows the test program.

Flexural Test

The most important process in the evaluation of the self-healing of concrete is to generate an adequate crack. Van Breugel reported that a crack with only less than 0.2 mm width could be expected to recover by self-healing [22]. This study used a crack mouth opening displacement (CMOD) by measuring a clip gauge set at the notch opening, and CMOD controlled the crack width during loading. Meng et al. reported that the effects of the notch-to-depth ratio are associated with the loading rate, and it is less than 10% at a deflection rate up to 1.25 mm/min [23]. But the loading speed of this study was 0.5–1.0 mm/min; therefore, the effect is insignificant.The self-healing performance was evaluated by three-times loading. The crack inducement (1st loading) was the process to make an adequate crack. The exact crack width could not be measured, but CMOD makes an almost constant crack width because the crack width and CMOD are linearly proportional [24]. The unloading point was set as CMOD 0.05 mm, which is a point after the peak load. After finishing the unloading of the crack inducement (1st loading), the 2nd loading was conducted. To prevent further crack propagation, the unloading point of the 2nd loading was the same as CMOD 0.05 mm. The next process was the healing. Specimens were healed in water and air, respectively, by case. Lastly, the 3rd loading was conducted. The 3rd loading was to evaluate the self-healing performance, and loading was performed, until the specimen was completely separated.

Flexural Stiffness

Self-healing evaluation by flexural strength implies the aging effect of an uncracked part. After casting, concrete has a constant hydration reaction. The flexural strength means the maximum load carrying capacity of the whole section, such as cracked and uncracked parts. In other words, taging effect of the uncracked part cannot be avoided. In order to accurately evaluate the self-healing performance, it is necessary to distinguish the recovery effect from the aging effect. However, it is not easy to separate the self-healing effect from the aging effect in the flexural strength comparison.

This study focused on the different initial behaviors of the load-CMOD curve of the 2nd and the 3rd loading. It is known that the load-CMOD gradient in the three-point bending test is proportional to the modulus of elasticity of the specimen [26, 27]. The initial behavior of the load-CMOD curve is related to the crack opening, so the change of the crack circumstance will be represented most sensitively. Figure 4 shows the initial behavior of the load-CMOD curve of the 2nd and the 3rd loading of PE-7–28-W and shows the different initial slopes by the loading. This study estimated the initial slope of the load-CMOD, which used data from the start point CMOD to (0.003–0.005) mm. The ratio between the initial slope of the 3rd loading and that of the 2n loading is known as the stiffness recovery effect. If the stiffness recovery effect is higher than 1.0, this means that the self-healing restored the flexural stiffness.

CHAPTER 3: METHODOLOGY & EXPREMENTAL STUDY

Before starting any research work some preliminary test or work has to be done in lab or at site. The detailed experimental procedure is discussed below:

- Collecting all the basic materials like cement, sand and aggregate as per Indian Standard Specification.
- Finding all the basic properties of cement, sand and aggregate by performing basic tests on it.
- Getting cement: sand: aggregate ratio by defining w/c ratio by IS mix design procedure. Casting of non-microbial concrete beams without addition of bacteria of size 150mm×150mm×700mm for performing different tests of 14 and 28 days.
- Casting of microbial concrete cube of size 150mm×150mm×700mm with different concentration of bacterial culture i.e. 15%, 25% and 50% for performing different tests on 14 and 28 days.
- Conducting slump test, pH test, EDTA test and water absorption test on bacterial concrete.

PRELIMINARY TEST ON FINE AGGREGATE SIEVE TEST

Sieve Analysis of the fine aggregate is carried out in the laboratory as per IS 383-1870. The sand is first sieved through 4.75mm sieve to remove any particle greater than 4.75 mm sieve and then washed to remove the dust. For performing the sieve test 1kg of fine aggregate is taken. The fine aggregates used for the experimental work is locally procured and comply with grading zone IV.

Table 3.1- Sieve Analysis of Fine Aggregate.

Sieve Size	Weight Retained	% weight Retained	Cumulative % weight Retained
4.75	0	0	0
2.36	8	0.8	0.8
1.18	32.5	3.25	4.05
850µ	39.5	3.95	8
600 µ	34.5	3.45	11.45
300 µ	310.5	31.05	42.5
150 µ	450.5	45.05	87.55
75 µ	111	11.1	98.6
Pan	14	1.4	100
			$\sum$= 352.95

Fineness Modulus of fine aggregate= 352.95/100 = 3.5295

Hence fine aggregate conforming to zone IV according to IS 383-1870 Clause 4.3 Table 4.

SPECIFIC GRAVITY

Specific gravity is defined as the ratio of unit weight of substance to the standard substance at standard temperature i.e. 4°C. This test is performed with the help of pycnometer.

1. Empty wt. of pycnometer (W_1)= 645.5 gm
2. wt. of pycnometer + wt. of fine aggregate (W_2)= 979.5 gm
3. wt. of pycnometer + wt. of fine aggregate + wt. of water (W_3)= 1745.0 gm
4. wt. of water filled in pycnometer up to full level (W_4)= 1537.0 gm

Specific Gravity G= 2.65

TEST ON COARSE AGGREGATE

SIEVE TEST

Crushed stone aggregate of maximum size 20 mm are used as coarse aggregate throughout the experimental study. The aggregates are washed to remove dust and dirt and are dried to surface dry condition. The aggregates are tested as per IS: 383-1970. For performing the sieve test 2kg of coarse aggregate is taken.

Table 3.1 Sieve Analysis of Coarse Aggregate

Sieve Size	Weight Retained	% weight Retained	Cumulative % weight Retained
20	97.5	4.875	4.875
16	408	20.4	25.275
12.5	807	40.35	65.625
10	498.5	24.925	90.5
6.3	176.5	8.825	99.375
5.6	4	0.2	99.575
4.75	2.5	0.125	99.7
Pan	6	0.3	100
			∑= 584.975

Fineness Modulus of fine aggregate= 584.975/100 = 5.85

Fig 1.3: Fine Aggregate

Fig 1.4 : Coarse Aggregate

SPECIFIC GRAVITY

Specific gravity is defined as the ratio of unit weight of substance to the standard substance at standard temperature i.e. 4ºC. This test is performed with the help of pycnometer.

1. Empty wt. of pycnometer (W_1)= 645.5 gm
2. wt. of pycnometer + wt. of coarse aggregate (W_2)= 979.5 gm
3. wt. of pycnometer + wt. of coarse aggregate + wt. of water (W_3)= 1745.0 gm
4. wt. of water filled in pycnometer up to full level (W_4)= 1537.0 gm

Specific Gravity G= 2.64

pH Test of Tap Water

pH is defined as the negative logarithm of hydrogen ion concentration. pH test is performed by pH meter. Fresh and clean tap water is used for casting the specimens in the present study. The water is relatively free from organic matter, silt, oil, sugar, chloride and acidic material as per Indian standard. pH of **tap water=** 8.08.

TEST ON CEMENT

Portland Pozzolana cement (PPC) is used for the present investigation. The cement is of uniform color i.e. grey with a light greenish shade and is free from any hard lumps.

Table 3.2- Test Results of Cement

S.NO	Characteristics	Obtained result	Standard values
1	Normal Consistency	37%	
2	Initial Setting Time	40 min	Should not be less than 30min

3	Final Setting Time	300min	Should not be more than 300 min
4	Fineness	6%	<10
5	Specific Gravity	3.15	

BACTERIAL CULTURE

Bacillus is used for this work which is extracted from CT5 strain. The inoculation of the bacteria Bacillus from CT5 strain is done by sequential order fulfilling all conditions of their growth.

INOCULATION PROCESS OF BACTERIAL CULTURE

First of all bacterial strain “BACILLUS CEREUS” that was in solid powder was converted in inoculum by mixing it in the broth solution.

It was done inside “laminar air flow” and suitable precautions were taken so that our bacteria do not contaminate.

It is then kept for one day inside BOD incubator for one day for the proliferation of bacteria. Inoculum was further increased by adding more broth solution so that our bacteria can be sufficiently grown.

Quantity of Nutrient Broth (NB) added per litre for inoculum=13gm.

PREPERATION OF BACTERIAL SOLUTION

For the preparation of 1 litre bacterial solution following steps were taken. As we are preparing 1 litre solution we need 500ml Nutrient Broth solution, therefore adding 6.5gm NB in 500 ml distilled water. The solution is then kept in autoclave to remove any contamination present in it. Concentration of $Ca(OH)2$ = 74.09gm/L Therefore adding 37.045gm $Ca(OH)2$ in 500ml of water. The pH of complete 1000 ml solution must be maintained between 9 to 12 which is the required pH for the growth of bacteria. The solution obtained is kept in shaker for 24hours at 27oC.

After 24 hours calcium acetate, urea and inoculum is added in the solution. The amount of these chemicals and bacterial strain added is calculated below.

Calcium acetate

We required 2.5ml of 25 mM acetate solution /100ml of culture. Therefor for 1000ml culture, acetate solution = 25ml. For 25 mM acetate- 0.4gm per 100 ml distilled water

Therefore for 25 ml solution acetate= 0.1gm

Urea-Concentration $40 \times X = 2 \times 100$ $X = 5$ml.

Inoculum is added as 10% of the total solution. After addition of all these three components in 1000 ml solution the final which comes out is known as Bacterial culture

This solution is kept in BOD Incubator for at least 3 days for proper growth of bacteria before using it in cube casting.

