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DETERMINATION OF BACTERIAL CONCRETE STRENGTH USING *Bacillus subtilis* AND LIGHTWEIGHT EXPANDABLE CLAY AGGREGATE

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ABSTRACT

This study examines the impact of bacterial concrete on strength and self-healing. Bacterial concrete has better compressive strength, permeability, corrosion resistance, chemical precursors, alkalinity resistance, and mechanical stress. *Bacillus subtilis* calcium lactate and spore powder effects are explored in this study, and the influence of this bacterial form on strength and self-healing capacity to crack repair. The *Bacillus subtilis* concentration 105 cells/mL is used in concrete with calcium lactate 0.3% of cement. In another trial, calcium lactates 0.3% and spore powder 0.5% of cement with *Bacillus subtilis* concentration of 105 cells/mL and lightweight expandable clay aggregate (LECA) is 30% replaced to the coarse aggregate used in concrete respectively. The conventional concrete and bacterial concrete cubes were molded with dimensions of 150 mm x 150 mm x 150 mm, cylinders with dimensions of 100 mm x 200 mm, and a beam with dimensions of 100 mm x 100 mm x 500 mm. These specimens were evaluated after 7 and 28 days of cure. The compressive, split tensile, and flexural strength of bacterial concrete was raised by 23%, 8%, and 7%, respectively when compared to conventional concrete. Thus, the experimental findings reveal that *Bacillus subtilis* at 105 cells/ml cells with 0.3% calcium lactate has a substantial impact on the strength and self-healing of bacterial concrete.

Keywords: Bacterial concrete, *Bacillus subtilis*, Calcium lactate, Lightweight expandable clay aggregate, Spore powder.

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INTRODUCTION

Concrete is often used as a construction material; nevertheless, minute cracks regularly create a continuous structure inside the concrete. Porosity is a result of continuous network construction, which finally leads the concrete to break. Fractures are a common issue with concrete. Concrete is a porous medium that is prone to many attacks, including chloride and carbon dioxide, due to pores. Repairing and retrofitting concrete buildings to reduce cement consumption by employing alternative cementation materials as a partial replacement for cement is a typical early degradation problem in many concrete infrastructures, such as carbonation and chloride attack. Concrete cracks may be repaired using a gentle method called self-healing concrete, which integrates microorganisms. Tensile cracks allow chemicals or water to flow into the concrete. The use of self-healing technologies on buildings may help to reduce the need for costly repairs. Concrete fractures may be prevented by using a unique self-healing technology. Using bacteria as a healing agent in concrete allows for the rapid development of a realistic self-healing technology through mineral deposition. Furthermore, employing calcite preparation and encapsulation technologies, structural concrete may be made stronger and more durable.

LITERATURE REVIEW

An eco-friendly bio-mineralization technology that can repair concrete fractures without human intervention is bacteria-based self-healing. When fractures occur in concrete, water penetrates and activates bacteria, causing precipitation and sealing the cracks. The biomineralization technique utilizes Bacillus pasteurii, a common soil bacterium with high urease production. When water seeps into the fractures in concrete, the bacteria contained in the material become active (Gandhimathi et al., 2012; Jing Xu, 2014). This approach works by causing microbially-induced calcium carbonate (CaCO3) precipitation (MICP).

Thermal power facilities generate fly ash as a waste product. In addition, these by-products are employed as supplemental cementitious materials in the building sector to alleviate some of the challenges associated with waste disposal. Cold bonding or pelletization may be used to make artificial lightweight aggregates from fly ash (Harilal, 2013). It is possible to make lightweight concrete with adequate strength by using lightweight particles. Concrete with a high bonding strength may be made using lightweight materials. It takes up less space and provides the same level of strength as standard concrete. As a result, the foundations are less strained by structural parts that are smaller in size. It also provides a lower weight-to-strength ratio for the manufacture of environmentally friendly concrete (Niyazi Ugur Kockal, 2011;

