

INVESTIGATION ON BACILLUS SUBTILIS

BACTERIUM CONCRETE

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ABSTRACT

*The many physiological circumstances that affect concrete structures cause a variety of durability problems, which ultimately cause the building to lose strength and sustain irreparable damage. The pore structure of concrete is the primary factor contributing to the degradation of the durability and mechanical properties of concrete. Recent years have seen the development of microbiologically induced calcium carbonate precipitation by bacteria as a method for consolidating various building materials as well as improving the characteristics of concrete and repairing concrete structures. The use of bacterial concrete, often known as bio-concrete, to improve the strength, mechanical properties, and permeation of concrete has been the subject of several studies in recent years. The study's objectives are to test and analyse the compressive strength of concrete cubes, determine the best dosage of bacteria to employ, and learn more about the characteristics of cracked specimens by using the ureolytic, gram-positive (spore-forming), facultative aerobic *Bacillus Subtilis*.*

1. INTRODUCTION

A unique variety of concrete called bacterial concrete has the capacity to repair itself on its own. A further benefit of bacterial concrete is that the addition of bacteria improves the characteristics of concrete in both natural and laboratory settings. According to prior studies, it has been discovered that microbiologically induced calcium carbonate precipitation technology has already been utilised to increase bricks' strength and consolidate sand. Given concrete's capacity for self-healing and other improvements, it is obvious that using this kind of agent would reduce environmental impact and cost. Because the other pre-determined materials for strengthening and durability improvements were bad for the environment, more expensive than bacterial concrete, and also needed maintenance on a regular basis.

The purpose of this study is to comprehend the importance of various microorganisms in concrete. The microbiologically induced calcium carbonate precipitation procedure improves

the concrete structures' tensile strength and longevity. It is primarily caused by a decline in the permeability of both water and chloride ions. Additionally, it aids in fusing the sand particles together so they behave like cement.

According to studies, calcite production, particle sedimentation blocking the channel, continuous cement particle hydration, and cement matrix expansion are some of the potential self-healing mechanisms. microbiologically induced calcium carbonate precipitation, which promotes mechanical property improvement and crack healing, is produced by biological processes and is pollution-free. The main determinants of microbiologically induced calcium carbonate precipitation are pH, the presence of a nucleation site, the concentration of calcium ions, and the amount of dissolved inorganic carbon. microbiologically induced calcium carbonate precipitation, which promotes mechanical property improvement and crack healing, is produced by biological processes and is pollution-free. The main determinants of microbiologically induced calcium carbonate precipitation are pH, the presence of a nucleation site, the concentration of calcium ions, and the amount of dissolved inorganic carbon.

The primary benefits of microbiologically induced calcium carbonate precipitation in concrete include an improvement in strength, low maintenance costs for the structure, resistance to freeze-thaw, high carbonation, which can assist reduce porosity and permeability, and a rise in resistance to chloride attack. The use of bacteria in concrete should be kept to a minimum since they are unsafe for human health, and there is no set design for bacterial concrete design mix, which, according to earlier studies, results in a cost increase of 7 to 30%. Numerous researchers have classified the employment of bacteria to create bacterial concrete as a biological tactic and have offered concepts for making bacterial or self-healing concrete.

2. MATERIAL AND METHOD

2.1 Material

2.1.1 Cement

43 grade of ordinary Portland cement used in the current investigation. The IS 4031: 1968 is used to determine the cement's characteristics, and the results are summarised in Table 1.

Table1. Properties of Cement

Properties	Values
Compressive strength	43 M Pa
Fineness	5 %
Initial Setting Time	30 minutes
Final Setting Time	10 hours
Standard Consistency	29 %
Specific Gravity	3.15

2.1.2 Fine Aggregate

The fine aggregate utilised was river sand. Table2 lists the characteristics of the fine aggregates.

Table 2. Properties of fine aggregate

Fine Aggregate	Values
Size	Passing through 4.75mm sieve
Bulk Density	1721 kg/m ³
Fineness Modulus	2.25
Specific Gravity	2.67

2.1.3 Coarse Aggregate

The coarse aggregate's parameters are listed in Table3, and tests were done to determine its specific gravity and fineness modulus in accordance with IS: 2386-1983.

Table 3.Properties of coarse aggregate

Coarse Aggregate	Values
Size	20 mm
Bulk Density	1674 kg/m ³
Fineness Modulus	6.23
Specific Gravity	2.81

2.1.4 Water

Since it actively participates in the chemical reactions involving cement to create the hydration product, calcium-silicate-hydrate (C-S-H) gel, water is a crucial component of concrete. According to Neville (2000), the amount of water added should be the bare minimum necessary for the chemical reaction of unhydrated cement, as the production of unwanted voids (capillary holes) in the hardened cement paste of concrete would result from adding more water than is necessary. Drinking water was employed in the current experiment for both concrete cube mixing and curing.

