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An Exploratory Investigation Using Bacterial as A Self-Healing Concrete

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Abstract. The results of this study show that using Subtilis microscopic organisms and applying concentrated effort to mending concrete as a way of break management to extend the operational life of a structural framework are both beneficial for the development of a robust framework. This article investigates a novel mechanism known as Microbiologically Induced Calcite Precipitation (MICP). Bacillus Subtilis is used in this situation together with its nutrients, which include nutritious fluids, calcium chloride dehydrates (CaCl2), sodium bicarbonate (NaHCO3), and ammonium carbonate (NH4Cl). The bacteria used was Bacillus Subtilis liquid form with a concentration level of 10,00,000 cells/ml in 30 ml of liquid form, and the mixing ratio used was (1:2 12: 5: 0.45). Three specimens of each test are used to evaluate the strength of the concrete combination: a cube measuring 300 x 300 x 300 mm is used for the compression test, a cylindrical mould measuring 612 x 12 inches is used for the split tensile strength test, a rectangular beam measuring 21 6 6 6 inches is used for the flexural strength test, and a square specimen measuring 3 x 6 inches is used to determine the moisture content. Each specimen used in the recovery process is 4 inches by 2 inches by 2 inches and has been intentionally fragmented. The experiment's findings show a notable improvement in the quality of microbe-infected or microbial concrete compared to conventional concrete, and as a result, caco3 precipitation may be seen after 3–4 weeks in Microcracks.

Keyword: Bacterial Concrete, Mechanical Properties, Curing Day's, Microbiologically Induced Calcite Precipitation (MICP), Bacillus Subtilis.

INTRODUCTION

Cement concrete is the most frequently used material in recyclable building projects. It is robust, easily available in the neighbourhood, durable, and versatile in usage and application. This composite material is created by mixing cement, water, and both coarse and fine particles, which solidify over time[1]–[3]. No matter how thoroughly the concrete mixture is worked, it also causes cracks. We are all aware that structures are vulnerable to breaking, which allows water to seep in and weaken the concrete, prompting expensive and sometimes dangerous repairs in the form of fracture capping to stop further damage[4], [5]. Recent

research has demonstrated the existence of self-healing or autogenously repairing cracks in concrete in a variety of ways[6]–[8]. Concrete only appears to be able to self-heal in microcracks that are 0.1-0.2mm wide or less. The self-healing mechanism may actually differ from one concrete mixture to another because the composition of a concrete mixture is so crucial. As demonstrated in this work, the process of fracture healing in the mortar of centuries-old brick buildings in Amsterdam canals was related to the breakdown and precipitation of CaCO3 inside the mostly lime-based mortar matrix. Fracture penetrating water interacts with hydrated lime components such calcium oxide and calcium hydroxide, causing co2 to be released into the environment in addition to dissolving calcium calcite particles present in the mortar matrix. The following reactions take place [9], [10].