Fig 1.5 : Bacterial Solution

MAIN INGREDIENT OF BIO-CONCRETE

The main components of bio-concrete are cement, sand, aggregate and addition of bacterial culture with its nutrient so that it can persist in unaesthetic medium of concrete.

FINDING RIGHT BACTERIA

The starting point of the research is to find bacteria capable of surviving in an extreme alkaline environment. Cement and water have a pH value of up to 13 when mixed together, usually a hostile environment for life most organisms die in an environment with a pH value of 10 or above. The search concentrated on microbes that thrive in alkaline environments which can be found in natural environments, Samples of endolithic bacteria (bacteria that can live inside stones) will be collected along with bacteria found in sediments in the lakes. Strains of the bacteria genus Bacillus will be found to thrive in this high-alkaline environment. It is found that the only group of bacteria that will be able to survive is the ones that produced spores comparable to plant seeds. Such spores have extremely thick cell walls that enable them to remain intact for up to 200 years while waiting for a better environment to germinate. They would become activated when the concrete starts to crack, food is available, and water seeps into the structure. This process lowers the pH of the highly alkaline concrete to values in the range (pH 10 to 11.5) where the bacterial spores become activated.

BACTERIA CEREUS

Bacillus cereus is a large, 1 x 3-4 µm, Gram-positive, rod-shaped, endospore forming, facultative aerobic bacterium. It was first successfully isolated in 1969 from a case of fatal pneumonia in a male patient and was cultured from the blood and pleural fluid. RNA comparison reveals Bacillus cereus to be most related to Bacillus anthraces, the cause of anthrax, and Bacillus thuringiensis, an insect pathogen used as pesticide. Although they have similar characteristics, they are distinguishable as Bacillus cereus is most motile bacteria. Bacillus cereus is mesophilic, growing optimally at temperatures between 20°C and 40°C, and is capable of adapting to a wide range of environmental conditions. It is distributed widely in nature and is commonly found in the soil as a saprophytic organism.

FUNCTION OF BACTERIA

Research has shown that autogeneous healing happens due to hydration of non-reacted cement particle present in the concrete matrix when comes in contact with ingress water resulting in closure of micro-cracks, studies also stated that only spore forming gram positive bacteria can survive in high pH environment of concrete sustaining various stresses. Therefore, bacterially induced calcium carbonate precipitation has been proposed as an alternative and environmental friendly crack repair technique. Microbial calcite precipitation is mainly due to urealytic activity and carbonate bio-mineralization of bacteria. The bacteria used in this research produce urease which catalyzes the hydrolysis of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}), First, 1mol of urea is hydrolyzed intracellular to 1mol of carbonate and 1mol of ammonia (Eq. (1)). $CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3$ (1) Carbonate spontaneously hydrolyses to form additionally 1mol of ammonia and carbonic acid (Eq. (2)). $NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$ (2) These products subsequently form 1mol of bicarbonate and 2mol of ammonium and hydroxide ions (Eq. (3) and (4)). $H_2CO_3 \rightarrow HCO_3^- + H^+$ (3) $2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-$ (4) The last 2 reactions give rise to a pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of carbonate ions (Eq. (5)). $HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \rightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$ (5) Since the cell wall of the bacteria is negatively charged, the bacteria draw cat ions from the environment, including Ca^{2+}, to deposit on their cell surface. The Ca^{2+} ions subsequently react with the CO_3^{2-} ions, leading to the precipitation of $CaCO_3$ at the cell surface that serves as a nucleation site (Eq. (6) and (7)) $Ca^{2+} + Cell \rightarrow Cell\,Ca^{2+}$ (6) $Cell\,Ca^{2+} + CO_3^{2-} \rightarrow Cell \rightarrow CaCO_3\downarrow$ (7)

TOOLS REQUIRED

- BOD Incubator
- Laminar Air Flow
- Orbital Shaker
- Auto Clave
- Incubator
- Micro Pipette
- Petri Plates
- Burner
- Pipette Tips
- Glass Rod, Flask, Measuring
- Cylinder

Fig 4.1 : Auto Clave

Fig 4.2 : Orbital Shaker

PROCESS OF TESTING

Different testing processes which involved in this report are, test for compressive strength, pH value determination, estimation of calcium carbonate precipitation by EDTA, Slump, Water absorption, crack analysis.

SLUMP

Slump test of concrete is carried out to check the workability of concrete. It is carried out in freshly mixed concrete. Mixed concrete is poured into the frustum of cone in three layers with 25 blows on each layer. The size of frustum is Upper diameter-100mm Lower diameter-200mm Height-300mm after pouring the concrete full into the frustum of raise the mould from the concrete slowly in vertical direction and measure the fall from the top of the concrete. Measure the slump as the difference between the height of the mould and that of height point of the specimen being tested.

Fig 4.3- Workability Apparatus

WATER ABSORPTION

One of the most important properties of a good quality concrete is low permeability, a concrete with low permeability resists ingress of water and is not as susceptible to freezing and thawing. Water enters pores in the cement paste and even in the aggregate.

For checking water absorption weigh the cube after demolding them.

This is called dry weight → W_1.

Put them in curing tank for 24 hours and then again weigh them.

This is called wet weight → W_2.

Water absorption is given by → W_2-W_1/W_1

CRACK ANALYSIS

As our concrete is self-healing concrete which autogenously repair its crack and for durability of concrete crack analysis is essential. Cracks in concrete can be made in 2 ways firstly realistic cracks and secondly standardized cracks; in our research work we are generating the standardized cracks. Standardized cracks are those cracks in which we pre-define the dimension of the crack in concrete. Thin copper plates of 0.3 mm thickness was incorporated in fresh concrete up to a depth of 10mm to 20 mm with a width of 5mm and during demoulding these copper plates are taken off. With passage of days visual examination of concrete samples is done and we can observe white colored precipitate of $CaCO_3$ on the crack made and surplus precipitate can be cleaned out.

Fig 4.4 Casting of beams

Fig 4.4 Curing of beams

Fig 4.4 Beam samples **Fig 4.5- Testing of beam**

CHAPTER 4: RESULTS AND DISCUSSIONS

Reinforced Concrete Beams (0% bacterial solution): Beams were tested on Flexural Testing Machine, the results are tabulated below

Table 4.1- Flexural Strength of Normal Beams

S.No.	Beam (0% bacterial solution)	Load (kN)	
		14days (Partial)	28days
1	B1-1	53.56	68.87
2	B1-2	52.76	67.63
3	B1-3	54.95	69.85
4	B1-4	-	73.45
5	B1-5	-	74.35
6	B1-6	-	75.20

Bio- Concrete Beams (15% bacterial solution): Beams were tested on Flexural Testing Machine, the results are tabulated below

Table 3.2- Flexural Strength of Beams with 15% bacterial solution

S.No.	Beam (15% bacterial solution)	Load (kN)	
		14days (Partial)	28days
1	B2-1	55.37	71.23
2	B2-2	56.76	72.87
3	B2-3	57.26	73.54
4	B2-4	-	75.43
5	B2-5	-	74.91
6	B3-6	-	74.59

Bio- Concrete Beams (25% bacterial solution): Beams were tested on Flexural Testing Machine, the results are tabulated below

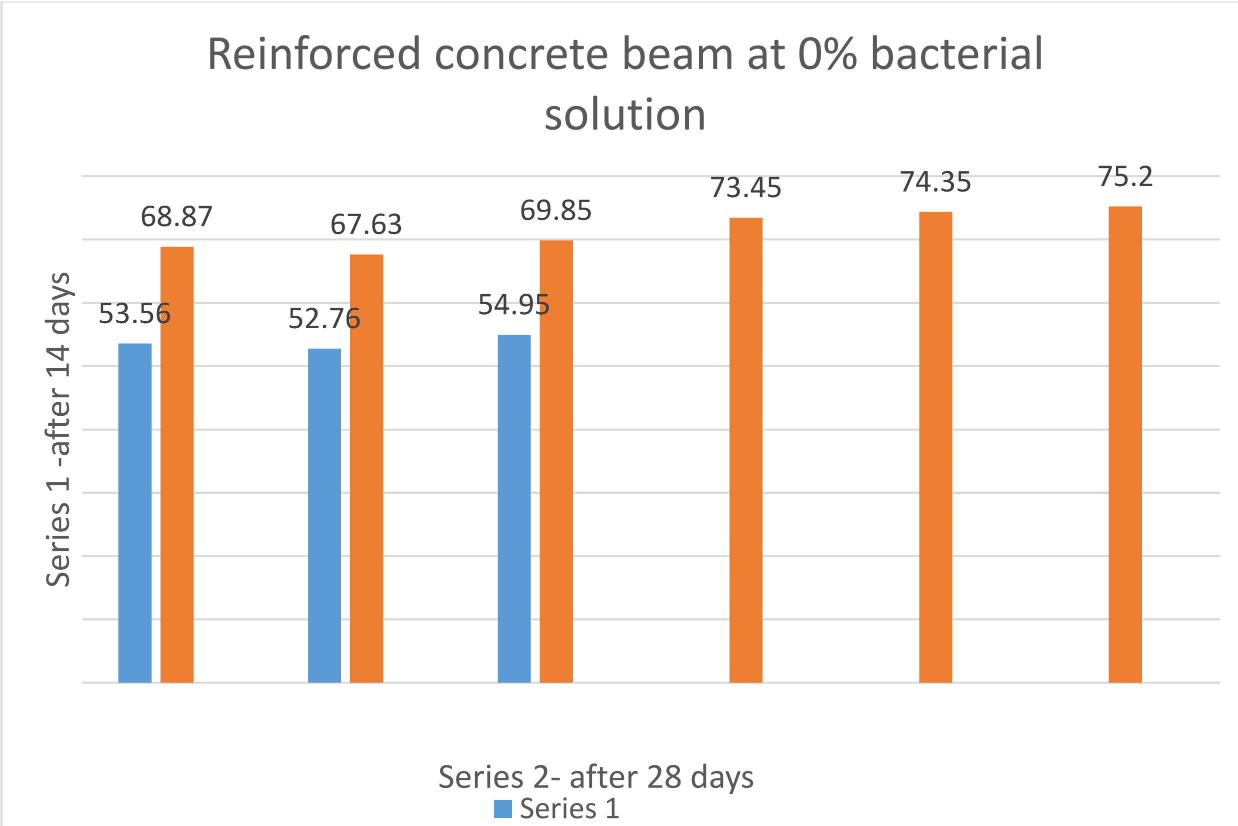
Table 3.2- Flexural Strength of Beams with 25% bacterial solution

