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# EXPERIMENTAL INVESTIGATION ON STRENGTH ASPECTS OF BACTERIAL CONCRETE

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***Abstract***

*Concrete is the primary construction and repair material of many structural systems. Today, many of the concrete structures, which have been exposed to aggressive environments, suffer from durability problems and fail to fulfill their design service life requirements. The problem is particularly serious in reinforced concrete structures where corrosion of reinforcing steel can impair their safety. The cost of the repair and rehabilitation of corrosion-damaged structures constitutes a large portion of the infrastructure expenditure. The limited knowledge of the field performance of corrosion damaged structures and the lack of systematic approaches for their inspection, maintenance and repair contribute to the increase of their life-cycle costs, and result in the loss of functionality and safety. A promising sustainable repair methodology based on application of mineral producing bacteria in cement/concrete is currently being investigated.*

*The main aim of this experimentation is to study the effect of the influence of Bacillus Sphaericus bacteria on the strength properties of normal and GGBS concrete. In GGBS concrete cement was replaced with two percentages (20% and 30%) with GGBS by weight. Different cell concentrations (0, 105, 107cells/ml) of bacteria were used in making the concrete mixes. Tests were performed for different strength properties at the age of 28 days. Test results indicated that inclusion of Bacillus sphaericus in normal and GGBS concrete enhanced the different strength properties of concrete. Maximum increase in compressive strength, split tensile strength, flexural strength, was observed with concentration 105 cells/ml of bacteria. This improvement in strength was due to deposition on the bacteria cell surfaces within the pores. The present work highlights the influence of bacteria on the properties of concrete made with supplementing cementing material like GGBS. Usage of bacteria like Bacillus sphaericus improves strength of normal and GGBS concrete through self-healing effect.*

***Keywords****- Bacterial concrete ,bacillus sphaericus, 0%,20%,30%GGBS(Ground Granulated Blast Furnace Slag), Compressive strength, Cell Concentration (0,$10^5$,$10^7$) cells/ml, Reference Mix, Scanning Electron Microscopy, Normal Concrete.*

## 1. INTRODUCTION

Concrete is the most widely used man made construction material in civil engineering world. It has specialty of being cast in any desirable shape but plain concrete however possesses very low tensile strength, limited ductility and little resistance to cracking. As a matter of fact, advancement in concrete technology has been generally on the strength of concrete. It is now recognized that strength of concrete alone is not sufficient, the degree of harshness of the environmental condition to which concrete is exposed over its entire life is very important. Therefore, both strength and durability have to be considered explicitly at the design stage. To do this, a durable structure needs to be produced. For concrete buildings, one of the major forms of environmental attack is chloride ingress, which leads to corrosion of the reinforcing steel and a subsequent reduction in the strength, serviceability and aesthetics of the structure. This may lead to early repair or premature replacement of the structure. A common method of preventing such deterioration is to prevent chlorides from penetrating the structure by using relatively impenetrable concrete. The ability of chloride ions to penetrate the concrete must then be known for design as well as quality control purposes. The penetrability of concrete is obviously related to the pore structure of the cement paste matrix. This will be influenced by the water–cement ratio of the concrete, the inclusion of supplementary cementing materials which serve to refine the pore structure and the degree of hydration of the concrete. The highly developed pore structure occurs due to greater amount of heat of hydration which intern depends on the age of concrete. This is especially true for concrete containing slower reacting supplementary cementing materials such as fly ash require a longer time to hydrate

### 1.1. GGBS

Ground-granulated blast-furnace slag (GGBS or GGBFS) is obtained by quenching molten iron slag (a by-product of iron and steel-making) from a blast furnace in water or steam, to produce a glassy, granular product that is then dried and ground into a fine powder.

### 1.2. APPLICATIONS

- GGBS is used to make durable concrete structures in combination with ordinary Portland cement and/or other pozzolanic materials.
- Two major uses of GGBS are in the production of quality-improved slag cement, namely Portland Blast furnace cement (PBFC) and high-slag blast-furnace cement(HSBFC), with GGBS content ranging typically from 30to 70%; and in the production of ready-mixed or site-batched durable concrete.
- Concrete made with GGBS cement sets more slowly than concrete made with ordinary Portland cement, depending on the amount of GGBS in the cementitious material, but also continues to gain strength over a longer period in production conditions. This results in lower heat of hydration and lower temperature rises, and makes avoiding cold joints easier, but may also affect construction schedules where quick setting is required.
- Use of GGBS significantly reduces the risk of damages caused by alkali silica reaction (ASR), provides higher resistance to chloride ingress reducing the risk of reinforcement corrosion and provides

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higher resistance to attacks by sulfate and other chemicals.