$$CaO + H_2O \rightarrow Ca(OH)_2$$

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

The newly produced products from the aforementioned processes that precipitate on the surface of cracks utilise a lot of energy and emit a lot of carbon dioxide, yet the cracks were able to mend as a result of the crack healing[11]. Contrarily, because the cement particles have already fully hydrated during the initial stages of production, concrete with a low cement content will not have a significant capacity for fracture healing. Such low-cost and ecological concrete is expected to be enhanced with an alternative self-healing mechanism to extend its endurance. Endolithic bacteria, alkaliphilic bacteria, and bacteria that produce minerals may all help to create this process. Particularly the bacteria from the latter producing groups appear to be more promising in terms of their capacity for self-healing. As a result, the concrete's strength properties, such as compression strength and flexural strength, are enhanced in addition to the fractures being repaired[12]–[14]. To increase the strength and durability of concrete, a range of microorganisms, including Bacillus subtilis, Cohnii, and Bacillus lichenformis, may be used. We have selected bacillus subtilis, bacillus cereus, and e-coli for our investigation because of how easily we can obtain them. These bacteria have the ability to produce endospores that act as barriers, enabling them to live in hostile conditions. Sources of calcium, phosphorus, and nitrogen are the nutrients needed by bacteria in order to precipitate calcite in their environment. When water enters into newly formed cracks, it helps the reaction of the nutrient with the bacterial components, which causes the precipitation of calcite[15]-[17]. These bacterial components are dormant in concrete. The most crucial factor to take into account is the change in porosity structure. The bio-cement used in this concrete has been shown to have greater strength when compared to standard concrete, despite the fact that altering the pore structure of concrete produces superior results in terms of limiting the intrusion of toxic chemicals into concrete, which may cause buildings to be destroyed. The urease-producing alkaphilic bacterium that is grown in nutrient medium and added to the concrete mix with the healing agent of calcium source has demonstrated a significantly higher compression strength than conventional concrete when compared to conventional concrete. Calcite precipitates are produced as a byproduct of the urease's breakdown, which also produces ammonia and caco3. The medium's pH is increased by ammonia discharge, which provides a favourable condition for calcium carbonate precipitation[18]–[20]. When calcite attaches to the ca ions in the media, caco3 crystals form and are then deposited in agar as a result of the binding reaction. The capacity of all three bacteria to precipitate calcite will help to speed the repair of small cracks and holes in concrete. According to earlier studies, the presence of microorganisms in the concrete has been demonstrated to increase the mixture's compression strength. Small cracks and holes in the concrete are sealed by calcite that has precipitated in the bacteria, increasing the concrete's compressive strength as a result. This is most likely caused by the buildup of calcite inside the gaps of the sand cement substrate and on the surfaces of microbiological cell walls, which clogs the pores. The application of this technique has also increased the concrete's resilience. Numerous factors, such as intracellular calcium, the amount of dissolved CO2, pH, and the accessibility of nucleation sites, affect caco3 precipitation [21], [22]. A fault-free self-healing system will be able to identify the flaw or fractures, which could cause the release of the healing agent at the proper site. Self-healing techniques are a fantastic choice for repairing microcracks in concrete. Micro-cracks on the

surface of concrete can be repaired utilising spalling healing techniques, and this method has proven successful. When bacteria are present, a previous, comparable layer that conforms to the creation of calcium carbonate will form on the surface of concrete fractures. The bacterium that was injected can survive in such an environment since concrete is a relatively alkaline substance. The filling of micro-cracks and the binding of other ingredients like gravel and sand in the concrete mix are both aided by microbiologically induced caco3 precipitation in concrete[23]. The strength and durability of concrete may be increased by the presence of organisms during the calcite precipitation process. By converting urea into ammonium and carbonate through the urea conversion process, the bacteria Sphaericus may precipitate chloride in an intensely alkaline environment. Concrete itself may repair minor fractures with a depth of less than 0.2 millimetres. However, concrete cannot repair itself if the fracture is larger than 0.2 mm in diameter, allowing dangerous substances to enter the building. The development of any fractures in self-healing concrete allows bacteria to emerge from their dormant state, which leads to the emergence of additional cracks. Carbonate enters fractures mostly during the self-healing process as a result of bacteria's metabolic activities. This aids in the healing of the fractures [24, 25].

Materials and Methodology

In the investigation, regular Ordinary Portland cement OPC of 43 grade cement was employed as the building material. The physical parameters of the cement are listed in Table 1 for your convenience. It is necessary to purchase the fine and coarse aggregates and crushed rock from a local seller, and their characteristics are shown in Tables1 correspondingly. All of the components have been tested in accordance with Indian specifications. The purpose of this study is to investigate and assess the efficacy of the bacterium Bacillus Subtilis in compression, flexural, splitting tension, and water absorption tests, as well as the usefulness of the bacterium in fracture sealant applications[16], [17], [26], [27].

Table 1: General Properties of Materials

Materials	Standard Consistency	Specific	Initial Setting Time	Final Settin Time	Finenes s	Zone	Water Absorption	Bulk Density
Cement	32%	3.02	39 min	10 hrs.	7%	-	-	-
Fine Aggregate		2.64			2.6	II	-	-
Coarse Aggregate	-	2.56	-	-	-	-	2%	1.56gm/cc

Preparation of Bacteria culture

bacterial culture produced by adding a few colonies from an earlier culture to a fresh growing medium. Sub-refined is used to increase the number of cells in a particular culture as well as to extend the life of microorganisms in a culture. Some of the ingredients in this recipe include urea, Bacillus subtilis, baking soda (NaHCo3), calcium chloride dehydrates (CaCl2), ammonium carbonate (NH4Cl), and nutritious broth. These nutrients are necessary for the bacterium to survive, and they also serve as its diet or main source of nourishment. The researchers used a biosafety cabinet, steriliser equipment, glassware, Erlenmeyer cups, inoculate wire, and other supplies [28], [29].