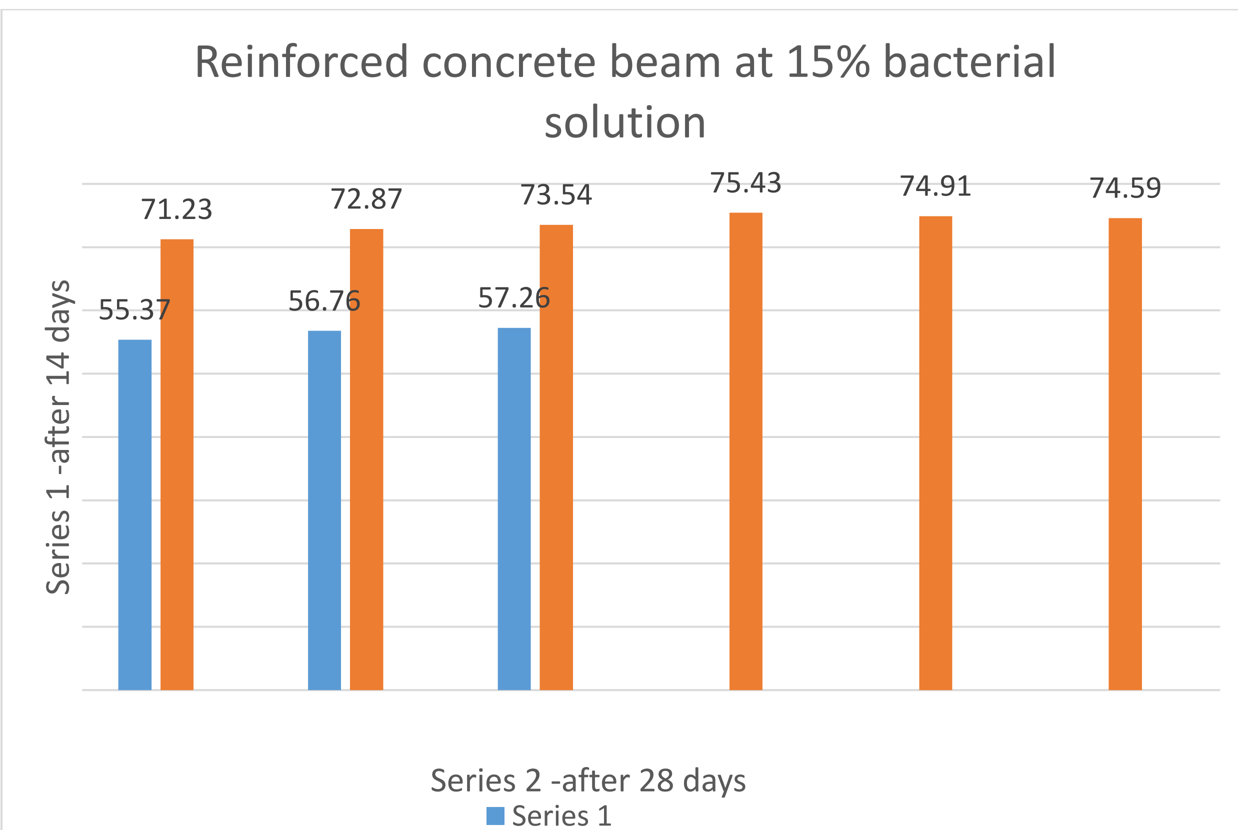
S.No.	Beam (25% bacterial solution)	Load (kN)	
		14days (Partial)	28days
1	B3-1	57.75	77.51
2	B3-2	58.20	78.76
3	B3-3	58.97	77.60
4	B3-4	-	75.86
5	B3-5	-	74.50
6	B3-6	-	76.39

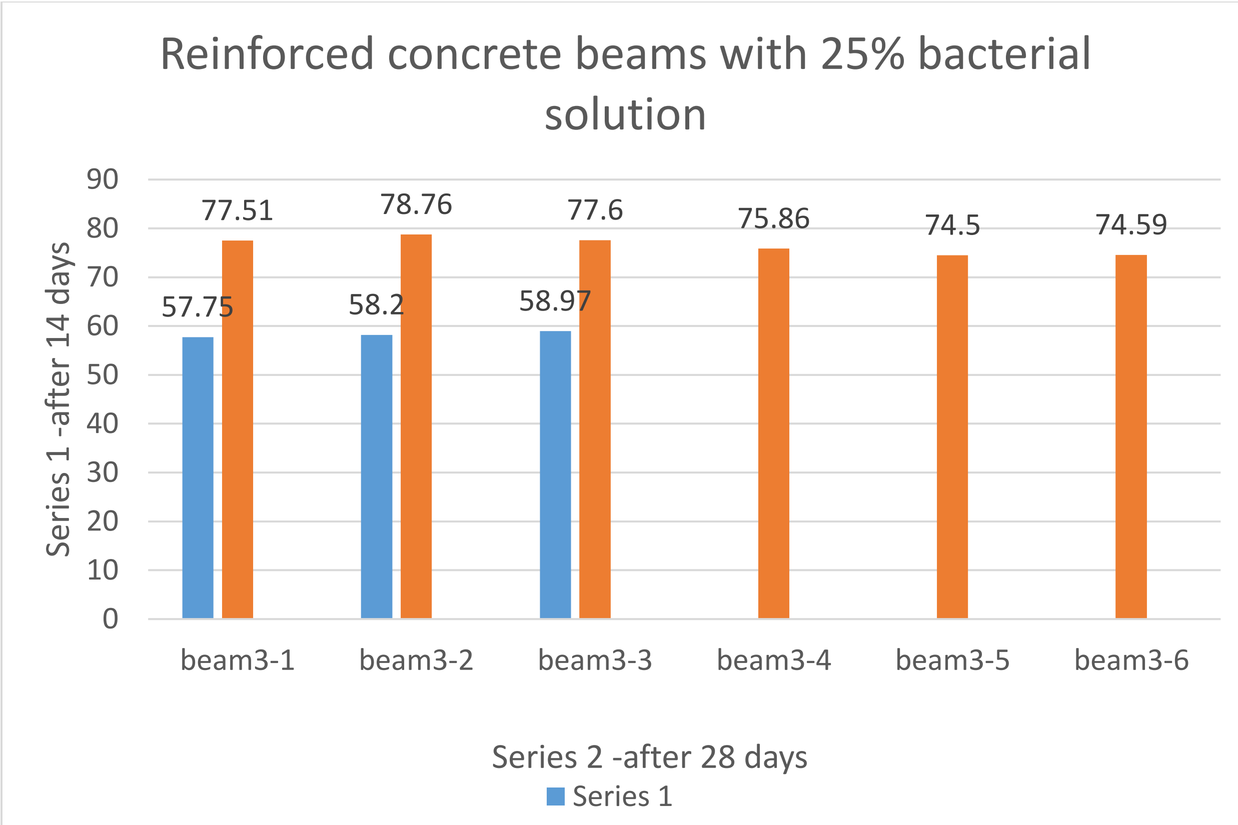
Bio- Concrete Beams (50% bacterial solution): Beams were tested on Flexural Testing Machine, the results are tabulated below-