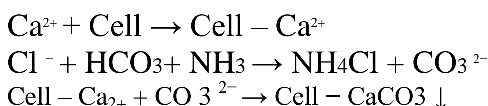
### 1.3. BACTERIAL CONCRETE

The "Bacterial 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). B. Sphaericus is used to induce calcite precipitation in concrete. 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. Bacterial cultures improve the strength of concrete and crack repair of concrete structures.

Calcite formation by Bacillus species is used in making bio-concrete, which can produce calcite precipitates on suitable media supplemented with a calcium source. The basic principles for this application are that the microbial urease hydrolyzes urea to produce ammonia and carbon dioxide and the ammonia released in surroundings subsequently increases pH, leading to accumulation of insoluble calcium carbonate. 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. These cracks will subsequently be rapidly plugged and sealed through metabolically mediated microbial calcium carbonate precipitation, hampering further ingression of water and other chemicals. As revived bacteria also need a suitable substrate that can metabolically be converted to calcium carbonate, this also needs to be part of the concrete compatibility.

#### 1.3.1. Working Of Bacterial Concrete

In natural environments, chemical CaCO3 precipitation ($Ca2^{+}$ + $CO3^{2-}$ $CaCO3^{-}$) is accompanied by biological processes, both of which often occur simultaneously or sequentially. This microbiologically induced calcium carbonate precipitation (MICCP) comprises of a series of complex biochemical reactions. As part of metabolism, B. Sphaericus produces urease, which catalyzes urea to produce CO2 and ammonia, resulting in an increase of pH in the surroundings where ions $Ca_2{}^{+}$ and $CO_3{}^{2-}$ precipitate as CaCO3. Possible biochemical reactions in medium to precipitate CaCO3 at the cell surface that provides a nucleation site can be summarized as follows.




$Cell - Ca_{2+} + CO\ 3^{\ 2-} \rightarrow Cell - CaCO3 \downarrow$

A dormant (alive but not growing) and viable (capable of working successfully) microorganism of certain number is induced in concrete during mixing. Bacterial spores immobilized in the concrete matrix will become metabolically active when revived by water and calcium media of concrete. The hollow space (microscopic level) will subsequently be rapidly plugged and sealed through metabolically mediated microbial calcium carbonate precipitation, hampering further ingression of water and other chemicals. When the concrete is mixed with bacteria, the bacteria go into a dormant state, alot like seeds. All the bacteria need is exposure to the air to activate their functions.Any cracks that should occur provide the necessary exposure. When the cracks form, bacteria very close proximity to the crack, starts precipitating calcite crystals. When a concrete structure is damaged and water starts to seep through the cracks that appear in the concrete, the spores of the bacteria germinate on contact with the water and nutrients. Having been activated, the bacteria start to feed on the calcium lactatenutrient. 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. As the bacteria feeds oxygen is consumed and the soluble calcium lactate is converted to insoluble limestone. The limestone solidifies on the cracked surface, thereby sealing it up. Oxygen is an essential element in the process of corrosion of steel and when the bacterial activity has consumed it all it increases the durability of steel reinforced concrete constructions. The last, but certainly not least, key component of the self-healing concrete formula is the bacteriathemselves. The most promising bacteria to use for selfhealing purposes are alkali philic (alkali-resistant) spore-forming bacteria.

#### 1.3.2. Bacillus Sphaericus

National chemical laboratory (CSIR-NCL) Pune, established in 1950 is a constituent laboratory of council of scientific and industrial research (CSIR) CSIR-NCL is a science and knowledge based research development and consulting organization. In the present study an attempt was made by using the bacteria Bacillus Sphaericus NCIM No.2478, which is a part of CSIR-NCL, Pune. Researchers with different bacteria proposed different bacterial concretes. Bacillus Sphaericus is obtained from NCIM Pune. The main advantage of embedding bacteria in the concrete is that it can constantly precipitate calcite. This phenomenon is called microbiologically induced calcite precipitation (MICP). Calcium carbonate precipitation, a widespread phenomenon among bacteria, has been investigated due to its wide range of scientific and technological implications. Bacillus Sphaericus NCIM No.2478 is a laboratory cultured bacterium and its effect on the strength and durability is studied here.

