BACTERIAL SELF-HEALING FOR SUSTAINABLE CONCRETE: A COMPARATIVE STUDY OF VEGETATIVE AND SPORE-FORMING BACTERIA

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Abstract- Affordability and availability of concrete is driving its use in building. Hence, sustainable, and durable concrete is needed. Cracks from excessive water, creep, or shrinkage cause concrete to fail. Crack creation reduces strength, and moisture alone or with a toxic chemical like sulfur can induce steel corrosion and concrete degradation, reducing the longevity of concrete. Hence, cracks must be filled, but mechanically repairing cracks, especially micro and deep cracks, is laborious and expensive and cannot be done in structural members that are not apparent. MICCP has been studied for concrete durability. This research covers self-healing characteristics using bacterial species; the effect of bio-concrete in prisms; and a comparative study using alkali-resistant spore-forming bacteria and vegetative bacteria. For concrete to self-heal, Bacillus subtilis and other alkali-resistant bacteria are added during mixing. This research indicates that Spore-forming bacteria performed better than vegetative bacteria. The crack remediation due to bacterial action was confirmed by performing SEM analysis.

Keywords- Self-Healing Ability of Concrete, Vegetative Bacteria, Spore Forming Bacteria, MICCP

1 Introduction

Concrete is inexpensive and commonly used in developing countries. As cracks form in concrete over time due to shrinkage, temperature, or other factors, they reduce the material's strength and weaken it, resulting in corrosion in steel-reinforced structures. When cracks exceed the allowed limit, they have a detrimental impact on the concrete. Yet, concrete and steel bars begin to deteriorate when cracks surpass the 0.4-mm limit [1].

Various mechanical methods can be employed to fill cracks in concrete, such as manually filling the crack with epoxy resin, which is expensive and cannot be used for small or deep cracks. In addition, regular oversight is required to monitor the formation of cracks and fill them mechanically. Therefore, mechanical crack filling cannot be performed on non-visible cracks developing in structural members [2]. An essential aspect of research is the improvement of the concrete industry in terms of its durability and self-healing capabilities utilizing natural or artificial means.

The self-healing action of concrete can be achieved through two distinct methods: intrinsic and bacterial self-healing [3]. Intrinsic self-healing of concrete refers to the concrete's inherent self-healing properties that can occur in the presence of water. Calcium carbonate is formed due to the hydration of un-hydrated cement that is present in the concrete. Cement hydration produces Ca (OH)2, which reacts with carbon dioxide in water to form CaCO3 crystals, allowing the concrete to close itself. In general, we can say that there is no added substance or material for self-healing properties, and the entire phenomenon is natural [4]. Several scholars have reported the development of autonomous self-healing. Wang Guiming et al. [5] revealed that SEM can be used to investigate the crystalline coating's self-healing mechanism in cementitious materials., which reveals that it not only fills mortar fractures and seals pores but also repairs cracks. E. Schlangen [6] explained in his investigations that experimental and numerical studies can explain how cracks that developed in early ages can be filled or repaired by future hydration processes. It has been found that even after one day of hydration, the sample began to regain its strength.

However, autonomous healing refers to the healing properties gained by introducing a healing substance into the cracks [7]. It has been observed that bacterial self -healing is restricted to average crack width of 0.8 mm. At this point, a crack self-heals, restoring its full strength and durability. Considering that intrinsic self-healing is limited to 0.4 mm, bacterial self-healing can provide complete results with high efficiency [8]. However, Nguyen Ngoc Tri Huynh et al. created bio-concrete to evaluate the compressive and flexural strength over largely induced artificial cracks. The cracks limit was 1-18mm and was evaluate over period of 24 hours. Their research indicated that compressive and flexural strength increased over small scale [9]. This research discusses the two most powerful healing phenomena: self-healing characteristics of alkali-resistant spore-forming bacteria and alkali-resistant vegetative bacteria. The Bacterial bio-concrete refers to the healing properties of concrete that are accomplished by employing a straightforward procedure to fill the cracks. The fundamental mechanism is accomplished by creating a concrete mixture containing a precursor such as calcium lactate (Ca(C3H5O2)2). When water enters the crack, bacteria start eating the Calcium lactate as food and start making calcium carbonate as a by-product, which accumulates in the crack to fill it. The microorganisms utilized in this type of concrete are alkali-resistant bacteria from the genus Bacillus. Bacteria from this category are the most ideal since they produce spores and can survive in harsh environments for more than 200 years. Because of its sustainable organic features, using bacteria as a healing mechanism is one of the best techniques for producing this type of concrete. Also, some precursor compounds result in an increase in concrete strength rather than a decrease in concrete strength. Microbially Induced Calcium Carbonate Precipitation (MICCP) has sparked a considerable interest as a viable, natural, and environmentally friendly technology for autonomous concrete repair, resulting in greater concrete durability. A substantial study has been undertaken on the use of MICCP to mend cracks in concrete [10].