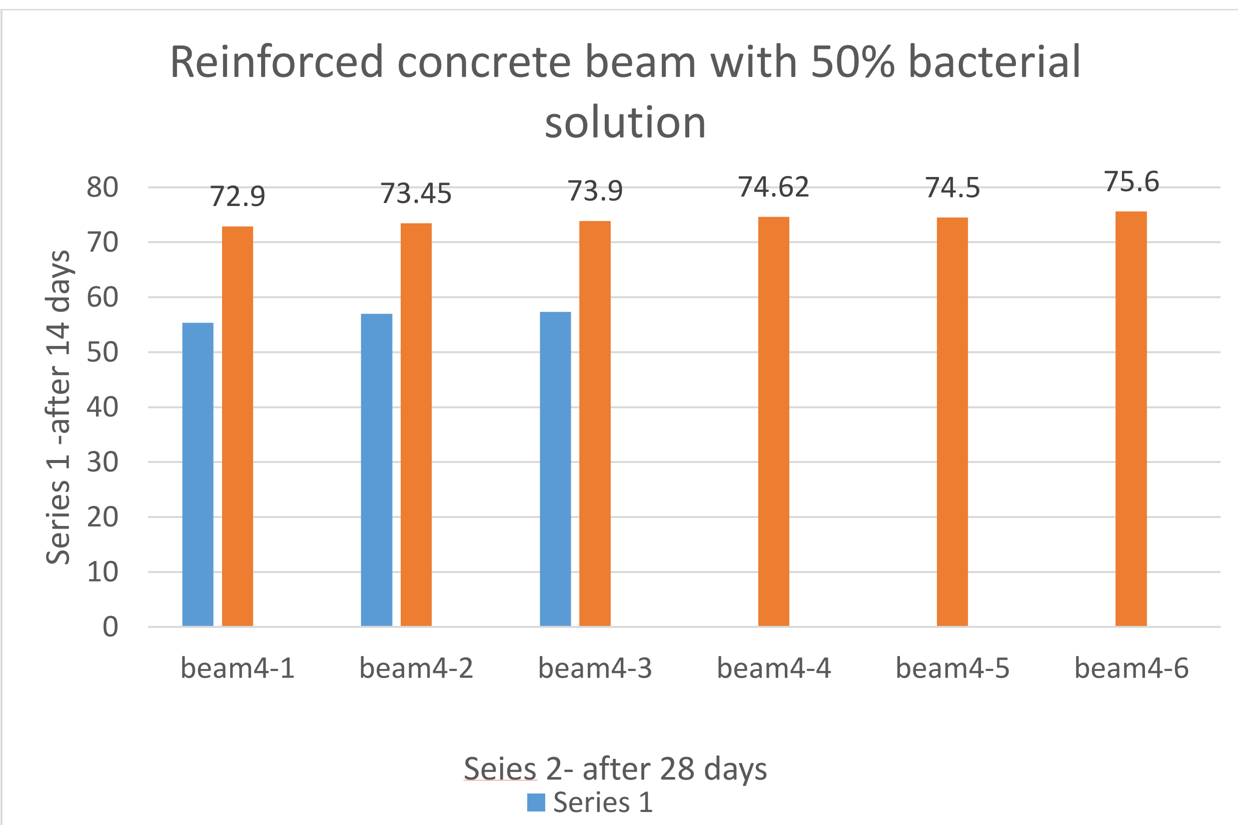
Table 3.2- Flexural Strength of Beams with 50% bacterial solution

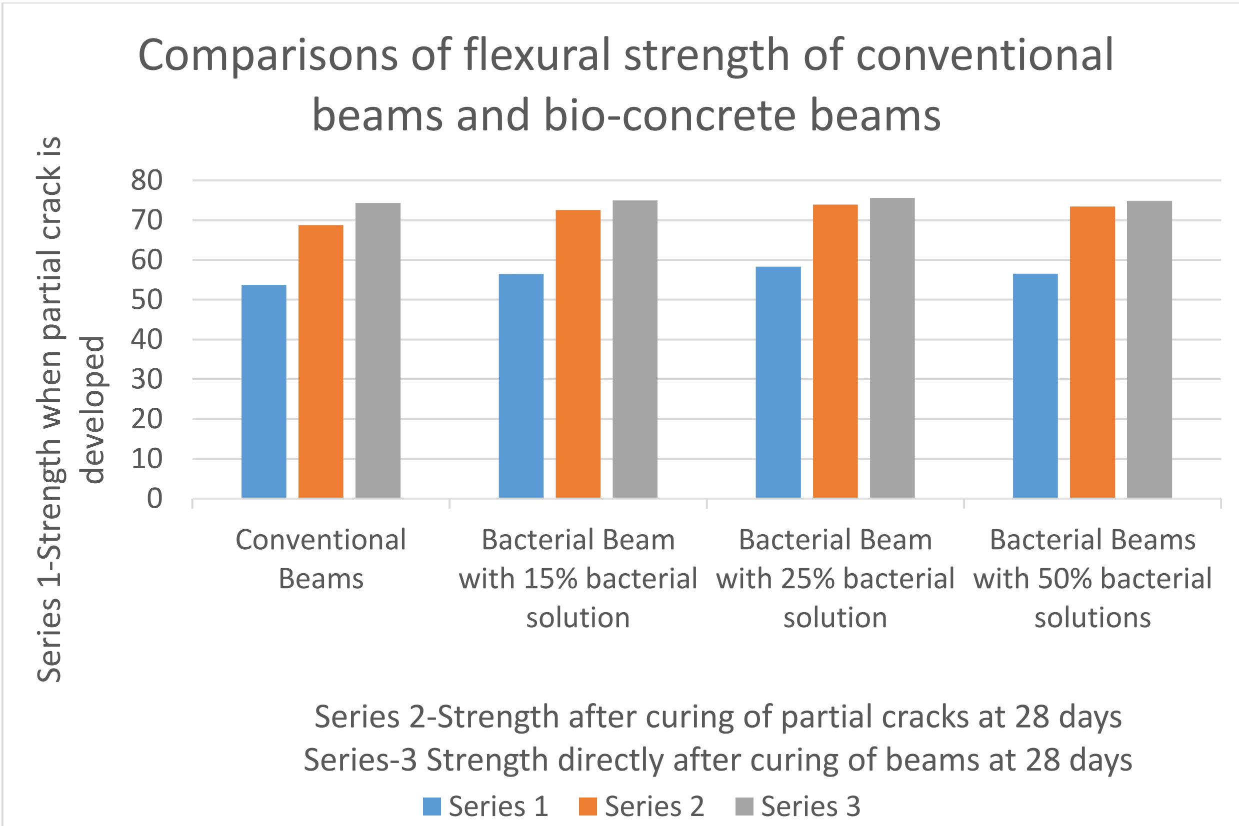
S.No.	Beam (50% bacterial solution)	Load (kN)	
		14days (Partial)	28days
1	B4-1	55.37	72.90
2	B4-2	56.98	73.45
3	B4-3	57.35	73.90
4	B4-4	-	74.62
5	B4-5	-	74.50
6	B4-6	-	75.60


Reinforced concrete beam at 0% bacterial solution
Series 1 -after 14 days
53.56
68.87
52.76
67.63
54.95
69.85
73.45
74.35
75.2
Series 2- after 28 days
Series 1


Reinforced concrete beam at 15% bacterial solution
Series 1 -after 14 days
55.37
71.23
56.76
72.87
57.26
73.54
75.43
74.91
74.59
Series 2 -after 28 days
Series 1


Reinforced concrete beams with 25% bacterial solution
90
80
70
60
50
40
30
20
10
0
Series 1 -after 14 days
77.51
78.76
77.6
75.86
74.5
74.59
57.75
58.2
58.97
beam3-1
beam3-2
beam3-3
beam3-4
beam3-5
beam3-6
Series 2 -after 28 days
Series 1


Reinforced concrete beam with 50% bacterial solution
80
70
60
50
40
30
20
10
0
Series 1 -after 14 days
72.9
73.45
73.9
74.62
74.5
75.6
beam4-1
beam4-2
beam4-3
beam4-4
beam4-5
beam4-6
Seies 2- after 28 days
Series 1


Comparisons of flexural strength of conventional beams and bio-concrete beams
Series 1-Strength when partial crack is developed
80
70
60
50
40
30
20
10
0
Conventional Beams
Bacterial Beam with 15% bacterial solution
Bacterial Beam with 25% bacterial solution
Bacterial Beams with 50% bacterial solutions
Series 2-Strength after curing of partial cracks at 28 days
Series-3 Strength directly after curing of beams at 28 days
Series 1
Series 2
Series 3

CHAPTER 5: CONCLUSIONS

- Introducing the bacteria into the concrete makes it very beneficial it improves the property of the concrete which is more than the conventional concrete.
- Bacteria repair the cracks in concrete by producing the calcium carbonate crystal which block the cracks and repair it.
- Many researchers done their work on the self-healing nature of concrete and they had found the following result that bacteria improves the property of conventional concrete such as increase in 13.75% strength increased in 3 days, 14.28% in 7 days and 18.35% in 28 days.
- Bio concrete is the best solution for the demand of sustainable concrete. Due to its ability of self-repair and durability. In future self-healing concrete is going to play the most important role in concrete technology.
- Bio concrete is eco-friendly and Enhance compressive strength and reduce the permeability.
- Bacterial concrete technology has proved to be better than many conventional technologies.
- Bio concrete enhance the life time of a structure by more than the expected value.

In the present study Bio-Concrete beams were casted with different percentages of bacterial solution, after the proper curing of beams were put to test at 14 days on Flexural Testing Machine by applying partial crack on it when load is applied till the initial cracks were observed on the beam surface. The partially cracked beams were again tested on Flexural Testing Machine at 28 days of curing and the rest of the beams samples were tested directly after 28 days. The results obtained clearly indicate that the load carrying capacity and hence the flexural strength of Bio-Concrete beam regains although beams were partially cracked. It is also observed that regain in strength is more for B3 in which 25% bacterial solution was used. This observation clearly shows the comparison between the conventional concrete beams and bacterial concrete beams for flexural strength of concrete, and also to optimize the better result on a particular percentage to resist lifelong structures.

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A STUDY ON FLEXURAL BEHAVIOUR OF BIO-CONCRETE BEAM

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ABSTRACT: Concrete is the most widely used construction material because of its high compressive strength, relatively low cost, etc. One adverse property of concrete is its sensitivity to crack formation as a consequence of its limited tensile strength. For that reason, concrete is mostly combined with steel reinforcement to carry the tensile loads. Although these rebar restrict the crack width, they Concrete is very sensitive to crack formation. As wide cracks endanger the durability, repair may be required. However, these repair works raise the life-cycle cost of concrete as they are labour intensive and because the structure becomes in disuse during repair. In the following years, several researchers started to investigate this topic. The "Bacterial Concrete" is a concrete which can be made by embedding bacteria in the concrete that can constantly precipitate calcite. This phenomenon is called "Microbiologically Induced Calcite Precipitation" (MICP). Bacillus Pasteruii, a common soil bacterium, can continuously precipitate a new highly impermeable calcite layer over the surface of an already existing concrete layer under suitable environmental conditions. Other bacteria species which can be used for the same purpose are cerus, Pasteurii, Cohnii and Filla.