#### 1.3.3. SCANNING ELECTRON MICROSCOPY

The most common SEM mode is detection of secondary electrons emitted by atoms excited by the electron beam. The number of secondary electrons depends on the angle at which beam meets surface of specimen that is on specimen topography. By scanning the sample and collecting the secondary electrons with a special detector, an image displaying the topography of the surface is created.

Calcite precipitation in concrete was carried out by SEM analysis. The specimens with bacteria did not develop any micro cracks, as they did not expand much unlike reference specimens. Furthermore, many calcite crystal faces show hollow, rod-like impressions of B. Sphaericus,

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where bacteria in contact with the calcite interfered with normal crystal growth.

## 2. OBJECTIVES

- To investigate the effect of variation of bacterial concentration on the strength properties of concrete.
- To investigate the effect of partial replacement of cement by GGBS on the strength properties of Bacterial concrete and Normal concrete.
- To study the mechanism of microbial calcite precipitation in concrete through "Scanning Electron Microscopy"

## 3. SCOPE

Concrete during service period cracks, which directly affects to the durability of structure. If crack widths needs to be repaired for better service life of structure, a reliable self healing method for concrete would lead to a new way of designing durable concrete structures. The development of the microbiologically induced calcite as a tool of concrete repair will provide the basis for an alternative and high quality concrete sealant that is cost. effective and environmentally safe. The scope of present work is to check the effect of bacteria in concrete and also the effect of GGBS when replaced with cement on the strength characteristics.

## 4. METHODOLOGY

### 4.1. Culture of Bacteria

**1. Sterilization:** Sterilization is done to achieve sterile environment by killing the microbes. It's done in an autoclave or pressure cooker at a temperature of $121^{0}$C for 25 to 30 minutes.
**2. Inoculation:** Inoculation is the streaking the bacteria on to the media with the help of an open loop.
**3. Incubation:** It is a done to grow and maintain the bacterial cultures.

### 4.2. Procedure for bacterial sub culturing

1. First sterilization of all the glass wares is done in an autoclave.
2. We then prepare the media for sub culturing by dissolving 28gm of nutrient agar in 1000 ml of distilled water. After the preparation the mouth of conical flask are closed with cotton plugs so as it doesn't get contaminated.
3. Contents of nutrient agar Beef extract: 10g, NaCl: 5g, Peptone: 10g, Agar: 20g.
4. Once again we sterilize the prepared media.
5. The sterilized media is allowed to cool down till it is warm. The media is then poured onto the Petri dishes.
6. The Petri dishes are kept under UV light for 30 min to provide further sterilization, i.e. to kill if any bacteria which are present in the Petri dishes.
7. Then we have to do inoculation step.
   a) First we take open loop and heat it near the flame to sterilize it.
   b) It is then dipped in the bacteria sample and streaked onto the agar filled Petri dishes in a zigzag manner.
8. The Petri dishes are kept in an incubator for 24 hours at a temperature of 37ºC.

### 4.3. Maintenance Of Stock Culture

Stock cultures of Bacillus Sphaericus were maintained on nutrient agar slants. The culture was streaked on agar slants with an inoculating loop and the slants were incubated at 37ºC. After growth, slant cultures were preserved under refrigeration (4ºC)until further use. Sub culturing was carried out for every 90 days. Contamination from other bacteria was checked periodically by streaking on nutrient agar plates.

### 4.4. Preparation Of Media To Culture Bacteria

1. First sterilization of all the glass wares is done in an autoclave.
2. We then prepare the media for sub culturing by dissolving 13gm of nutrient broth in 1000ml of distilled water. After the preparation the mouth of conical flask are closed with cotton plugs so as it doesn't get contaminated.
3. Ingredients of Nutrient Broth gms/litre.
   (a) Peptic digest of animal tissue 5.0g
   (b) Sodium chloride 5.0g
   (c) Beef extract 1.5g
   (d) Yeast extracts 1.5g
   (e) Final pH (at 25°C) 7.4±0.2
4. Once again sterilization step is carried out for the prepared media.
5. The sterilized media is allowed to cool.
6. The conical flask are kept under UV light for 30 min to provide further sterilization, to kill if any bacteria which are present in the conical flask.
7. Then we do inoculation step
8. First we take open loop and heat it near the flame to sterilize it.
9. It is then dipped in the bacteria sample and streaked onto the prepared media.
10. The streaked sample is kept in an incubator for 24 hours at a temperature of 37ºC.

