ABSTRACT:
When the loads is applied on any structures cracks in concrete are formed which allow water and other chemicals to enter thus making it vulnerable which leads to unwanted corrosion of the steel reinforcement and deterioration of concrete structure. For these project we use M25 grade of concrete because of its superior properties. As mentioned above in cases like this where there is a formation of cracks there is an acute need of Self-healing concrete to achieve this we added BACILLUS SUBTILLUS which is a GRAM POSITIVE bacterium to the mixture. Along with this bacteria concrete mixture we use chemical compound CALCIUM LACTATE (C6H10CaO6) which is used to activate the healing procedure. To test this theory we made 2 concrete blocks of dimension 150x150x150mm.

KEYWORDS—Self healing bacteria, Bacillus Subtilis, Calcium Lactate.

I. INTRODUCTION
Concrete is 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 rebars restrict the cracks width they are mostly not designed to completely prevent cracks formation.

Cracks endanger the durability of concrete structure as aggressive liquid and gases may penetrate in to these cracks and may cause damage. When cracks appear in concrete structure and water start to seep in through, the spores of the bacteria starts microbial activities on contact with water and oxygen. In the process of precipitating calcite crystals through nitrogen cycle the soluble nutrients are converted in to insoluble CaCo3. CaCo3 solidifies on the cracks surface thereby sealing it up.

The consumption of oxygen during metabolic biochemical reaction to form CaCo3 help in arresting corrosion of steel because the oxygen is responsible to initiate the process of corrosion there by increasing the durability of steel reinforced concrete structures.

II. MATERIALS & METHODS
A. Materials
Following materials are used for making Concrete:

a) Cement – Ordinary Portland cement of 53 grade is used. The cement has been tested for various properties has been tested for various properties as per IS 4031-1988

b) Sand – Sand size less than 4.75mm size were used as time aggregate (Sp.gravity 2.69).

c) Coarse Aggregate – Hard granite broken stones of size 20mm were used as coarse agg. (Sp.gravity-2.75).

d) Water – Locally available portable water confirming to IS 456 is used.

e) Micro-organism – Bacillus Subtilis bacteria used.

III. BACTERIAL CONCRETE
A. Classification of bacteria:
Bacteria is generally classification in three category Basis on shape, Basis on gram stain and Basis on oxygen demand which is shown in figure.

Figure 1 Classification of Bacteria

Classification of Bacteria

Shape

Gram Stain

Oxygen Demand

Various types of bacteria used in concrete-
There are various type of bacteria were used in construction area from literature review.

1. Bacillus Pasteurii.
2. Bacillus Sphaericus
3. Escherichia Coli
4. Bacillus Subtilis
5. Bacillus Cohnii
6. Bacillus Pseudofirmus

C. How does concrete works-

Self-healing concrete is a product that will biologically produce lime stone to heal cracks that appear on the surface of concrete structure. Specially selected types of bacteria genus Bacillus, along with calcium based nutrients known as calcium lactate & nitrogen 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 hundred years. However, when a concrete structure is damaged water start 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 feeds oxygen is consumed and the soluble calcium lactate is converted to insoluble limestone. The lime stone solidifies on the crack surface, thereby sealing it up. It mimics the process by which bone fractures in the human body are naturally healed by osteoblast cells that mineralize to reform the bone. The consumption of oxygen during the bacteria conversion of calcium lactate to limestone has an additional advantage. Oxygen is an essential element in the process of corrosion of steel and when bacterial activity has consumed it all and increase the durability of steel reinforced concrete construction.

D. Bacillus Subtilis Description –

Bacillus Subtilis is a Gram – Positive bacterium rod - shaped and catalase positive. It was originally named vibrio subtilis by Christian Gottfried Ehrenberg and renamed bacillus subtilis by Ferdinand Colm in 1872. Bacillus subtilis are typically rod shaped and are about 4 to 10 micrometer long and 0.25 to 1 micrometer in diameter with a cell volume of about 4.6 FL at stationary phase. As with other members of the genus bacillus, it can form an endospore to survive extreme environmental condition of temperature and desiccation.

E. Reproduction –

Bacillus subtilis can divide symmetrically to make two daughter cells (Binary Fission), or symmetrically producing a single endospore that can remain viable for decades and is resistant to unfavorable environmental condition such as drought, salinity, extreme pH, radiation. It is found in soil, water, air decomposing plant matter.

F. Uses –

Bacillus Subtilis is widely used laboratory studies, but more for genetic research as oppose to health research.

1. Bacillus Subtilis is to produce many antibiotics such as Diffcidiin, Oxidydiffcidin, Bacilli and Bacitracin which is helpful in treating bacterial skin infections and preventing infection in minor cuts and burns.
2. As an important source of industrial enzymes and polymers.
3. As a Probiotic.