2.1.5 *Bacillus Subtilis* Bacterium

Bacillus Subtilis was chosen because it generates calcium carbonate and is widely available. A gram-positive, catalase-positive bacterium known as Hay *Bacillus* or Grass *Bacillus* is also found in soil and the gastrointestinal tract of ruminants and people. *Bacillus Subtilis*, a rod-shaped member of the genus, can produce a hard, protective endo-spore, which enables it to withstand harsh climatic conditions. The well-studied Gram-positive bacterium, as well as a model organism for research on bacterial cell development and chromosome replication.

A typical soil bacteria called *Bacillus subtilis* has the ability to create calcite precipitates when given the right medium and a calcium supply. Using liquid media, the bacteria were cultivated in accordance with the supplier's instructions. 5.0 g of peptone and 3.0 g of meat (beef) extract per litre of distilled water made up the medium used to grow bacteria. 1.5% agar was then added to create a solid medium for the stock culture. To promote sporulation, 0.01 g MnSO₄ H₂O was added to this medium, and the pH was raised to 7.0 using 1 N HCl. Prior to cooling to room temperature, the mixture was autoclaved for 20 minutes at 121 degrees Celsius to disinfect it (25°C). *Bacillus Subtilis* cultures were made using lyophilized bacteria that had been activated, whereas all subsequent tests used cultures made using subcultures. Keep in mind that the entire culture process was carried out under sterile conditions. Following that, cultures were cultured for 72 hours at 30°C and 130 rpm in a shaker incubator. After that, the 72-hour-old growing culture was centrifuged to extract the germs inside.

2.2 Mix design

The process of choosing appropriate concrete ingredients and figuring out their relative proportions with the goal of creating concrete with a particular minimum strength and durability as affordably as possible is known as mix design. As per IS 10262, we made M40 grade concrete for our investigation. The concrete presents Cement = 474 kg/m³, Water (Net mixing) = 110 kg/m³, Fine aggregate (SSD) = 748 kg/m³, Coarse aggregate (SSD) = 1 085 kg/m³, Chemical admixture = 4.74 kg/m³, Free water-cementitious materials ratio = 0.327.

Table 4. Mix details of Bio-concrete

S.No	Mix	Bacterial	Cement	Fine	Coarse	Water	chemical
		solution	kg/m ³	aggregate	aggregate	kg/m ³	admixture
		ml		kg/m ³	kg/m ³		kg/m ³
1	Mix1	0	474	748	1085	110	4.74
2	Mix2	15	474	748	1085	110	4.74
3	Mix3	30	474	748	1085	110	4.74
4	Mix4	45	474	748	1085	110	4.74
5	Mix5	60	474	748	1085	110	4.74

2.3 Methods

2.3.1 Test on Fresh Concrete

Slump Cone Test

Workability of concrete determines by using slump cone test. This method is very easiest method for onsite work. Workability of geopolymer concrete observed as per IS 1199-2000. Slump cone equipment is used to conduct the slump cone test. The cone that is utilised for the slump cone test is 30 cm tall, with bottom and top diameters of 20 cm and 10 cm, respectively. As shown in Figure1, the steel tamping rod has a 16 mm diameter, is 60 cm long, and is rounded at one end. The 400x400mm metal base plate offers a level and secure platform for carrying out the test¹.



Fig 1. Slump cone apparatus

After the geopolymmer concrete has been properly mixed, the interior of the mould is cleaned and lubricated to make it simple to remove the cone and stop concrete from sticking to its surface². The base is positioned on a horizontal, smooth surface. Three layers of the prepared concrete mix are added to the container, and each layer is tamped 25 times with the tamping rod. The top surface of the mould is levelled once it has been fully filled, and then it is carefully pulled upward and the droop is measured right away. The height difference between the mould and the concrete's highest point is used to calculate the slump value.

2.3.2 Harden concrete test

Compressive strength

The compressive strength plays an important role in structural design. 15cm × 15cm × 15cm cube cast and cured, tested by using universal testing machine. The test was workout by using IS 516-1959 guide lines. The breaking load on cube is divided by compressed area is called as compressive strength. The specimen with universal testing machine shown in figure 2.



Fig 2. Testing of cubes in CTM

3.0 RESULT AND DISCUSSION

3.1 Slump Cone Test

Workability of bacterial concrete is very important think. The concrete achieve optimum workability means handling operation is very easy. Very lower workability concrete handing operation is very hard. Workability of bacterial concrete observed by using slump cone test. Mix 1 to Mix 5 slump cone test adopted. The slump value shown in figure 3.