2 Experimental Procedures

2.1 Materials

To test the self-healing capabilities, concrete specimens with dimensions of 500x100x100 mm were prepared. Specimens were cured for 24 hours at room temperature after molding. To evaluate the mechanical qualities, 150x300 mm cylinders were also prepared. All specimens were prepared in accordance with ASTM C31/C31M standards. Tables 1-3 provide details of the mix designs used in this research. The compositions of the concrete trails had a set parameter. The total time for mixing the concrete was set at 10 minutes.

Table 1 Types of Materials

Type	**Materials**
Cement	P.O 42.5 Ordinary Portland cement named "Paidar Cement" (ASTM C-150 TYPE 1)
Water	Domestic Water
Fine Aggregates	River sand, Absorption Rate; 2.5060
Coarse Aggregates	Crushed stone Aggregates, Absorption Rate; 2.29
Precursor Compound	Calcium Lactate with the specified percentage of 1% of dry weight of cement
Autogenous Compound	Bacillus Subtilus; alkali resistant bacteria of genus Bacillus.

Table 2 Standard Consistency Value of Cement

Sr. #	Wt. of water (W_2) gm.	%Age Wt. of water	Penetration mm
1	140	28	10

Table 2.1 Settling times of Cement.

Initial setting time	Final setting time
218 mins (3.64 hours)	514 mins (8.55 hours)

Table 2.2 Composition Values of Cement

Cao	SiO2	Al203	Fe2O3	MgO	SO3	Na20	P2O5	MnO
63.5%	19%	5%	3%	2.25%	2.5%	0.4%	0.25%	0.4%

Table 3 Properties of Fine and Coarse Aggregates

Fine Aggregates	Fineness Modulus	Specific gravity (Apparent)	Water Absorption %	Apparent Density (kg/m3)	Bulk Density (kg/m3)	Porosity %
	2.5877	2.4903	2.5060	1345.01	1346.15	0.0651 %
Coarse Aggregates	**Fineness Modulus**	**Specific gravity (Apparent)**	**Water Absorption %**	**Apparent Density (kg/m3)**	**Bulk Density(kg/m3)**	**Porosity %**
	6.5588	2.81	2.29	708.56	1349.32	0.17 %

Moreover, Figure 1 and 2 shows each size level of fine and coarse aggregates.