### 4.5. Growth Curve of Bacteria

1. We first Set and calibrate the spectrophotometer. To do this we set the wavelength knob to 600 nm for measuring Bacillus Sphaericus. Then, adjust the meter needle to zero by rotating the zero control knobs.
2. Blank the spectrophotometer. To do this we insert a test tube containing the media that we are using i.e. plain nutrient broth sample (called a blank) into the sample holder. Adjust the meter needle to read 100% transmittance by rotatingthe light control knob.
3. Remove the blank from the instrument.
4. Shake the conical flask containing the bacterial sample to ensure uniform distribution of the bacteria in the media. Pour this sample in the tube. Insert the tube into sample holder.
5. Read and record the percentage transmittance and the optical density (OD) of the culture.

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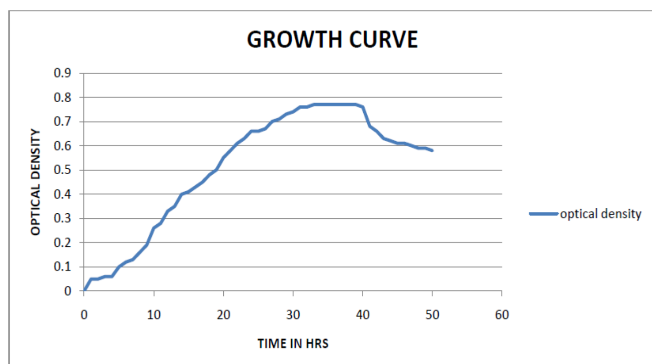
6.Steps 4 to 5 were repeated after every one hour. We have studied the growth curve of Bacillus Sphaericus and found out that growth phase begins at 8 hours and continues up to 24 hours and reaches.




### 4.6. Inference Made By The Growth Curve

Bacterial growth follows three phases. When a population of bacteria first enters high-nutrient environment that allows growth, the cells need to adapt to their new environment. The first phase of growth is the lag phase, a period of slow growth when the cells are adapting to the high-nutrient environment and preparing for fast growth. The lag phase has high biosynthesis rates, as proteins necessary for rapid growth are produced. The second phase of growth is the logarithmic phase (log phase), also known as the exponential growth. The rate at which cells grow during this phase is known as the growth rate, and the time taken for the cells to double is known as the generation time. During log phase, nutrients are metabolized at maximum speed until one of the nutrients is depleted and starts limiting growth. The final phase of growth is the stationary phase and is caused by depleted nutrients. The cells reduce their metabolic activity and consume non-essential cellular proteins.

## 5. EXPERIMENTAL INVESTIGATION

In this experimental work, a total of 60 numbers of concrete specimens were casted. 15 cubes, 15 beams, 15 cylinders and 15 shear specimens.
The mix design of concrete was done according to Indian Standard guidelines M 20 grade. The standard sizes of

- Cube specimens for testing Compressive Strength of dimensions 150mm×150mm×150mm.
- Cylindrical specimens for testing Split Tensile Strength of diameter 150mm and length 300mm.
- Beam specimens for testing flexural strength of dimensions 100mm×100mm×500mm

### 5.1. Tests On Fresh Concrete

Slump test most commonly used method consistency of concrete which can be employed either in laboratory or in site. Apparatus consist of slump cone made up of steel 200mm bottom diameter, 100mm top diameter and 300mm height. The slump obtained from this method was 50mm.