IV. METHODOLOGY

The methodology for producing a self-healing concrete involves the following steps:
1. Selection and cultivation of bacteria.
2. Preparation of test specimens.
3. Characterization studies
   a) X-ray diffraction
   b) Scanning Electron
   c) Microscopy (SEM)
   d) Thermo-Gravimetric
   e) Analysis (TGA)
   f) Compressive Strength
   g) Tensile Strength Testing
   h) Ultrasonic Pulse Velocity

1. Selection of Bacterial Species–
Spore forming alkali-resistant bacteria can be isolated from its source. Bacterial strains such as Bacillus pasteurii, Escherichia coli, Bacillus sphaericus, Bacillus subtilis, Bacillus cereus etc., are commonly used for research works. Initially these bacteria are obtained from the source and first cultured in a solid media and then transferred to nutrient broth (liquid media) which is sterile and kept shaking in an incubator.

2. Measurement of Bacterial Cells
Concentration of bacterial cells is measured by Haemocytometer and optical density could be found by spectrophotometer analysis before adding bacteria to cement composites. Gram staining method was used to determine the morphology of the bacterial strains and the bacterial cultures were tested for urealytic activity and also calcium carbonate precipitation. Before addition to cement mixture for test specimen preparation, bacteria should be cleaned from culture residues by repeated centrifugation and resuspension of obtained cell pellet in a clean tapwater. Ureolytic bacteria such as B. sphaericus could precipitate CaCO3 by conversion of urea into ammonium and carbonate. Strains of B. Subtilis were used in the formation of calcium silicate gelby means of
adsorbing silicate using chemically modified B. subtilis (CMBS). It is found that thereis 28% improvement in compressive strength of CMBS incorporated concrete compared to control concrete with optimum concentration. The matrix of fresh concrete is highly alkaline particularly due to the formation of portlandite (calcium hydroxide) which is after calcium silicate hydrate quantitatively the most important hydration product of ordinary Portland cement (OPC). Matrix capillary water of young concrete is typically characterized by pH values between 11 and 13. Bacteria added to the concrete mixture thusdo not only have to resist mechanical stresses due to mixing but should also be able to withstand a high alkalinity for prolonged periods. Most promising bacterial agents for incorporation in the concrete matrix therefore appear to be alkaliphilic (alkaliresistant) spore-forming bacteria. As the concrete matrix is toxic due to ingress oxygen (diffusion through matrix capillaries) incorporated bacteria also need to be oxygen tolerant. Such aerobic alkaliphilic spore-forming bacteria occur within the genus Bacillus, and several representatives of these were therefore selected to test their applicability as healing agent in concrete. 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 most organisms die in an environment with a pH value of 10 or above. These bacteria concentrated on microbes that thrive in alkaline environments which can be found in natural environments. Samples of endolithic bacteria (bacteria that 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. Different types of bacteria which can survive in such a high pH environment is mentioned. It is found that the only group of bacteria that will be able to survive is the ones that producespores 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.

3. Preparation of Test Specimens

Bacterial concrete casted by using ordinary Portland cement mixed with bacterial concentration 106 cells/ml of water. Conventional concretesamples are also casted in parallel. The specimens are cured under tap water at room temperature and tested at 7, and 28 days.

4. Characterization Studies

The formation of calcite by means of biomineralization can be analyzed by using various characterization techniques or methods. These techniques are specialized or involve all modes of microbial analysis like imaging, diffraction and spectroscopy, including light, X-rays, neutron orelectron as primary radiation. To conduct the above studies, samples should be collected from the tested mortar or concrete specimens in the form of powders or broken pieces.

a) X-ray diffraction - This test is performed to indicate the presence of calcite. Higher the peak values obtained, higher is the presence of calcite. Hence it can be said that microbial precipitated calcite improves the performance of cement composites.

b) Scanning Electron Microscopy (SEM): The deposition of calcite inside the micro cracks of concrete by bacteria is analyzed under SEM. The increase in compressive strength of concrete can be examined by doing SEM analysis. To determinewhether the increase in compressive strength of these specimens with bacteria and sand in their cracks could be attributed to the microbial calcite precipitation, the crack samples with the highest strength values are examined.

c) Compressive Strength and Tensile Strength: Compressive strength of cement paste, mortar and concrete with bacteria is performed using automatic compression testing machine. Split tensile strength concrete with bacteria is performed.

d) Ultrasonic Pulse Velocity: The time taken for the pulse to pass through the concrete is measured by electronic measuring circuits.