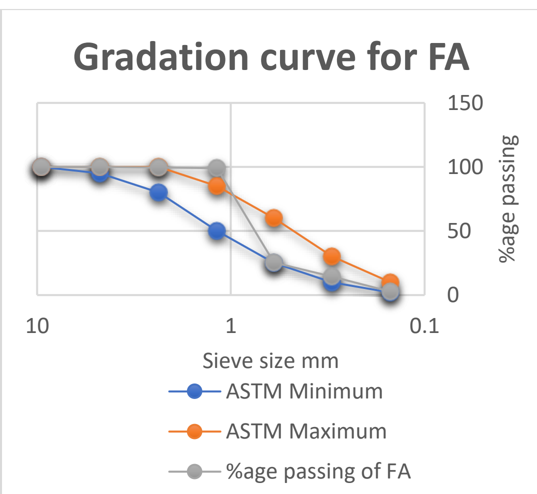

Figure 1 Gradation Curve for FA

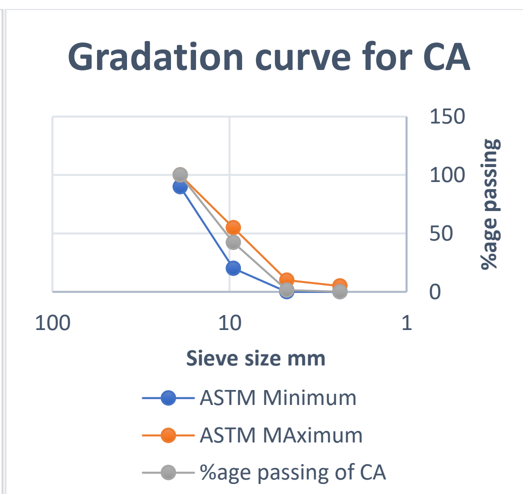

Figure 2 Gradation Curve for CA

2.2 Bacterial Cultures Protocol

An Alkali Resistant Bacteria was selected considering the pH of concrete. As normal pH of concrete ranges from 11 to 13 which is quite high for bacteria to survive, an alkali bacterium was needed for self-healing of concrete. This research uses Bacillus Subtilus in both vegetative and spore forming culture for their comparative study and to evaluate healing ability of concrete as shown in figure 3. The strains of Bacillus Subtilus were isolated and the medium for their culture is generated. Then the culture was inoculated and incubated. After the incubation for the required amount of time, culture was diluted with N-broth medium and then it was centrifuged in falcon tubes. The OD was determined at 600nm by using spectrophotometer and pellets were dissolved in required amount of controlled distilled water to obtain the bacterial water to be used in concrete. The strains of bacteria were cultured as per specification with the concentration to be achieved as 10^6 cells/ml. The concentration at the time of synthesis was enhanced so that it can be diluted at the time of use age.

Figure 3 Bacterial Solution

2.3 Preparation Of Micro-cracks

After 24 hours of curing, three-point bending method was used to induced cracks and a smart crack gauge of 4-mm range was used to measure the width of cracks. SEM was used to bring in the images. A sample binarized image for measuring the self-healing progress is shown in Fig. 4. The bacteria self-healing of the concrete samples was ultimately characterized by measuring the overall healing area of the crack.

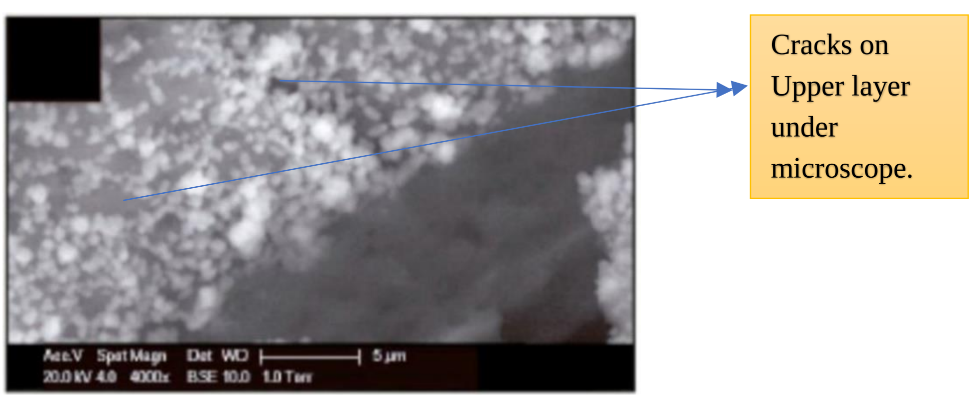

Figure 4 SEM

3 Research Methodology

The methodology of this research involved the use of cement, sand, aggregates, precursor compound, and bacterial water in a non-encapsulated way to produce bio-concrete. The selection of cement, sand and aggregates was made through ASTM Codes involving ASTM C-150 TYPE 1, ASTM C144 and ASTM C33/C33M. Fixed mix design ratio of 1:1.5:3 and water-cement ratio of 0.49 was used to produce Samples. The precursor compound, calcium lactate was added at a specific percentage of 1% of Cement Weight to the dry mixture. The selected bacterial strain was cultured in a nutrient-rich medium, and the bacterial water was then harvested and added to the concrete mixture during the batching process. The precursor compound, typically calcium lactate or calcium acetate, was also added to the mixture to provide a source of calcium ions for the bacteria to convert into calcium carbonate. The mixture was then placed into molds and cured under optimal conditions to allow the bacteria to consume the nutrients and produce calcium carbonate, which acted as a binding agent. The bio-concrete was then tested for strength and durability using standard methods such as compressive strength, water absorption, Modulus of Rupture and Self-healing Progress. Overall, this research aimed to investigate use non-encapsulated bacterial water and precursor compounds in the production of bio-concrete, with the potential to develop sustainable and durable construction materials.

