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Bio Concrete Innovation: Review on Confirmatory Testing for Precipitation Formed by Bacillus Bacteria.

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Abstract: Bacillus Subtilis microorganism, which is incorporated in concrete, impacts the eco-friendly self-healing process in the current project. In this technique, bacteria Bacillus Subtilis are mixed in concrete which are having calcium lactate as their food from concrete and when these bacteria get in contact with the atmosphere, they use water, oxygen, and carbon dioxide from the surrounding environment and produce a precipitate of calcium carbonate (CaCO3) (limestone) which seals the cracks and enhances compressive strength of concrete. Here in this paper, we will discuss whether the precipitation is due to bacillus bacteria or other means. There are several types of tests by which we can confirm the origin of precipitation. Here we will discuss the calcium carbonate test, gram staining test, and confirmatory test for CaCO3.

Index Terms – precipitation, calcium carbonate, gram staining, etc.

I. Introduction

A proper solution for preventing crack growth is Bio concrete. Since these bacteria normally create spores with specialized cells capable of resisting strong mechanical forces and hostile environments. Spore-forming bacteria allied with the genus Bacillus Subtilis are often used as healing agents for crack repairs. The formation of calcium carbonate is affected by several variables such as pH, and inorganic compounds dissolved in it; the presence of calcium ions throughout is directly meant for the self-healing process. Instead of external application, these bacteria and organic materials are mixed into concrete during the mixing process for improved action at depth. These bacteria can survive in concrete for over 200 years. If concrete gets damaged, bacteria will rise and multiply when water will come in contact. Hence, bio-concrete has the capability to save annual maintenance when utilized in different construction projects.

1.1. DEFINITION

"Bio concrete is a self-healing path using bacteria that can fill the cracks in concrete by forming calcium carbonate precipitation through biomineralization." The mechanism of self-crack healing starts when the concrete crack occurs. The water will then leak through the formed cracks and capillary pores of the concrete. by consuming water and nutrients inside the concrete matrix, the growth of bacteria will take place and the limestone (CaCO3) produced can fill the cracks. The bacteria can be added to the concrete mix to restrain the growth of cracks by calcium carbonate precipitation. In comparison with other conventional healing materials, such as silicon-based polymers and acrylic resin, bacteria are preferable. Calcium carbonate has good thermal compatibility and mechanical property with cementitious composites.

1.2. PURPOSE

In the field of bio concrete, many researchers have done a lot of work to increase the strength and durability of concrete. They have briefly investigated the increase in compressive strength, tensile strength, etc. They worked with different proportions of bacterial strains since there is no standard code provision yet done for it. So, the purpose of the work is to find out a standard proportion of bacterial strain which will be sufficient for the specific grade of concrete. Hence there will be no need for repetitive work along different quantities of bacteria.

1.3. NEED OF STUDY

The current situation about the degradation of concrete and the economic impact of the maintenance and repair of concrete structures have drawn attention to processes of concrete deterioration, and to the methods to delay or even to get rid of concrete degradation. The service environment and properties of concrete determine the risk of damage and the speed at which it can develop. Most of the physical and chemical disintegration mechanisms of concrete are related to aggressive substances present in an aqueous solution. An important measure to protect concrete against damage is then declining the uptake of water. Surface treatment is an important consideration in limiting the infiltration of water and harmful components into concrete. Nowadays lots of organic and inorganic products are available in the market for the protection of concrete surfaces, such as a variety of coatings, water repellents, and pore blockers. These conventional means of protection, however, show besides their favorable influences also several disadvantageous aspects such as:

1. Different thermal expansion coefficients of the treated layers.
2. Degradation over time.
3. Need for constant maintenance.

Additionally, the use of some solvents contributes to environmental pollution. To partially offset these disadvantages, more ecologically, friendly methods have been suggested. Within this framework, using bacteria for carbonate mineralization has been proposed as a novel and environmentally friendly strategy for the protection and remediation of concrete.

1.4. OBJECTIVES

1. To examine the use of bio concrete for building construction.
2. To investigate the physical and mechanical properties of bio-concrete blocks.
3. To study the efficiency of Bacillus Subtilis bacteria to heal concrete blocks.
4. To study cost comparison in bacterial concrete blocks and conventional concrete blocks.
5. To study the proportionality of bacteria mixed with concrete to achieve the standard mix.