Figure 1: Serial Dilution

Figure 2: Culturing of Bacteria

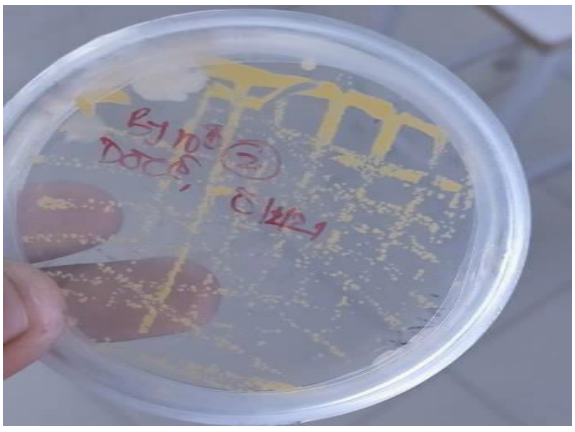

Figure 3: Growth of Bacteria

The bacteria multiply and remain suspended in great numbers on the culture medium. The culture is produced using a nutrient broth that also includes baking soda, NH4Cl, urea broth, and cacl2 die of dehydration. In an autoclave set to 121 degrees Celsius and 151 pounds of pressure, the culture media is sterilised. The 2.10 grammes of nutritive broth[30], 1.50 grammes of baking soda[31], 7 grammes of ammonium carbonate, 7 grammes of urea broth, and 5 grammes of cacl2 dehydrate should all be diluted in 1 litre of water. Give it a good swirl. Disinfect the tool used to induce Bacillus Subtilis bacteria before using it to reduce the risk of infection. Before employing the blended nutrients, vapour sterilise them in the steriliser equipment for 30 minutes at 151 ps (pressurised vapour) pressure. From previously prepared agar plates/test tubes, colonies of microorganisms will be extracted using an

inoculating needle. Before beginning the experiment, prepare the Bacillus Subtilis bacteria and the sterilised nutrition broth in the biosafety hood. Use the inoculating wire, and before using it, make sure the loop is red-hot. To make sure there are no extra contaminants inside the tube opening, test tube apertures are heated to high temperatures[32], [33]. To reduce infection, the capillary tube should be tightly closed with cotton. To enable the Bacillus Subtilis germs to multiply and colonise the test tubes and Erlenmeyer jars, place them in the steriliser for eighteen to twenty-four hours at room temperature.

Gram Staining

Gram staining is a method used in the microbiological lab to divide microorganisms into Gram-positive and Gram-negative groups based on the physical properties of their cell membranes. Gram-negative results are depicted by a pink-red tint, while Gram-positive results are displayed by a purple-blue hue. In the process of gramme staining, cells are first fixed with heat before being stained with crystal violet, a fundamental dye that is absorbed by all bacteria about equally. The plates are then promptly rinsed with 95 percent alcohol (detained), stained with safranin to restore the colour to its normal shade, then counterstained with I2-KI combination to set the staining[34]. Contrarily, Gram-positive bacteria retain the violet dye from the beginning of the experiment while Gram-negative organisms are decolored by the organic solvent and display the pink counterstain. When it comes to germs, the ability of the organism's cell wall to preserve the colour crystal violet serves as a differentiation between Gram-positive and Gram-negative microbes. Before publishing their findings, the scientists used the Gram method to be sure that no additional types of microorganisms had entered the samples. A microscope was used to study the Gram-stained cereus in Figure 2[35, 36].

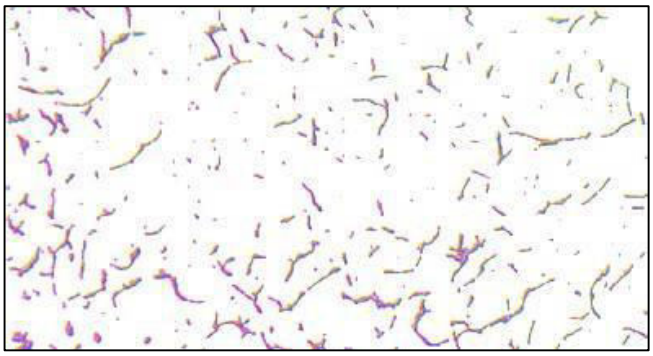

Figure 4: Gram Staining

Using high performance concrete of M60 grade, the mixed construction was finished in line with the IS code. Eight distinct mixed ratios were used, one of which was a control mix devoid of any bacterial components. The combined amounts of the substances are displayed in Table 2. There are only two types of combinations available: one that contains only bacteria, and the other that combines bacteria with MBS. Cubes and cylinders with dimensions of 150 mm, 150 mm X 150 mm, and 150 mm x 300 mm, respectively, were used for the casting process. The cubes started to distort after a day and were kept for curing. According to IS: 516-1959[37], the split tensile and split compressive strengths were assessed after seven, twenty-eight, fifty-six, and ninety days after curing, respectively.