Keywords: MICP, Bacillus Subtilis, Bacillus Pasteurii, Bio Concrete.

I. INTRODUCTION

Concrete can bear up with compression load, but the material is weak in tension. Hence, steel reinforcement is provided and the steel bars take over the load when the concrete cracks in tension. However, the development of cracks in the concrete pose a problem. Due to reasons like freeze-thaw reactions, shrinkage, low tensile strength of concrete etc., cracks develop during the process of concrete hardening resulting in weakening of the buildings. Due to lack of permeability, water droplets seeping inside can damage the steel reinforcement present in the concrete member. When this phenomenon occurs, the strength of the concrete decreases causing the decay of structure. Synthetic materials like epoxies are used to remediate, but costly, not compatible and need time to time maintenance. Using chemicals is also causing damage to the environment. The need for an environment friendly and effective alternate crack remediation technique leads to the development of using the bio mineralization technique in concrete. Here we are incorporating calcite precipitating bacteria to concrete in certain concentrations so that the bacteria will precipitate calcium carbonate when it comes in contact with water and this precipitate will heal the cracks. Micro biologically Induced Calcite Precipitation (MICP) is the process behind bio mineralization. The basic principle in the process is that the microbial urease, hydrolyses urea, to produce ammonia and carbon dioxide and the ammonia released in surroundings subsequently increases the pH, leading to accumulation of insoluble calcium carbonate. Thus, this self-healing system can help in achieving a massive cost reduction in terms of health monitoring, damage detection and maintenance of concrete structures which assures a safe service life of the structure.

II. LITERARTURE REVIEW

1).Hendrik et al. (1998)[1]. Generally, cement after responding with water during the primary reaction engenders calcium silicate hydrated (C-S-H) gel and calcium hydroxide in aqueous form. Existence of pozzolanic material inside the concrete triggers Secondary hydration and fabricates additional C-S-H gel that accelerates and augments the strength of final concrete. Though, the pozzolanic materials such as silica fume are exclusive and limited. Besides, the cement manufacture released about a tonne of greenhouse gases (GHG) for every tonne of cement

2). **V.Krishnan et al. (2001):** This paper has been informed that MICP it's the mechanism used for healing crack and fissure by using biotechnology and they use B. pasteruii. They observed that because of the production of calcite is in the natural way the MICP is cost effective. Durability, stiffness and strength of concrete is enhancing by the use of bacterial solution in concrete. They use XRD and SEM analysis to measure the amount of precipitation of calcite and pictured. Finally, they observed that the several chemical attacks and the shrinkage enhance because of introducing of bacteria in various medium of concrete.

3). Annie Peter.J et al, (2004): In order to understand the structural behaviour of SCC & CVC in hardened state, reinforced concrete (RC)beams of size 150mm x 400mm x 3000mm with similar concrete strength and identical reinforcement were cast, tested and compared the structural behaviour such as load-deflection characteristics, crack-widths, spacing of cracks, number of cracks, crack pattern, ultimate load- carrying capacity, moments-curvature relationship, longitudinal strain in both concrete and steel. Beyond the peak load stage, CVC beams showed no drop in load with increased deformation while SCC beams showed drop in load with increased deformation. Crack widths were within the limits specified by IS 456 at all load stages. The average crack widths of both the types of beams were comparable.

4). Lakshmanan.N, Devdas Manoharan (2004): Concrete is an absolutely essential component of construction materials used in infrastructure and most buildings. However, concrete is sometimes exposed to substances that can attack it and cause deterioration. The corrosion of the concrete is caused by the interaction between biological and chemical process .Recent research has shown that specific species of bacteria can be useful to enhance the durability and strength of concrete structure. The microbial concrete presents a potentially enormous lengthening in service-life of infrastructure, substantially reduces the maintenances costs. This paper outlines the basic mechanism involved in enhancing the strength and durability of concrete.

5).Prasad et al. (2006): Cracking, strength loss and disintegration strikes concrete when endangered to acid attack, is one of the concrete durability aspects i.e. chemical attack. The acidic strike is subjective to the dissolution processes of the cement paste components. The acid attack menace can be diminished by hindering the 26 corridors present inside the concrete formation. Calcium Carbonate, from microorganism as grout material can shrink the pores and mend the concrete durability.

6). Willem De Muynck et al, (2008): Short comings of conventional surface treatments have drawn the attention to alternative techniques for the improvement of the durability of concrete. This paper report the effects of bacterial carbonate precipitation on the durability of mortar specimens with different porosity. An increased resistance towards freezing and thawing was also noticed. The results obtained with their bio deposition treatment were similar as those obtained with conventional surface treatments.

7). H.M Jokers and Erik (2009): Concrete structures usually show some self-healing capacity, i.e. the ability to heal or seal freshly formed micro-cracks. This property is mainly due to the presence of non-hydrated excess cement particles in the materials matrix, which undergo

Delayed On secondary hydration upon reaction with ingress water. In this research project we develop a new type of self-healing concrete in which bacteria mediate the production of minerals which rapidly seal freshly formed cracks, a process that concomitantly decreases concrete permeability, and thus better protects embedded steel reinforcement from corrosion. Further development of this bio-concrete with significantly increased self-healing capacities would represent a new type of durable and sustainable concrete with a wide range of potential applications.

8). Jonkers et al. (2010); Van Tittelboom et al. (2010), Wiktor and Jonkers(2011); Wang et al. (2012).Consequently, a more natural way to instigate self-healing mechanism is to use bio-materials, Such as microorganisms which work on the basis of bio mineralization. Since, most compatible substance with concrete compositions is calcium carbonate; the techniques of conventional treatment of crack can be replaced by microbial metabolic pathways.

9). Dhami et al (2012): Epoxy treatments are currently used but they are harmful for environment and health as lethal fumes emanated may cause skin and breathing issues. These treatments usually encompass chemical constituents such as epoxy resins, chlorine infused rubbers, waxes, polyurethane, acrylics and siloxane. While passive treatments are appropriate for numerous standing concrete constructions, they have countless restrictions which deter their practice. Some of the shortcomings in the use of chemical treatments have inferior weather endurance, moisture vulnerability, low heat confrontation, un-sustainability, poor adhering with concrete, exposure to decomposition and de-lamination with time and diverse thermal expansion coefficients between concrete and chemical treatments.

10). Vekariya M. et-al(2013): This have defined the bacterial concrete, its classification and types of bacteria, chemical process to fix the crack by bacteria, advantages and disadvantages and possibilities of application of MICP and advantages by switching it over epoxy resins.

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Tensile and compressive strength by B. subtilis type of bacteria for 3, 7 and 28 days. In addition to above technique fly ash was partially added in the place of cement. The cement is replacing by 10% and 30% fly ash in concrete mix tests were did generally found that cement when replacing by 10% fly ash attained maximum strength with and without bacteria than normal concrete.

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III. CONCLUSION

In the present study Bio-Concrete beams were casted with different percentages of bacterial solution, after the proper curing of beams were put to test at 14 days on Flexural Testing Machine by applying partial crack on it when load is applied till the initial cracks were observed on the beam surface. The partially cracked beams were again tested on Flexural Testing Machine at 28 days of curing and the rest of the beams samples were tested directly after 28 days. The results obtained clearly indicate that the load carrying capacity and hence the flexural strength of Bio-Concrete beam regains although beams were partially cracked. It is also observed that regain in strength is more for B3 in which 25% bacterial solution was used. This observation clearly shows the comparison between the conventional concrete beams and bacterial concrete beams for flexural strength of concrete, and also to optimize the better result on a particular percentage to resist lifelong structures.

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6.2 IMPACT FACTOR

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AN EXPERIMENTAL STUDY ON STRENGTH BEHAVIOUR OF BIO-CONCRETE CUBES CYLINDERS AND BEAMS

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ABSTRACT: Concrete is the most widely used construction material because of its high compressive strength, relatively low cost, etc. One adverse property of concrete is its sensitivity to crack formation as a consequence of its limited tensile strength. For that reason, concrete is mostly combined with steel reinforcement to carry the tensile loads. Although these rebar restrict the crack width, they Concrete is very sensitive to crack formation. As wide cracks endanger the durability, repair may be required. However, these repair works raise the life-cycle cost of concrete as they are labour intensive and also the structure becomes in disuse during repair. In view of the above, several researchers started to investigate this topic. The "Bacterial Concrete" is a concrete which can be made by embedding bacteria in the concrete that can constantly precipitate calcite. Cracking in the surface layer of concrete mainly reduces its durability, since cracks are responsible for the passage of liquids and gasses that could potentially contain deleterious substances. When micro-cracks migrates towards the reinforcement, not only the concrete itself may be damaged, but also corrosion occurs in the reinforcement due to exposure to water and oxygen, and possibly CO_2 and chlorides too. Micro-cracks are therefore the main cause to structural failure. One way to circumvent costly manual maintenance and repair is to incorporate an autonomous self -healing mechanism in concrete. One such an alternative repair mechanism is currently being studied, i.e. a novel technique based on the application of bio-mineralization of bacteria in concrete. Synthetic polymers such as epoxy treatment etc. are currently being used for repair of concrete are harmful to the environment, hence the use of a biological repair technique in concrete is focused. In the present paper, an attempt is made to incorporate dormant but viable bacteria in the concrete matrix which will contribute to the strength and durability of the concrete. Water which enters the concrete will activate the dormant bacteria which in turn will give strength to the concrete through the process of metabolically mediated calcium carbonate precipitation. Concrete, due to its high internal pH, relative dryness and lack of nutrients needed for growth, is a rather hostile environment for common bacteria, but there are some extremophiles spore forming bacteria may be able to survive in this environment and increase the strength and durability of cement concrete. Overview of bioengineered concrete using bacterial Bacillus Cereus and its enhanced mechanical and durable characteristics will be briefly described in this paper.
Keywords: MICP, Compressive, tensile and Flexural strength bacteria solution, media.