1.5. SCOPE OF STUDY

1. By adopting this method, the durability of concrete can be increased by self-healing the cracks.
2. As we are using organic materials as healing agents, they do not have any adverse effects on the environment.
3. Adopting a new idea of bacterial healing approach for healing purposes can reduce the cost of repairing as a conventional method is costlier.
4. The time required for regular maintenance of the structure is more as compared to bio concrete. Also, the efforts of laborers are reduced by these techniques.

II. LITERATURE REVIEW

Salifu E., Gutteridge F., and Wittie K. in the year, 2021 revealed attention to microbial biotechnology transitioning towards eco-friendly cementitious technology. It approaches to create low-cost, low-carbon microbial-based products like bio-bricks, bio-concrete, etc.

Yang Wang, Jun Feng, and Bingcheng Chen in 2021, aimed at the formation of novel self-healing concrete consisting of fly ash, quartz sand, Superplasticizers, and Polyvinyl Alcohol (PVA) fiber. They found a strength recovery of 14% for repeated bending for a crack range of 0.3mm.

Gupta and Naval, 2020, noted that in humid environments, strength decreases due to the penetration of chemicals through microcracks in the structure. It is better to repair these cracks at early age stages and for that purpose, bio concrete is the best alternative. This is because every micro crack in the structure cannot be detected by any other means.

Adam Souid, and David Elliott, 2019, studied how to encapsulate the bacteria. It compares the incubation methods for prepared specimens, I. Soil incubation. II. Water incubation. The results showed that soil incubation is better than water incubation for healing purposes.

Tripathi, Arora, and Shrivastava 2017, used the bacteria Bacillus cereus. They tested 3 different bacterial concentrations in Portland cement. It was discovered that the addition of bacteria significantly increases the strength of concrete.

Khaliq and Ehsan 2015, worked on the process of incorporation of bacteria. The incorporation is done with the carrier compound for bacteria such as Lightweight aggregates (LWA) and Graphite Nanoplatelets (GNP). This incorporation made the inner core bulkier and hence compressive strength increases.

Mion Luo, Qian, and Li 2015 studied the factors affecting the crack-repairing capacity of bacteria. The Bacteria used was Bacillus sphaericus. Effects due to width, availability of microbes, age of the crack, etc. were checked in this study. They found that there is a delay in crack healing if the width of cracks increases and the same for the age of the crack. The availability of microbes at the place of damage influences the efficiency of healing.

III. METHODOLOGY

1. Direct method

- Direct Mixing
- Mixing by encapsulation of bacteria with a protective membrane

DIRECT METHOD: -

In the direct method, while mixing concrete, the bacteria and calcium lactate are poured. The bacteria and calcium lactate do not change the normal properties of concrete when mixed. When the formation of cracks occurred in the structure, the bacteria are exposed to external climatic conditions. When water encounters this bacterium, they germinate and feed on calcium lactate and produce limestone and seal the cracks. In the encapsulation method, the bacteria, and calcium lactate as food for bacteria, are closed inside treated clay pellets. These clay pellets are then mixed with concrete. The amount of clay pellets added to concrete is about 6%. For concrete structures made with bacterial concrete, when a crack occurs in the structure and clay pellets are broken, the bacteria germinate and consume the calcium lactate in the presence of water, oxygen, and carbon dioxide, and form crystalline limestone, which seals the cracks. Minor cracks about 0.8mm in width can be treated by using bacterial concrete. Among these two methods encapsulation method is commonly used, even if it is costlier than the direct method.

PROCEDURE: -

1. This study utilizes a systematic literature review and analyzes how the use of Bio concrete can be adopted for the construction of buildings in the construction industry.
2. First of all, the concrete blocks will be cast with different proportions of Bacillus bacteria incorporated within them and without them.
3. Then artificial cracks will be formed on the concrete blocks.
4. Along with curing the observations will be taken for 7, 14 & 28 days to examine the healing of cracks.
5. The bacteria will use calcium lactate to feed on and produce a precipitate of calcium carbonate ($CaCO_3$) in presence of atmospheric water and carbon dioxide.
6. The comparison will be done based on the percentage healing of cracks to determine the most suitable mix proportion which will serve as the standard proportion for any work.