OUTCOME AND DISCUSSION

Compressive Strength

The compression strength of the concrete was determined using the dry proportions of the

ingredients in accordance with the mixture design guidelines (cement, sand, & coarse aggregate). To reach the correct cell concentration when mixing bacterial concrete, a pure culture containing 10,000 000 cells/ml of water must be added. As part of our study, we used a technique known as specific technique of combining, in which microorganisms are combined immediately with water. The cubes should be put accurately on the equipment, and the sample must be precisely aligned with the cylindrical shape seated plate before running the test. To bring the cube down, a constant force of 140 kg/cm2 per minute will be given to the specimen in an axial direction until it collapses. When a sample fails, the ultimate load where it breaks is considered to be the compression load. The results of the controlled concrete as well as the bacterial concrete are recorded and compared. A comparison is done with concrete using all 3 kinds of bacterial concrete for seven days, twenty-eighth days, Fifty-six days and Ninety days.

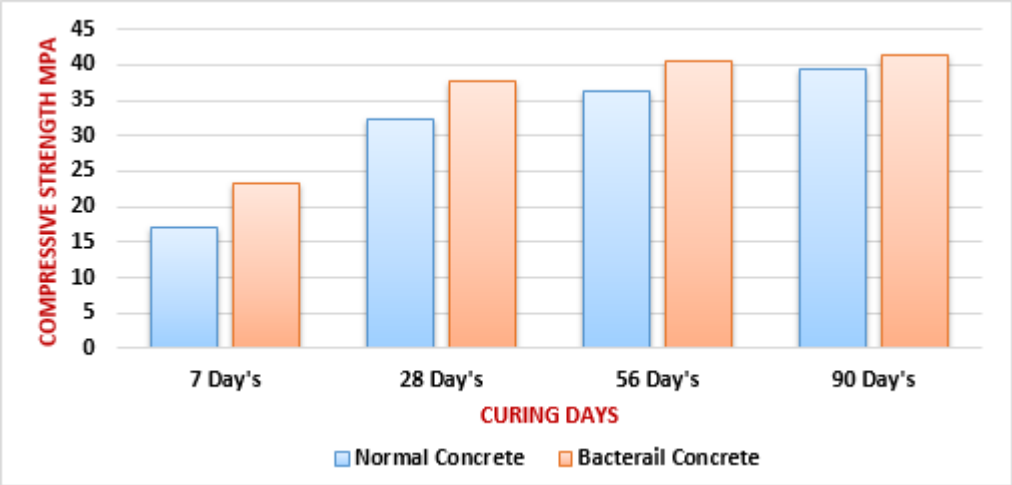

Figure 5: Compressive Strength of Concrete at Different Curing intervals

Split Tensile Strength

The cylindrical moulds used for the tensile tests are 150mm x 300mm in size. To test how well they functioned, cylinders were cast with 0%, 10%, and 30% fly ash as well as with and without a microbiological culture. Following three, seven, twenty-eight, fifty-six, and ninety days of curing, cylinders were tested for tensile strength in the tensile strength equipment shown in figure 4. Graphs are used to display the results of the tensile strength testing (Figure 6). The results of the testing revealed that the bacterial concrete was stronger than regular concrete.