1. INTRODUCTION

It is special type of concrete invented by a group of micro biology researchers under the head of Henk Jonkers.Bio-concrete is specially made to increase the life span, strength, and durability of concrete structures by the self-healing action of that concrete. The Bio-concrete is dead concrete that awakens when crack appears. Then they produce limestone that fills the crack in a matter of weeks. In the recent years MICCP (microbiologically induced calcium carbonate precipitation) by the bacteria considered as an environment friendly method to enhance the properties of concrete, also for the repair of concrete structure and to consolidate different construction materials. This project presents a review of different researches in the recent years on the use of bacterial concrete/bio-concrete for the enhancement in the durability, mechanical and permeation aspects of concrete. The selection of bacteria was according to their survival in the environment such as B. pasteurii, Bacillus subtilis and Bacillus cereus which are mainly used for experiments by different researchers for their study. The condition of growth is different for different types of bacteria. For the growth of bacterial solution which is to be used in bio-concrete were put in medium containing different chemicals at a particular temperature for a particular period of time. It contains studies on different bacteria's, their isolation process, different approaches for addition of bacteria in concrete, their effects on compressive strength and tensile strength and flexural strength of concrete containing bacteria.

Principle of self-healing concrete-

Self-healing concrete was invented by Henk Jonkers, a microbiologist and professor as Delft University of Technology in the Netherlands. Jonkers began developing self-healing concrete in 2006. After three years of experimenting, he found the perfect healing agent –bacillus. "You need bacteria that can survive the harsh environment of concrete," Jonkers said in an interview with CNN. "It's a rock like, stone-like material, very dry."

Bacillus is a perfect match for the job. The bacteria will thrive in a high-alkaline conditions of concrete and produces spores than can live up to four years without any food or oxygen. Jonkers finalized his creation by adding calcium lactate to the limestone concrete mixture in order to feed the bacillus so that they can produce limestone to repair cracks in the concrete. "It is combining nature with construction materials," Jonkers said. "Nature is supplying us a lot of functionality for free. In this case, limestone-producing bacteria. Self-healing concrete could solve the problem of concrete structures deteriorating well before the end of their service life. Concrete is still one of the main materials used in the construction industry, from the foundation of buildings to the structure of bridges and underground parking lots. Traditional concrete has a flaw, it tends to crack when subjected to tension.

Objective:

The main objectives of the work are-

- To compare the compressive, tensile and flexural strength of normal concrete and bio concrete.
- To develop self- healing bio concrete using biological based techniques.

2. FUNCTION OF BACTERIA

Research has shown that autogeneous healing happens due to hydration of non-reacted cement particle present in the concrete matrix when comes in contact with ingress water resulting in closure of micro-cracks, studies also stated that only spore forming gram positive bacteria can survive in high pH environment of concrete sustaining various stresses. Therefore, bacterially induced calcium carbonate precipitation has been proposed as an alternative and environmental friendly crack repair technique. Microbial calcite precipitation is mainly due to urealytic activity and carbonate bio-mineralization of bacteria. The bacteria used in this research produce urease which catalyzes the hydrolysis of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}), First, 1mol of urea is hydrolysed intracellular to 1mol of carbonate and 1mol of ammonia (Eq. (1)).

$CO(NH_2)_2 + H_2O$ --- $NH_2COOH + NH_3$ (1)

Carbonate spontaneously hydrolyses to form additionally 1mol of ammonia and carbonic acid (Eq. (2)).

$NH_2COOH + H_2O$ ---- $NH_3 + H_2CO_3$ (2)

These products subsequently form 1mol of bicarbonate and 2mol of ammonium and hydroxide ions (Eq. (3) and (4)).

H_2CO_3 -------- $HCO_3^- + H^+$ (3)

$2NH_3 + 2H_2O$ ----- $2NH_4^+ + 2OH^-$ (4)

The last 2 reactions give rise to a pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of carbonate ions (Eq. (5)).

$HCO_3^- + H^+ + 2NH_4^+ + 2OH^-$ ------ $CO_3^{2-} + 2NH_4^+ + 2H_2O$ (5).

Since the cell wall of the bacteria is negatively charged, the bacteria draw cat ions from the environment, including Ca^{2+}, to deposit on their cell surface. The Ca^{2+} ions subsequently react with the CO_3^{2-} ions, leading to the precipitation of $CaCO_3$ at the cell surface that serves as a nucleation site (Eq. (6) and (7))

$Ca^{2+} + Cell \rightarrow Cell\ Ca^{2+}$ (6)

$Cell\ Ca^{2+} + CO_3^{2-} \rightarrow Cell\ CaCO_3 \downarrow$ (7)

Several bacteria have the ability to precipitate calcium carbonate. These bacteria can be found in soil, sand, natural minerals. This strain showed a high urease activity, a continuous formation of dense calcium carbonate crystals and a very negative zetapotential. Self-healing concrete is a product that will biologically produce limestone to heal cracks that appear on the surface of concrete structures. Specially selected types of the bacteria genus Bacillus, along with a calcium-based nutrient known as calcium lactate, and nitrogen and phosphorus, are added to the ingredients of the concrete when it is being mixed. These self-healing agents can lie dormant within the concrete for up to 200 years.

3. MATERIALS

3.1 Concrete and concrete specimens

Pozzolana Portland cement of M20 was used for all mixtures. Depending on the type of experiment, concrete samples were chosen for reasons of practical convenience. Standardized concrete cubes (150×150×150mm) prepared, were grounded in powder form for pH, EDTA and SEM experiments. For M20, the Cement: Sand: Aggregate ratio should bein1:1.5:3 i.e.1partofcement, 1.5part of sand and 3 part of aggregate.IS: 456-2000 has recommended that minimum grade of concrete shall be not less than M20 in reinforced concrete work. Design mix concrete is preferred to nominal mix. If design concrete cannot be used for any reason on work for grade M20 or lower, nominal mixes may be used with the permission of engineer-in charge, which however is likely to involve a higher is likely to involve a higher cement content.

3.2 Micro-organism and growth conditions

A thermophilic, anaerobic microorganism Bacillus Cereus isolated from CT5 strain was used in this study. This microorganism was cultured anaerobically in a modified medium (pH 11) before adding to the cement sand and aggregate concrete. Bacillus microorganism grown in Nutrient Broth (NB) medium having pH of 11 was used to study their effect on concrete. 3.3 Mix proportion Microorganism in different ways was added to concrete. The cement to sand to aggregate ratio was used as 1:1.26:2.56 (by weight), and water to cement ratio was fixed at 0.45. A cube mould of 150 mm was used, as per IS 4031-1988 [11]. Three different cases including non-bacterial treated and bacterial treated were studied. 3.4 Culture media Bacillus Cereus was cultivated under aerobic batch conditions in a culture media.100 ml culture media was prepared.0.65 gms of Nutrient broth was added in 50 ml of water and kept for autoclaving. 3.7045 gms of calcium hydroxide was added in 50 ml of water. Both the above solutions were mixed together and a complete 100 mL solution were obtained. 2.5 mL of calcium acetate and 5 mL of urea were added in 100 mL of solution.0.5 % of bacterial strain were added in 100 mL of solution to obtain compete bacterial culture. The culture so obtained was kept in shaker for 24 hrs.

3.3 PREPERATION OF BACTERIAL SOLUTION-

For the preparation of 1 litre bacterial solution following steps were taken-

- As we are preparing 1 litre solution we need 500ml Nutrient Broth solution, therefore adding 6.5gm NB in 500 ml distilled water.
- The solution is then kept in autoclave to remove any contamination present in it.
- Concentration of $Ca(OH)_2$ = 74.09gm/L
- Therefore adding 37.045gm $Ca(OH)_2$ in 500ml of water.
- The pH of complete 1000 ml solution must be maintained between 9 to 12 which is the required pH for the growth of bacteria. The solution obtained is kept in shaker for 24hours at 27^oC.
- After 24 hours calcium acetate, urea and inoculum is added in the solution.

Bacteria Cereus Culture

Bacterial Solution

4. METHOD OF INVESTIGATION

4.1 Compressive Strength

This test was carried out on six specimens of each sample in which three of them were tested at 7 days and rest three were tested at 28 days with following the procedure described by compression testing machine. Compressive strength measurements were carried out using five tones German Bruf Pressing Machine with a loading rate of 100 kg/min. The compressive strength is frequently used as a measure of the resistance because this strength is the most convenient to measure. The cement mortar/concrete properties of various ingredients of the mixture are usually measured in terms of their compressive strength. The compressive strength of concrete/mortar is usually determined by submitting a specimen of constant cross-section to a uniformly distributed increasing axial compression load in a suitable testing machine.