### 5.2. Tests On Hardened Concrete

#### 5.2.1. Compressive Strength of Concrete

For evaluating the compressive, specimens of dimensions 150X150X150mm were prepared .They was tested on compressive testing machine as per IS: 516:1959. The compressive strength is calculated by using the equation,

$$F = P/A$$

#### 5.2.2. Split Tensile Strength of Concrete

For evaluating the tensile strength, cylindrical specimens of diameter 150mm and length 300mm were prepared. Split tensile test was carried out on 2000kN capacity compression testing machine as per IS 5816:1999. The tensile strength of is calculated using the equation,

$$F = 2P/\pi DL$$

#### 5.2.3. Flexural Strength of Concrete

For evaluating the flexural, beam specimens of dimensions 100mmX100mmX500mm were prepared. The two point loading were placed at a distance of 133mm and bottom was placed at a effective span of 400 mm. The load was applied without the shock and the increasing continuously at uniform rate. The flexural strength of the specimen shall be expressed as the modulus of rupture Fb. The modulus of rupture was calculated as follows,

$$F = PL/(bd^2)$$

#### 5.2.4. Casting of Specimens

Cement, fine aggregate and coarse aggregate were weighed and mixed according to mix proportion for M25 grade of concrete i.e. 1: 1.735: 2.85. Cement was replaced by GGBS in different percentages such as 0%, 20% and 30%. For this dry mix required amount of microorganisms with media (water which contains bacterial concentrations as 0, 105,107 cells/ml of mixing water) (w/c=0.48) are added and thoroughly mixed in a machine mixture. This concrete was poured into moulds required for strength assessment. Pouring of the concrete into the moulds was done in three layer sand hand compaction was done. Then the mould was placed on vibrator and sufficient vibrations were given so that the entrapped air was expelled out. After 24 hours the specimens were demoulded and transferred to curing tank wherein they were allowed to cure for 28 days.

| W/C Ratio | Cement | Fine Aggregates | Coarse Aggregate |
|---|---|---|---|
| 192 | 399.16Kg/m$^3$ | 692.91 Kg/m$^3$ | 1137.57Kg/ m$^3$ |
| 0.48 | 1 | 1.735 | 2.85 |

## 6. EXPERIMENTAL RESULTS

The strength results obtained from the experimental investigations are showed in tables. All the values are the average of the three trails in each case in the testing program of this study. The results are discussed as follows.

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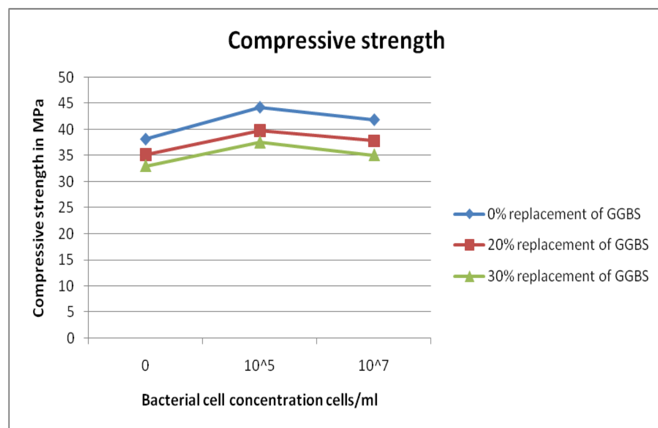
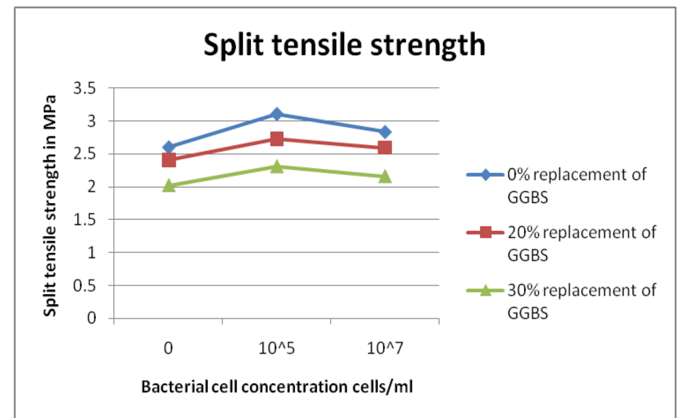
### 6.1. Compressive Strength Results

| Description of concrete | Bacteria concentration (cells/ml) | Average compressive strength (Mpa) | Percentage increase in compressive strength w.r.t. reference mix |
|---|---|---|---|
| 0% replacement of GGBS | 0 | 38.20 | - |
| | $10^5$ | 44.29 | 15.94 |
| | $10^7$ | 41.91 | 9.71 |
| 20% replacement of GGBS | 0 | 35.11 | - |
| | $10^5$ | 39.77 | 13.27 |
| | $10^7$ | 37.77 | 7.57 |
| 30% replacement of GGBS | 0 | 33.03 | - |
| | $10^5$ | 37.55 | 13.68 |
| | $10^7$ | 35.11 | 6.29 |