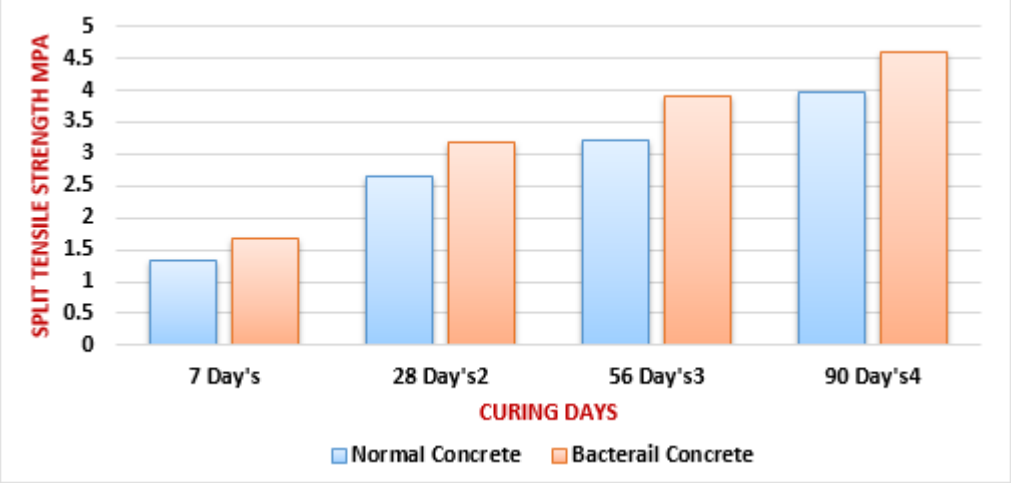

Figure 6: Split Tensile Strength of Concrete at Different Curing intervals

Flexural Strength

A set of Moulds measuring 100 mm by 100 mm by 500 mm is employed in this instance, and following cleanup, the Moulds are thinly covered with Moulds petroleum products. To ensure that no water escapes during stuffing, a corresponding coating of petroleum products for moulds should be used between the surface contact along each border of the moulds & the base plate. A little layer of mould lubricant should be placed to the interior sides of the manufactured moulds to prevent concrete from sticking to their surfaces. The precise weights of the necessary amounts of cement, fine aggregate, and equivalent coarse aggregate for the specific mix are also determined concurrently, prior to the concrete being poured. The fine aggregate and cement were carefully combined in a hand mixer to guarantee that the colour of the slurry remained uniform throughout. The mixer must then be filled with a specific amount of coarse aggregate before being turned on to create a homogeneous dry mixture. The microbe mixture was then added along with the appropriate amount of water, and the mixture was continued for about 3 to 5 minutes to ensure a uniform mixture was generated.

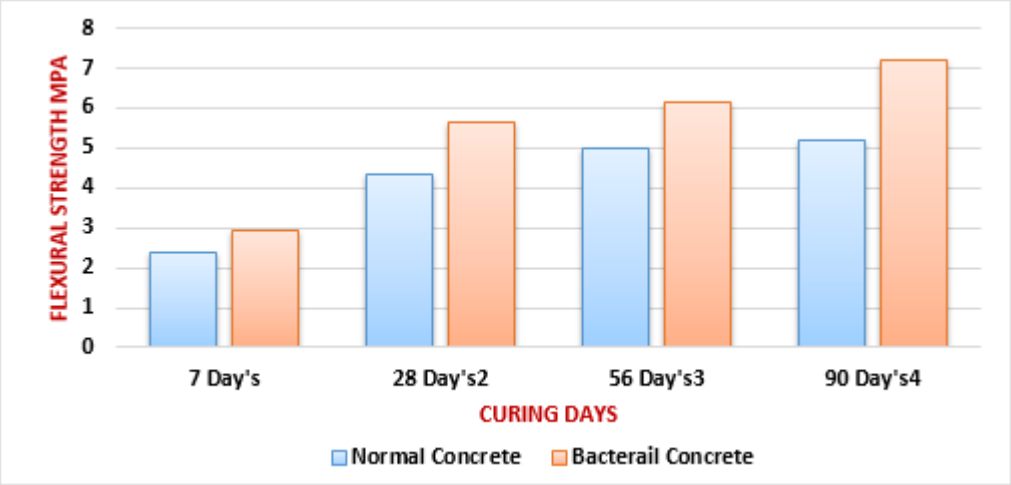

Figure 7: Flexural strength of Concrete at Different curing intervals

CONCLUSION

When the experiment is taken into consideration, it demonstrates the expansion of microbial species that the addition of microorganisms Solution-Bacillus Subtilis to concrete demonstrates improvements in a variety of properties of the concrete. These improvements can be seen in a compression strength of concrete test, a split tensile strength test, and a flexural strength test, among other things. Because the bacteria are able to reproduce within the research facility, it is possible that they do not pose a threat to the health of either humans or animals. This may be demonstrated if adequate precautions are taken. According to the findings of the study, the introduction of microbial species into cement improves both its performance and its toughness. As a consequence of these findings, the utilisation of this kind of microorganisms for the self-healing process in concrete may result in expensive, sturdy, and long-lasting structures.

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