4.2 Flexural Strength

The durability of concrete/mortar is related to the characteristics of its pore structure. Furthermore, permeability of concrete/mortar is dependent on the porosity and the connectivity of the pores. The degradation mechanisms often depend on the way potentially aggressive substances can penetrate the cement-based material, possibly causing damage. The more open the pore structure and connectivity of the pores, the more vulnerable it is to degradation caused by penetrating substances. One of the predominant causes of the corrosion of steel in cement-based structures is chloride attack. Chloride ions may be present in a cementitious material either as a result of aggressive ions ingress or incorporation of the aggressive ions during concrete/mortar preparation. Chloride ions may also penetrate from external sources such as seawater or dicing salts. In the marine environment, the in Chloride is bound if it has reacted with cement and is free if it is available in pore solution. Bio-cementation in OPC lowers chloride ingress and permeability into the cement matrix. This is due to the refinement of the pore structure. Chloride ions penetrate a pore system and form chloride salts which may crystallize within the pores inducing internal cracks. The cracks affect the mechanical and durability properties of concrete/mortar.

4.3 Scanning electron microscopy (SEM)

The scanning electron micrographs of freshly fractured specimens were taken with Inspect S (FEI Company, Holland) equipped with an energy dispersive X-ray analyzer (EDAX) at the accelerating voltage of 200 V to 30 kV. SEM micrographs were obtained using a Jeol JSM5600LV apparatus. Samples were gold coated with a JFC1200 fine coater prior to examination.

4.4 pH

pH test was conducted on powder specimen. Water and powder sample were taken in 1:9 ratios and kept for a overnight. Then the pH of each individual case was determined by using pH meter. Third important factor is ph which influences calcite precipitation. Urea hydrolysis will occur only when urease enzyme will attain specific pH values which would result in Calcite precipitation, which is subjective to pH. Many researchers have proclaimed that the optimum pH in favor of urease is 8.0, beyond which the activity of enzyme dwindles. An increased pH is imperative for ammonia creation via urea hydrolysis.

4.5 EDTA

Powder samples, 0.5 gms of each individual case in addition with 3 ml HCl, 4 ml NaOH and 43 ml water were titrated against EDTA. Hydroxy napthol blue indicator was used. Variation in solution colour from pink to blue confirms the presence of calcium carbonate in the solution.

4.6 Slump Value Test

Mixed bacterial concrete was poured into the frustum of cone in three layers with 25 blows on each layer. The size of frustum is Upper diameter-100mm Lower diameter-200mm and Height-300mm After pouring the concrete full into the frustum of raise the mould from the concrete slowly in vertical direction and measure the fall from the top of the concrete. Slump as the difference between the height of the mould and that of height point of the specimen being tested

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5. RESULTS AND DISCUSSIONS

5.1 Compressive strength and Tensile Strength

Control cubes and microbial cubes for four different percentage of addition of bacterial culture such as C100 – specimens were mixed with 100 % bacterial culture in place of water cement ratio.; C75- specimens were mixed with 25 % water and 75% bacterial culture; C50-specimens were mixed with 50% water and 50% bacterial culture; C25-specimens were mixed with 75% water and 25% bacterial culture After casting, curing of cubes readings were taken on each 7 and 28 days. The results obtained from the testing are tabulated in Table 1.It can be seen that increase in strength is maximum at 7 days in all the cases. It shows that till 7 days, bacteria grow properly and has completely adopted the atmosphere inside concrete cube. As a bacterium grows, calcium carbonate precipitation starts and increases the compressive strength of concrete. Since the bacteria grow only in aerobic condition, the maximum precipitation occurs on the surface of concrete. Slowly after 7 days increase in compressive strength were observed due to formation of calcite layer. Layer of calcium carbonates so obtained as shown in Fig 1 on concrete cube is known as microbial induced calcite precipitation. A significant increase of 39%, 41%, and 38.88% were observed for different cases i.e. case1, case2, case3 and case4 at initial 7 days in comparison to control case respectively. After 28 days it was observed that, the effective increase in compressive strength was less as compared to 7 days strength. Because once all the pores on surface is blocked, anaerobic condition arises in the concrete and the activity of bacteria slows down, with bacteria converting themselves in to spores. A very important point can be seen from Table1, which no matter in what percentage w/c ratio were replaced with bacterial culture, it shows same increase in strength for7 and 28 days. This means microbial effect influence concrete in same manner whether it is used in any percentage.

5.2 Flexural Strength

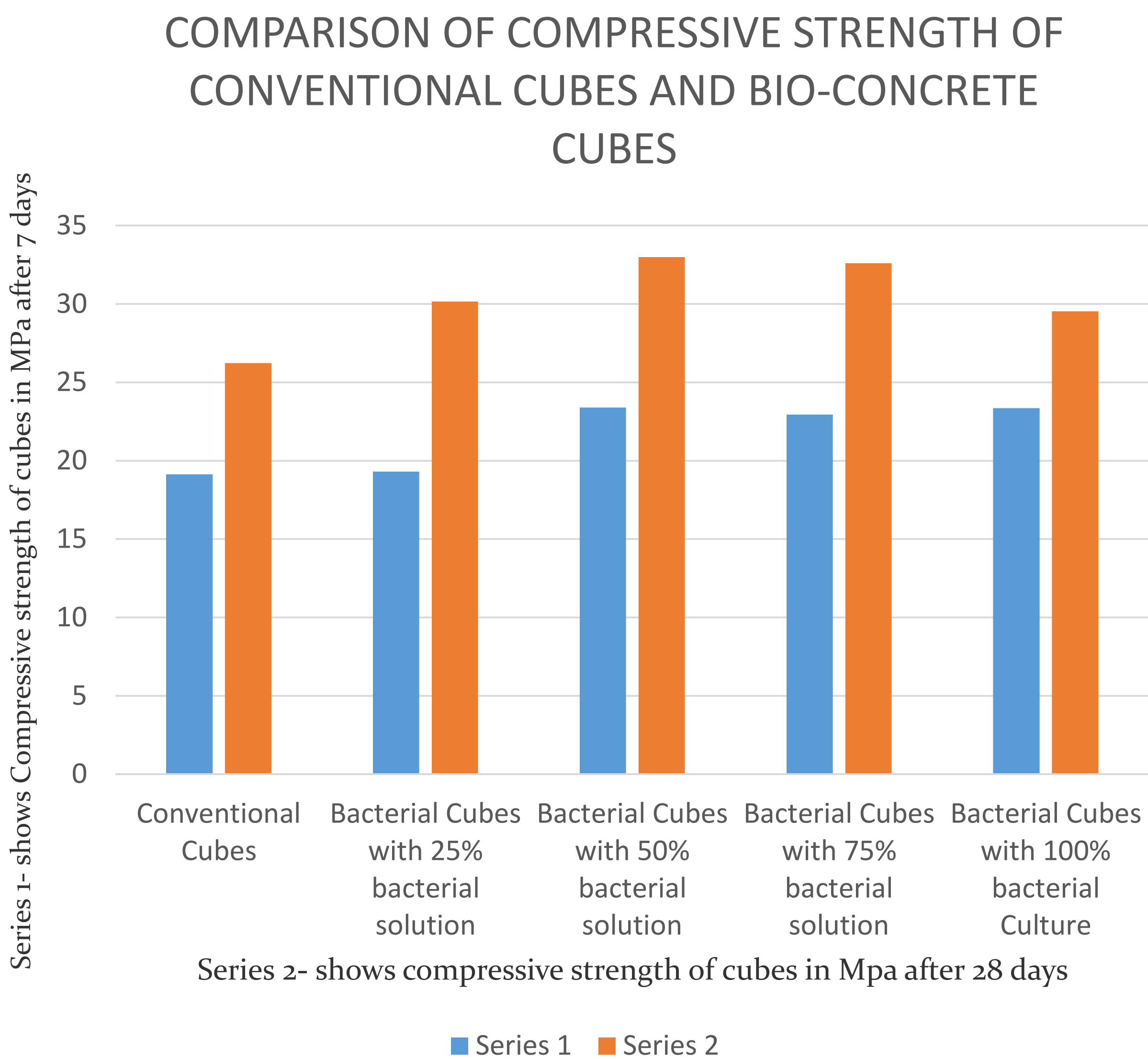
In the present study Bio-Concrete beams were casted with different percentages of bacterial solution i.e. 15%, 25% and 50% , after the proper curing of beams were put to test at 14 days on Flexural Testing Machine by applying partial crack on it when load is applied till the initial cracks were observed on the beam surface. The partially cracked beams were again tested on Flexural Testing Machine at 28 days of curing and the rest of the beams samples were tested directly after 28 days. The results obtained clearly indicate that the load carrying capacity and hence the flexural strength of Bio-Concrete beam regains although beams were partially cracked. It is also observed that regain in strength is more for B3 in which 25% bacterial solution was used. This observation clearly shows the comparison between the conventional concrete beams and bacterial concrete beams for flexural strength of concrete, and also to optimize the better result on a particular percentage to resist lifelong structures.