V. EXPERIMENTAL WORK

A. Mix design for concrete blocks

a) Concrete mix Design - The process of selecting suitable ingredients of concrete and determining their relative amounts with the objective of producing a concrete of required strength, durability and workability as economically as possible is termed the concrete mix design.

b) Requirements of concrete mix design - The requirements which forms the basis of selection and proportioning of mix ingredients are:

- The minimum compressive strength required from structural consideration.
- The adequate workability necessary for full compaction with the compacting equipment available.
- Maximum water-cement ratio and/or maximum cement content to give adequate durability for the particular site conditions.
- Maximum cement content to avoid shrinkage cracking due to temperature cycle in mass concrete.

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c) **Mix Proportion designations**- The common method of expressing the proportions of ingredients of a concrete mix is in the terms of parts or ratios of cement, fine and coarse aggregates. For e.g., a concrete mix of proportions 1:2:4 means that cement, fine and coarse aggregate are in the ratio 1:2:4 or the mix contains one part of cement, two parts of fine aggregate and four parts of coarse aggregate. The proportions are either by volume or by mass. The water-cement ratio is usually expressed in mass.

d) **Factors to be considered for mix design**- The grade designation giving the characteristic strength requirement of concrete. The type of cement influences the rate of development of compressive strength of concrete. Maximum nominal size of aggregates to be used in concrete may be as large as possible within the limits prescribed by IS 456:2000.

e) **Procedure**-
- Determine the mean target strength $f_t$ from the specified characteristic compressive strength at 28-day $f_{ck}$ and the level of quality control.

$$f_t = f_{ck} + 1.65 S$$ (1)

where $S$ is the standard deviation obtained from the Table of approximate contents given after the design mix.
- Obtain the water cement ratio for the desired mean target using the empirical relationship between compressive strength and water cement ratio so chosen is checked against the limiting water cement ratio.
- The water cement ratio so chosen is checked against the limiting water cement ratio for the requirements of durability given in table and adopts the lower of the two values. Estimate the amount of entrapped air for maximum nominal size of the aggregate from the table.
- Select the water content, for the required workability and maximum size of aggregates (for aggregates in saturated surface dry condition) from table.
- Determine the percentage of fine aggregate in total aggregate by absolute volume from table for the concrete using crushed coarse aggregate.
- Adjust the values of water content and percentage of sand as provided in the table for any difference in workability, water cement ratio, grading of fine aggregate and for rounded aggregate the values are given in table.
- Calculate the cement content from the water-cement ratio and the final water content as arrived after adjustment. Check the cement against the minimum cement content from the requirements of the durability, and greater of the two values is adopted.
- From the quantities of water and cement per unit volume of concrete and the percentage of sand already determined in steps 6 and 7 above, calculate the content of coarse and fine aggregates per unit volume of concrete from the following relations:

$$V = \text{absolute volume of concrete} = \text{gross volume (1m}^3) \text{minus the volume of entrapped air}$$

$$S_c = \text{specific gravity of cement}$$

$$W = \text{Mass of water per cubic meter of concrete, kg}$$

$$C = \text{mass of cement per cubic meter of concrete, kg}$$

$$p = \text{ratio of fine aggregate to total aggregate by absolute volume}$$

$$f_a, C_a = \text{total masses of fine and coarse aggregates, per cubic meter of concrete, respectively, kg, and}$$

$$S_{fa}, S_{ca} = \text{specific gravities of saturated surface dry fine and coarse aggregates, respectively}$$

- Determine the concrete mix proportions for the first trial mix.
- Prepare the concrete using the calculated proportions and cast three cubes of 150 mm size and test them wet after 28-days moist curing and check for the strength.
- Prepare trial mixes with suitable adjustments till the final mix proportions are arrived at.

By considering above mentioned parameters we did mix design for concrete blocks and the details are as follows:

<table>
<thead>
<tr>
<th>WATER</th>
<th>CEMENT</th>
<th>SAND</th>
<th>AGGREGATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.42</td>
<td>1</td>
<td>1.13</td>
<td>2.72</td>
</tr>
</tbody>
</table>

- The grade of concrete used in our project work is M25 with the mix ratio 1:1.13:2.72.
- Water Cement ratio used is 0.42.
- 2 blocks of grade 25 specimen of size 150x150x150mm were casted.

Figure 2 Curing of concrete blocks for 14 days
After curing the concrete blocks for 14 days, Compression test was performed. A load of 580 KN was applied on the concrete block to create cracks.

VI. CONCLUSION

A. Enhancement of compression strength, reduction in permeability, water absorption reinforced corrosion have been seen in various cementitious and stone materials.

B. Cementation by this method is very easy and convenient for usage. This will soon provide the basis for high quality structures that will be cost effective and environmentally safe but, more work is required to improve the feasibility of this technology from both an economical and practical viewpoints.

C. The greatest improvement in compressive strength occurs at cell concentrations of $10^5$ cells/ml for all ages. The study showed that a 25% increase in 28 day compressive strength of cement mortar was achieved.

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