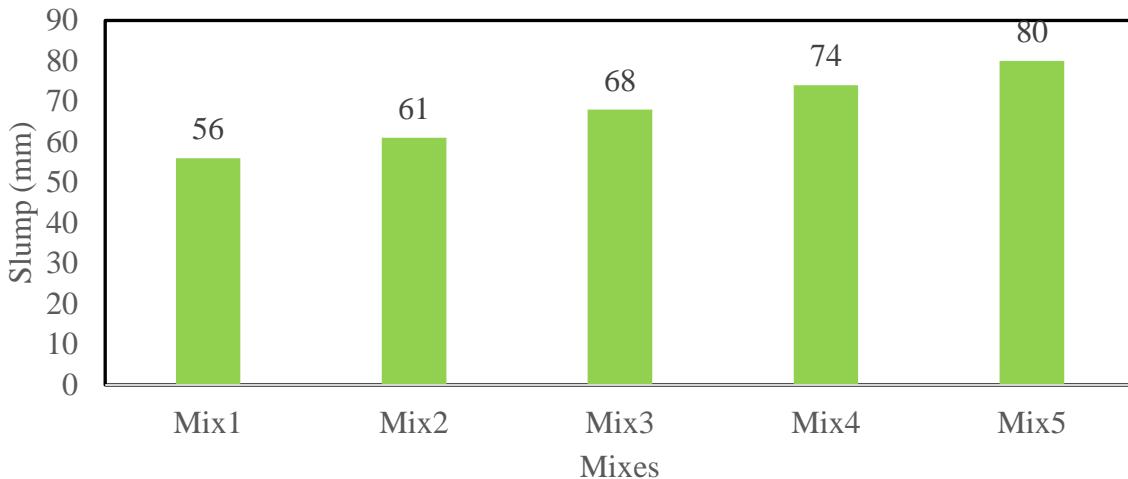


Fig3. Comparison of slump value

Slump tests have been run initially on concrete M-40 that is both healthy and contaminated with bacteria³⁻⁵. This test is carried out to ascertain the concrete's workability, which is a representation of the material's strength. The capacity of concrete to be mixed easily, which again depends on the amount of water and aggregate quality, is referred to as workability. The slump test value in this study work runs from 56 to 80 mm.

3.2 Compressive strength test

Each mix 3nos of 15cm × 15cm × 15cm cube specimens casted and tested. Totally 30 cubes adopted for compression test. Compression load obtained from compression testing machine. And we calculated compressive strength is equal to compression load by compressed area.

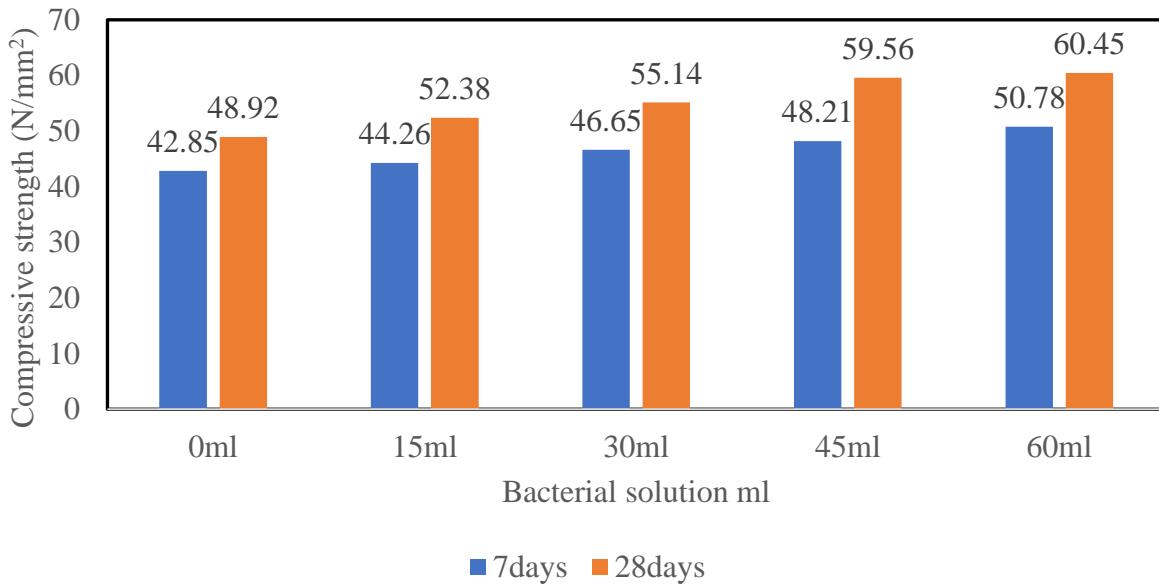


Fig4. Compressive strength of bacterial concrete

The above result shows that the percentage of *Bacillussubtilis* solution increased at the time compressive strength of geopolymers concrete also increased⁶⁻⁷. Mix 2 compressive strength increased 10% compare to Mix 1. The optimum compressive strength achieved Mix 560ml *Bacillussubtilis* solution mix. Figure 4 shows that 7 and 28 days compressive strength. Figure 4 described compressive strength of bacterial concrete.

4. CONCLUSION

With the addition of bacteria, it was shown that the compressive strength increased. This increase is mostly attributable to the deposition of microbially induced calcium carbonate precipitation on the microorganism cell surfaces and inside the mortar pores. It was shown that bacterial cell concentrations up to 106 cells/ml increased the compressive strength of regular mortar. At 106 cells/ml, the greatest increase in compressive strengths was attained. Comparing bacterial concrete made with *Bacillussubtilis* for 7 days and 28 days to conventional concrete, the compressive strength of the 45ml and 60ml samples increased by a higher proportion.

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