Gomathi, 2015). An EDS examination showed that concrete specimens contained calcite deposition in the form of CaCO3 precipitation, indicating that bacteria could survive in expanding clay aggregates for an extended period. In addition, a visual examination of the healing of the fractures showed that the bacteria floating in the water had survived and assisted in the self-healing process (Jonkers et al., 2011). Lightweight aggregate concrete (LWAC) should be utilized to reduce building weight. Furthermore, scientists are investigating the usage of microorganisms in reinforced concrete projects. Bacillus Cereus, *Bacillus subtilis*, Bacillus Sphaericus, Bacillus Pasteurii, and many more bacterium species might be utilized in this job. Many investigations have revealed the optimal concentration for different species of bacteria and alternative treatment of concrete surfaces using precipitation of bacterial concrete (Mondal et al., 2016; Willem et al., 2008). Many researchers have investigated the mechanical properties of bacterial concrete. Bacillus Cereus, *Bacillus subtilis*, Bacillus Sphaericus, Bacillus Pasteurii, and various other bacteria have been studied for their impact on concrete. The bacterial cell walls may be responsible for the improved mechanical characteristics of concrete. Furthermore, the Bacillus Sphaericus and Bacillus Pasteurii bacteria were demonstrated to improve the mechanical qualities of concrete (Pei et al., 2013; Jagannathan et al., 2017). Lightweight concrete's split tensile strength must be considered in the design requirements; hence this study focuses more on that aspect. The healing of the cracks remedies the tensile cracks, and the percentage of load after healing is considered. Traditional bacteria concrete may be replaced with lightweight bacterium concrete to provide an eco-friendly and effective bio-sustainable material. Using the encapsulation process and direct application of bacteria, researchers have investigated the manufacture of bacterial concrete. Lightweight aggregate partially replaces coarse material in the encapsulation procedure. The bacteria may be applied directly to the concrete and encapsulated to repair the bacterial concrete's structural cracks (Balam et al., 2016; Alghamri et al., 2016). The strength of concrete was tested using both natural and manufactured sand. Researchers estimate that employing manufactured sand may increase the strength of concrete (Khartode and Kulkarni, 2016a, 2016b) These organisms should be able to influence carbonate precipitation through the delivery of the urease protein. Until super-saturation is achieved, the potential of microorganisms to repair themselves as self-healing specialists was explored and proved effective in this precipitation process (Andalib et al., 2016; Luo et al. 2015; Siddique et al. 2016).

METHODS AND MATERIALS

The materials required to make bacterial concrete are explained further down.

Cement

The building industry relies heavily on cement since it is the binding element in concrete and hardens when water is added. Table 1 shows the parameters of OPC 53 grade cement tested as per IS:12269-1987 requirements.

Table 1: Properties of cement

Properties	Values
Fineness (m^2/kg)	3.20
Specific gravity	3.15
Initial setting time (minutes)	60
Final setting time (minutes)	240
Standard consistency	3.07

Sand

Natural river sand of 4.75 mm in size is used. Sand has a specific gravity of 2.94 and a water absorption capacity of 1.69. As a result, sand has a fineness modulus of 2.61, making it zone II according to IS: 383-1970.

Aggregate

In concrete, the aggregate component is a ferocious and porous one. In this experiment, an angular aggregate from a nearby quarry passed through a 20 mm IS sieve and was retained on the 12.5 mm IS sieve. The aggregate has a specific gravity of 2.85 and water absorption of 1.57. IS: 383-1970 is used to measure the physical properties.

Light Weight Expandable Clay Aggregate

Rotary kilns are used to dry and fire the clay with low lime content, resulting in Light Weight Expandable Clay Aggregate (LECA). Maxit Group factories across Europe produce lightweight aggregate. Lightweight expanding clay aggregate is seen in Figure 1. High particle strength is generated in certain manufacturers with a bulk density of 800 kg/m^3. The greater the particle density, the greater the strength of the particle.