EXPERIMENTAL WORK: -

A) MATERIAL REQUIRED FOR THE PROJECT

(I) Cement.
(II) Graded Fine Aggregate.
(III) Graded Coarse Aggregate.
(IV) Water as per design Ratio.
(V) Bacteria culture.

B) SELECTED BACTERIA FOR THE PROJECT – Bacillus Subtilis

C) PREPARATION OF BACTERIA CULTURE –

1. Culture of bacteria to be prepared in the Biotechnology laboratory. (3 Days)
2. Preparation of bacteria using culture and nutrient broth by placing in a shaker with incubator at 37oC.

D) PREPARATION OF BACTERIAL CONCRETE –

❖ BY DIRECT APPLICATION –

In the direct application method, the bacterial solution is poured into concrete directly while the mixing of concrete is done.

E) CASTING OF CUBES. Size of cube: 150mm x 150mm x150mm

F) CURING OF CUBES.

G) CUBES TEST (AFTER 7 / 14 / 28 DAYS).

H) OBSERVATIONS FOR PRECIPITATION.

I) CHECK FOR PRECIPITATION

J) INTERPRETATION OF RESULT.

- **EXPERIMENTAL WORK -**

CHECK FOR PRECIPITATION:

- TEST FOR PRECIPITATE RELEVANCE
  1. Calcium carbonate titration with EDTA solution
  2. Gram Staining Test
  3. Confirmatory test for $CaCO_3$

After the formation of the precipitate, it is collected from the crack surface. Then it is crushed to powder form and added to water to form a smear for the testing process.

1. **Calcium carbonate titration with EDTA solution.**

- Test procedure:
  1. Prepare a sample of precipitate with 50 ml of water.
  2. Add 2ml of water NaOH (1.0 normal)
  3. Now add 2-3 drops of Urochrome black T which turns the water sample color changes to purple.
  4. Begin the titration of the prepared solution with 0.01M EDTA solution.
  5. The color changes will appear after a specific volume of EDTA titration.
  6. The color changes from Violet to Sky Blue.
  7. The formula for calculating calcium content

Amount of $CaCO_3$ = (Burette reading x 1000 x 0.4) / (Sample taken)

- Results –

Data for calculating $CaCO_3$: Burette reading – 10ml

The sample was taken – 50ml

Therefore;

Amount of $CaCO_3$ = (10 x 1000 x 0.4) / (50)

= 80mg/l

• Conclusion – From the above experiment we can conclude that the sample prepared from precipitate on the crack surface of concrete block is calcium carbonate.

2. **Gram Staining Test.**

- Test procedure:
  1. Take a clean, grease-free slide.
  2. The smear of suspension is prepared on the clean slide with a loopful of samples.
  3. Air dry and heat fix.
  4. Now pore Crystal Violet and keep it for about 30 seconds to 1 minute and rinse it with water.
  5. Gram's iodine is suspended on a slide for 1 minute and washed with water.
  6. Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
  7. Safranin is added for about 1 minute and washed with water.
  8. Air dry it and Observe under the Microscope.

- Result and Conclusion – Violet-colored spots were observed under a microscope which indicates that the stain present is of gram-positive bacteria.

Hence, as the Bacillus Subtilis bacteria are Gram-positive we can conclude that the smear prepared from precipitate contains Bacillus Subtilis bacteria.

3. Confirmatory test for CaCO3

- Test for Calcium:
  1. A sample of precipitate is mixed with water.
  2. Added some amount of HCL acid.
  3. Took some amount of this sample in a test tube.
  4. Now added Ammonium Oxalate to the test tube sample.
  5. After the addition of Ammonium oxalate "White Precipitate" is formed at the bottom.

- Test for Carbonate:
  1. Take a sample of precipitate with water and added Dilute HCL to it.
  2. In addition, a Bubble of CO2 is released.

- Result and Conclusion – From the above two tests we can conclude that,
  1. The white precipitate formed at the bottom of the test is an indication of Ca2+ ions concentration.
  2. The bubble formation indicates the presence of carbonate ions in the sample.

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