To trace the progress of bio-concrete with respect to controlling specimens, slump cone tests, compression tests, modulus of rupture tests, and scanning electronic microscopy tests were conducted. A slump cone test was conducted to determine the workability of concrete. However, results indicated that concrete had true slump, as the slump of the conventional concrete was recorded at 76 mm, while the slump for bio-concrete was 50mm, as shown in Figure 5.

Figure 5 Slump Cone Test

4 Results

4.1 Effect of Bacterial Cultures on Compressive Strength of Concrete

The compressive strength of control specimens and bio-concrete specimens was measured after 7- and 28-days curing. The size of the tested concrete cylinders was 150 x 300 mm, and an unconfined compression test was conducted.

Figure 6 Compressive Strength Setup

Figure 7 Cylindrical Specimen during Testing

The 7-day testing and 28-day testing shows a increase in compressive strength of specimens.

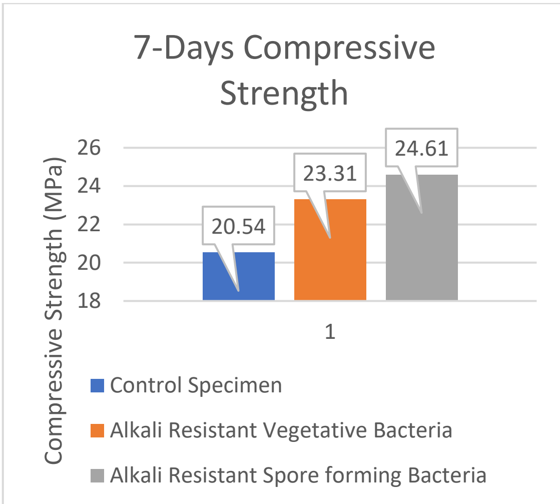

Figure 9 7-Days Compressive Strength Test

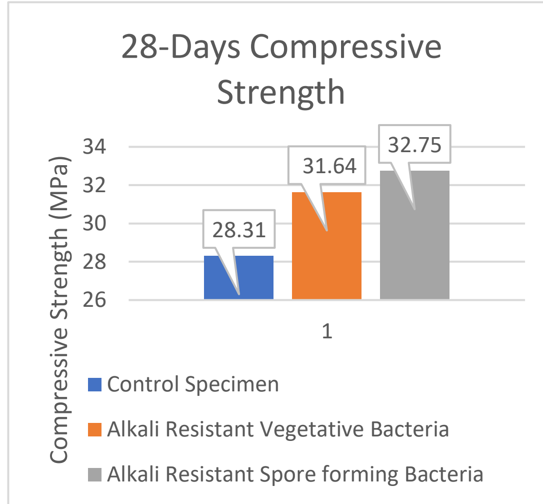

Figure 10 28-Days Compressive Strength Test

It is evident from the graphical representation of compressive strength that the Alkali Resistant Vegetative specie of Bacillus Subtilus increases compressive strength by nearly 14% after 7 days of curing, while the Alkali Resistant Spore forming specie of Bacillus Subtilus increases compressive strength by nearly 20%. Similarly, the compressive strength test after 28 days revealed an increase of nearly 13% for the vegetative species of Bacillus Subtilus and nearly 16% for the spore-forming species. This demonstrates conclusively that the use of microbes in concrete increased its compressive strength through the precipitation of calcium carbonate, as supported by a second analysis using scanning electron microscopy.