Figure 1: Lightweight expandable clay aggregate

A LECA of high density has a far better mechanical strength than a coffee-density material when created at the same location and using the same

raw materials. When new cement is mixed with lightweight aggregate, it strongly connects with the material's rough and porous surface. Table 2 lists the LECA's characteristics.

Table 2: Properties of LECA

Properties	Values
Specific gravity	0.58
Water absorption	18.27%
Impact value	49.65%

Bacteria

The bacteria to be introduced might be liquid (broth) or solid (powder) (agar). The liquid form was selected for use in concrete. The bacteria must be cultivated in a specified medium. The broth solution is made by dissolving 13.0 g of nutritional broth in 1000 ml of distilled water. Concrete microorganisms feed on these substances. It took 20 minutes to autoclave the media. Then the pure bacterial culture was injected into the broth. The inoculated broth medium was held at 37o C for 24–48 hours. The calcium lactate and spore powder materials were added to the concrete mix at 0.3% and 0.5% by weight of cement, respectively. The Vidya Pratishthans School of Biotechnology in Baramati provided the bacterial pure culture strain. *Bacillus subtilis* with 10^5 cells/mL bacterial solution is employed for this study.

Water

Normal water for drinking purposes is employed in concrete conforming to IS 456-2000.

Methods

Mechanism of Self-Healing

Cracks in regular and lightweight bacterial concrete are self-healing because CaCO3 precipitation forms on the surface. Microbially Induced CaCO3 Precipitation may be used to seal the fractures (MICP). The CO2 in the calcium hydroxide reacts with the calcium carbonate on the surface of the fractures to generate calcium carbonate.

$$CO2 + Ca\ (OH)2 \rightarrow Ca\ CO3 + H2O \quad \text{(i)}$$

$$Ca\ (C3H5O2)2 + 7\ O2 \rightarrow CaCO3 + 5\ CO2 + 5\ H2O \quad \text{(ii)}$$

This procedure is more efficient since the concrete contains active metabolic transformations of calcium nutrients and microorganisms (Klaas van Breugel, 2012). Carbonate ions are formed due to a rise in the pH of the specimens used for self-healing.

$$Ca2+ + cell \rightarrow cell\text{-}Ca2+ \quad \text{(iii)}$$

$$Cell\text{-}Ca2+\ CO3^{2-} \rightarrow cell\text{-}Ca\ CO3 \downarrow \quad \text{(iv)}$$

Cell walls of bacteria are negatively charged, which attracts cations from the environment. The precipitation of CaCO3 by Ca^{2+} ions, which then accumulate on the cell surface, is an example of this mechanism in action (Wasim Khaliq, 2016).

Mix proportion

IS 10262-2009 is used to create the concrete mix presented in Table 3.

Table 3: Details of the concrete materials' mixing ratios (Kg/m^3)

Mix	Cement	Sand	Aggregate	LECA	Water	Spore powder	Calcium lactate
CC	492.9	750.52	1106.37	nil	197.16	nil	nil
BC+LECA	492.9	750.52	774.46	331.91	197.16 (10^5 cells/mL)	2.25	1.35
BC	492.9	750.52	1106.37	nil	197.16 (10^5 cells/mL)	nil	1.35

Experimental investigation

The procedure of fabrication and testing of concrete are discussed below.

Batching

The batching has been done by volume or by mass of concrete ingredients. Traditionally, batching is completed by volume, but mass batching was employed for accuracy during this study.

Mixing

There are two approaches to incorporate bacteria into concrete for microbially-induced calcium carbonate precipitation (MICP):



Nutrient broth With bacteria

Figure 2: Bacteria growing in the Nutrient broth

Direct application

First, the measuring jars were sterilized in an oven at a temperature of roughly 1000 °C for five minutes. Then, the bacterial concrete was mixed directly in the jars (Figure 2). A measuring jar was then filled with 105-cell-per-mL *Bacillus subtilis* and calcium lactate

(0.3% of cement), and then the solution was introduced straight to water. Next, a bacterial solution was added to the water, and the water was agitated well before being poured into the concrete for immersion. This technology may be used to produce normal bacteria-based self-healing concrete (Wasim Khaliq, 2016).