5.3 pH measurement

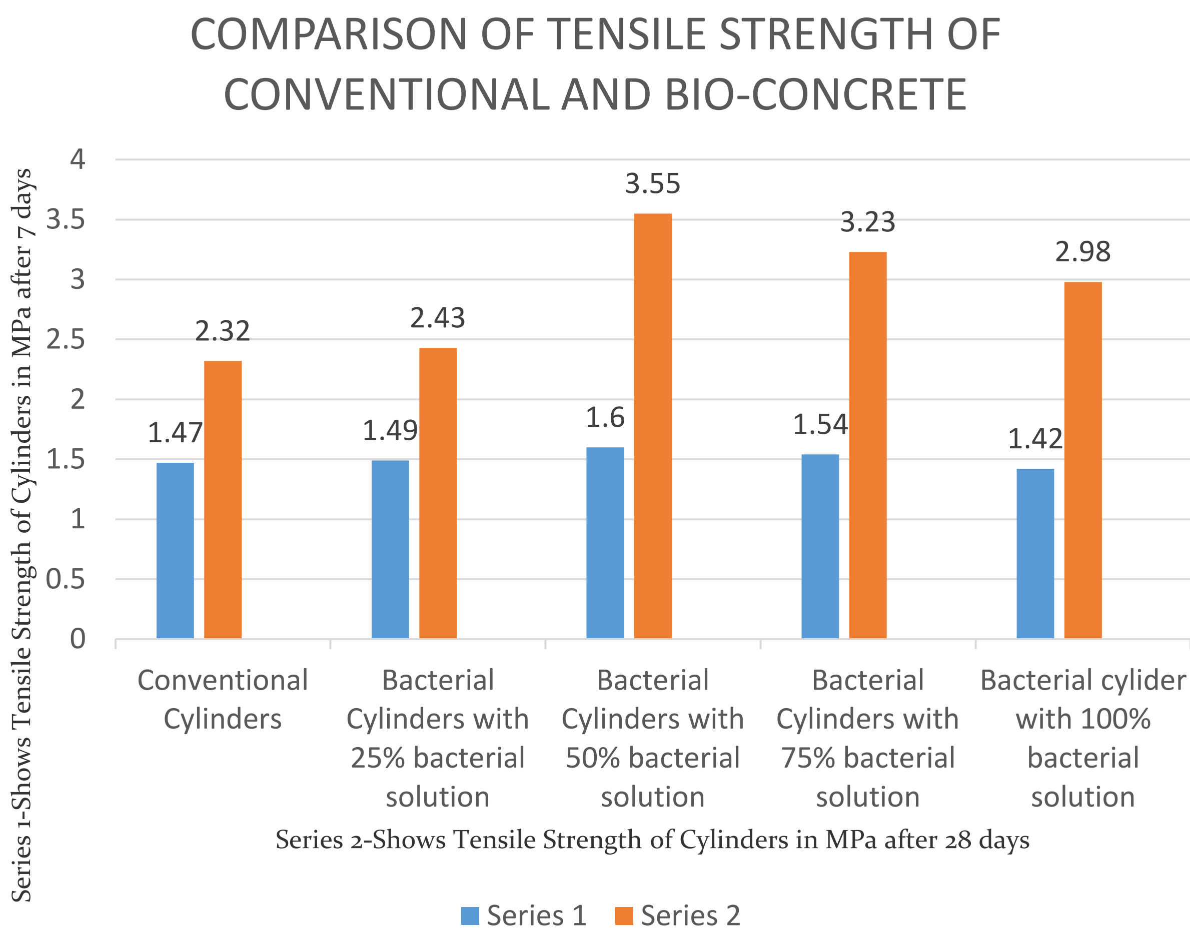
For determining pH of the given samples, crushed concrete obtained after conducting compressive strength was taken were converted in to powdered form which can pass through 300μ sieve. Powdered sample was mixed with water in ratio of 1:9 in test tube and left for overnight, next day this sample was tested by pH meter to get pH of the mix.

5.4 Slump Test Result

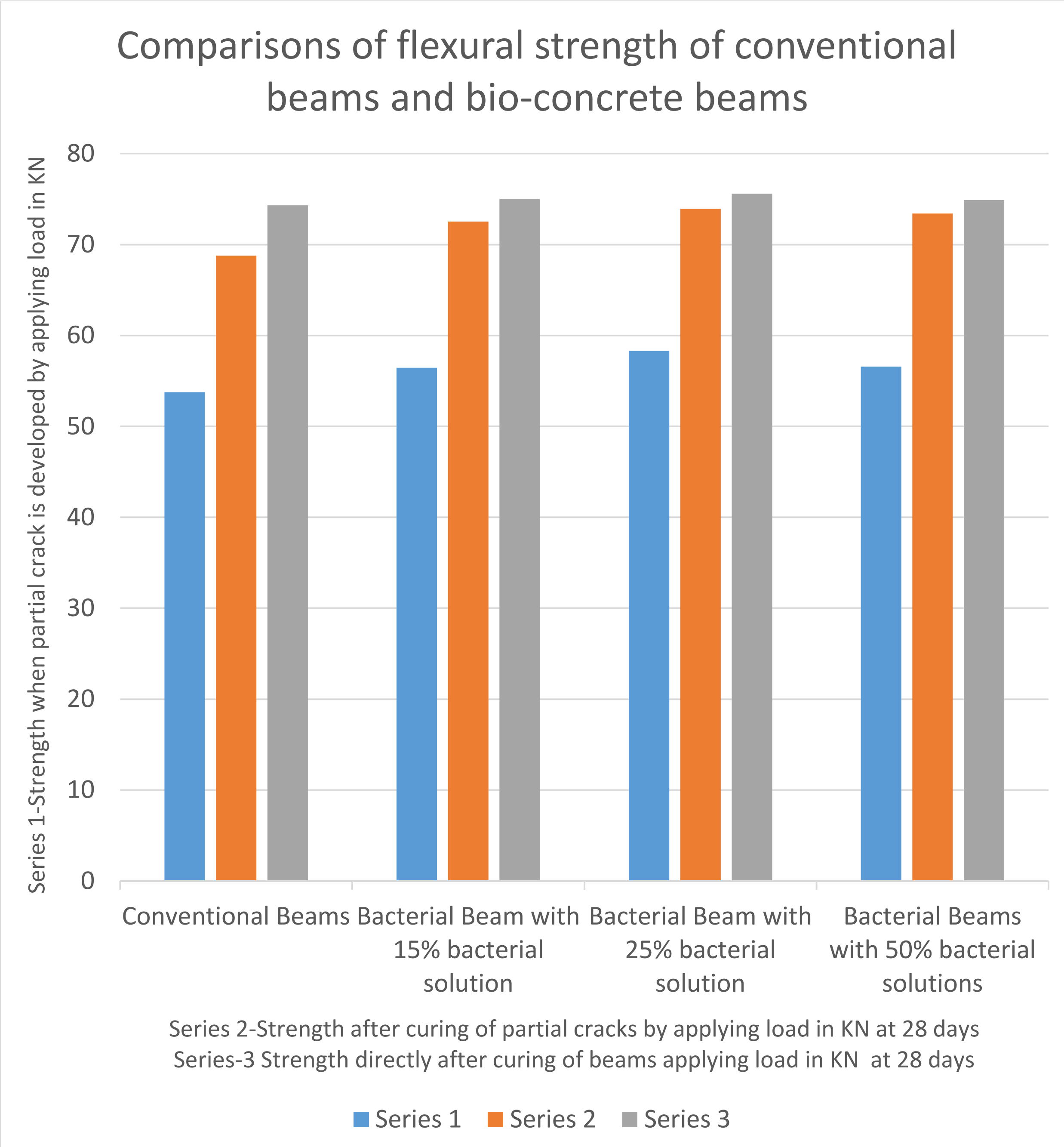
Decrease in height of concrete to that of mould was noted and it was found to be 114mm for conventional concrete and 65, 63, 67and 68 mm for bacterial concrete for C100, C75, C50 and C25 respectively.



Bar Graph of Compressive strength of various cube samples



Bar Graph of Tensile strength of various cylindrical samples



Bar Graph of flexural strength of various beam samples

6. CONCLUSION

On the basis of the above results it is clearly shown that there is an increment in the compressive and tensile strength of concrete cubes and cylinders and the calcium carbonate precipitation also starts forming on surface.

Fig-Calcium carbonate precipitation formed

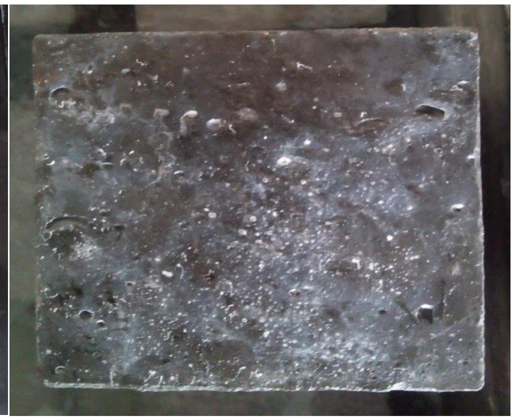


Fig-Pores start filling up by calcium carbonate

If we compare all the five results case i.e. producing the best result is third case in which the concentration of bacterial solution is 50% and rest is normal water. If we look at the strength after seven days for 100% bacterial solution the increment in strength is 22% and for 75% solution it is 19.96% but there is an immense increment in strength after seven days for 50% bacterial solution i.e. 23%. The results follow the same trend for the 28 days 50% case has the maximum compressive strength. The third case is also suitable from the economic point of view.

The test results of Bio-Concrete beams clearly indicate that the load carrying capacity and hence the flexural strength of Bio-Concrete beam regains although beams were partially cracked. It is also observed that regain in strength is more for B3 in which 25% bacterial solution was used. This observation clearly shows the comparison between the conventional concrete beams and bacterial concrete beams for flexural strength of concrete.

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