4.2 Effect of Bacterial Culture on Tensile Capacity of Concrete

The modulus of rupture test was conducted on 100 mm x 100 mm x 510 mm prisms. 28 days was the curing period for both the control and bio-concrete samples. Using the following relationship, the Modulus of Rupture was computed.

$$\boldsymbol{Stress} = \frac{\boldsymbol{Pl}}{\boldsymbol{bd^2}}$$

The following are the results of the Modulus of Rupture test performed on prismatic specimens.

Figure 11 Prismatic Specimen after Testing

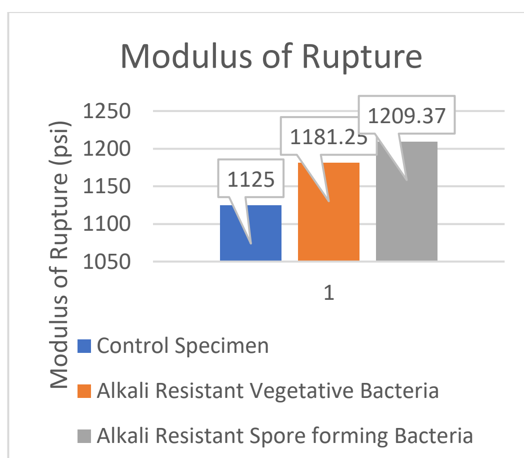

Figure 12 Test Results

The Modulus of Rupture test findings demonstrate that the use of self-healing concrete did not significantly boost the tensile capacity of the concrete. This is because, in comparison to its compressive strength, concrete's tensile strength is significantly lower, ranging from 8 to 14 percent. Since the tested prisms broke suddenly (as depicted in the image above), this explained why the concrete's tensile strength didn't improve much.

4.3 Scanning Electron Microscopy (SEM).

Scanning Electron Microscopy (SEM) was used to analyze the minerology of the deposited calcium carbonate caused by the induction of bacteria.

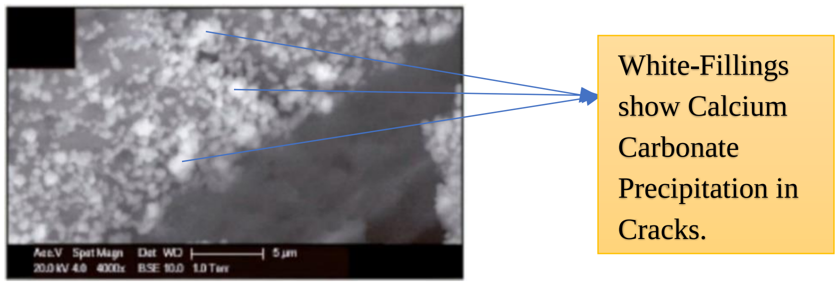

Figure 13 SEM Results (Healed Microcracks)

White Crystals, showing in Figure 13, demonstrated the concrete self-healing ability. The specimens tested for compressive strength analysis were cut into little 37 × 37 mm cubes and then SEM examination was done on them. The Figure 12 depicts the outcome of the SEM study. This result from the SEM confirms the filling of a microcrack and justifies the precipitation of calcium carbonate precipitation.

5 Conclusions

Following conclusions are drawn after deeply analyzing and studying the experimental results obtained by different test:

1. Locally developed Concrete incorporated bacteria can produce copious amount of minerals which can help in filling of cracks.
2. The Bio-concrete or Self-Healing concrete resulting from the application of Vegetative specie of Bacillus Subtilus increased the 7-days compressive strength by almost 14% while the 28-days compressive strength was increased by the percentage of almost 12%.
3. Similarly, the Bio-concrete or Self-Healing concrete by using Spore-forming specie of Bacillus Subtilus increased 7-days and 28-days compressive strength by 20% & 16% respectively.
4. The increase in the compressive strength is due to the accumulation of Microbiologically Induced Calcium Carbonate precipitation.
5. The increase is tensile strength found by Modulus of Rupture test was not much. This was because of low tensile strength of concrete and due to the abrupt failure of the specimens tested for tensile strength.
6. Microbiological crack remediation is more efficient in shallow cracks than in deeper cracks, primarily because the microorganisms grow more actively in the presence of oxygen.
7. The results from the SEM Analysis further confirms that the increase in compressive strength is due to precipitation of Calcium Carbonate and confirmed crack filling was observed.

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