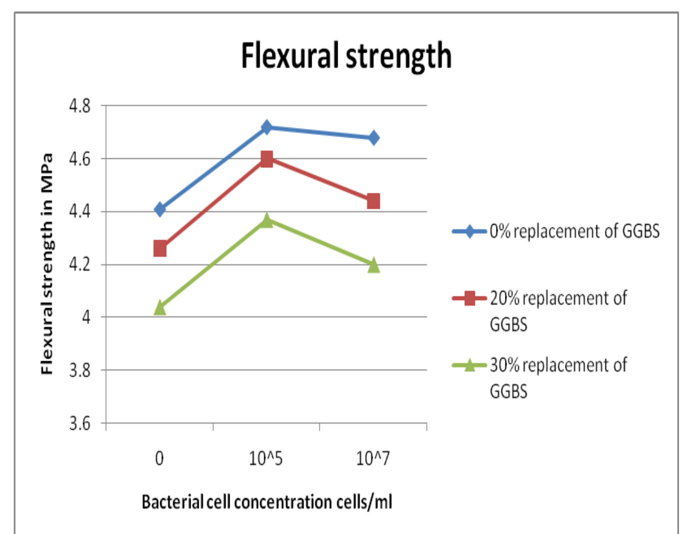

### 6.2. Split Tensile Strength Results

| Description of concrete | Bacteria concentration (cells/ml) | Average Tensile strength (Mpa) | Percentage increase in Tensile strength w.r.t. reference mix |
|---|---|---|---|
| 0% replacement of GGBS | 0 | 2.60 | - |
| | $10^5$ | 3.10 | 19.23 |
| | $10^7$ | 2.83 | 8.84 |
| 20% replacement of GGBS | 0 | 2.40 | - |
| | $10^5$ | 2.73 | 13.75 |
| | $10^7$ | 2.59 | 7.91 |
| 30% replacement of GGBS | 0 | 2.02 | - |
| | $10^5$ | 2.31 | 14.35 |
| | $10^7$ | 2.16 | 6.93 |




### 6.3. Flexural Strength Results

| Description of concrete | Bacteria concentration (cells/ml) | Average Tensile strength (Mpa) | Percentage increase in Tensile strength w.r.t. reference mix |
|---|---|---|---|
| 0% replacement of GGBS | 0 | 2.60 | - |
| | $10^5$ | 3.10 | 19.23 |
| | $10^7$ | 2.83 | 8.84 |
| 20% replacement of GGBS | 0 | 2.40 | - |
| | $10^5$ | 2.73 | 13.75 |
| | $10^7$ | 2.59 | 7.91 |
| 30% replacement of GGBS | 0 | 2.02 | - |
| | $10^5$ | 2.31 | 14.35 |
| | $10^7$ | 2.16 | 6.93 |

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## 7. CONCLUSIONS

The following conclusions are drawn based on the experimental investigation conducted.

- Bacteria Bacillus Sphaericus plays a significant role in increasing the compressive strength of normal concrete by up to 15.94% and GGBS concrete up to 13.27%, for 20% and 13.68% and 30% GGBS as replacement of cement respectively as compared to reference concrete at a particular cell concentration i.e. at 105cells/ml.
- Bacteria Bacillus Sphaericus plays a significant role in increasing the split tensile strength of normal concrete by up to 19.23% and GGBS concrete up to 13.75%, for 20% and 14.35%, for 30% GGBS as replacement of cement respectively as compared to reference concrete at a particular cell concentration i.e. at 105 cells/ml.
- Bacteria Bacillus Sphaericus plays a significant role in increasing the flexural strength of normal concrete by up to 7.03% and GGBS concrete up to 7.98%, for 20% and 8.16%, for 30% GGBS as replacement of cement respectively as compared to reference concrete at a particular cell concentration i.e. at 105 cells/ml.
- The increase in compressive strength, split tensile strength and flexural strength is mainly due to consolidation of the pores inside the normal and GGBS concrete with bacteria induced calcium carbonate precipitation.
- Bacillus Sphaericus can be produced in the laboratory which is proved to be safe and cost effective.

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