Encapsulation method

Concrete may be encapsulated by submerging lightweight particles in a bacterial solution in place of the conventional aggregates. The primary benefit of this encapsulation procedure was the successful sealing of concrete interior fractures. Bacteria are suspended in nutrient broth media-prepared water. For spore production, light aggregates are submerged in a bacterial solution. After 24 hours in the bacterial solution, the lightweight aggregates were allowed to dry. To make light concrete, lightweight aggregates were then employed.

Casting and curing

In casting and curing, moulds were cleaned with oil and then filled in three layers, each of which was tamped down with the tamping rod once the concrete had been properly mixed. After 24 hours, the specimens were put in a curing tank after being placed over a table vibrator for table vibration. Casting specimens required the use of moulds with proportions like 150 mm x 150 mm x 150 mm, 100 mm x 200 mm cylinders, and a 100 mm x 100 mm x 500 mm concrete beam.



Compressive strength Split tensile strength Flexural strength

Figure 3: Testing of concrete specimens

Testing of specimens

After curing, the concrete specimens were taken out of the tank (figure 3). Then the load is progressively and continuously applied to the specimen until it fails. Then, the heaviest load was reported. Whenever feasible, readings were recorded after 7 and 28-day curing intervals

on the controlled and bacterial concrete sample. Axial compressive load and deflection are two parameters that are assessed throughout the testing process.

RESULTS AND DISCUSSION

Behaviour Bacterial Concrete

The cracks formed in concrete are healed by using cultured bacteria (calcium lactate). Figure 4 shows that the cracks in the concrete specimen are healed by inducing carbonate precipitation, an environmentally friendly crack repair technique. The cultured bacteria are giving good leads to the self-healing of concrete.



Before healing After healing

Figure 4: Self-healing of cracks by adding cultured bacteria in concrete

The technique known as the encapsulation method was employed to repair the cracks formed in concrete. Figure 5 shows the cracks in the concrete specimen healed by inducing some parts of the lightweight expandable clay aggregate to be impregnated with twice the calcium lactate solution and bacteria spores, which are environmentally friendly crack repair techniques. In addition, the spore-powder bacteria are also giving good results in the self-healing of concrete. It is found that lightweight concrete has a higher capacity to repair internal and exterior fractures in the concrete when comparing bacterial specimens.



Before healing After healing

Figure 5: Self-healing in cracks by adding spore powder (bacteria) in concrete

Compressive Strength

Specimens of the concrete cast and allowed to harden for various periods, such as 7 and 28 days, were analyzed. Then, in a compressive testing machine, the specimens were put under the heaviest possible pressure. Figure 6 shows the specimens' average compressive strengths. The compressive strength of bacteria with *Bacillus subtilis* is above standard concrete and lightweight weight aggregate concrete with *Bacillus subtilis*, as shown in Table 4. The compressive strength of *Bacillus subtilis* bacterial concrete increases by 23.46% and 14.39% at 28 days compared to standard concrete and *Bacillus subtilis* with lightweight aggregate (LECA) concrete, respectively. Cement paste and fly ash have developed a strong connection. It is via this process that the internal fractures may be bridged. Compared to conventional bacterial concrete, lightweight concrete has higher internal bonding strength and greater workability, similar to compressive strength.

Table 4: Different types of concrete's compressive strength

Control Mix (M20)	Conventional concrete (CC)	Bacterial concrete with *Bacillus subtilis* and Lightweight aggregate (BC+LECA)	Bacterial concrete with *Bacillus subtilis* (BC)
	7 days Compressive Strength in MPa		
	17.10	19.84	22.30
	28 days Compressive Strength in MPa		
	25.50	29.17	31.48

Split Tensile Strength

Concrete cylinder specimens with diameters of 100 mm and depths of 200 mm were tested for split tensile strength at curing ages of 7 and 28 days. Horizontally held specimens were used to induce tensile fractures. Afterward, after the initial failure, the specimen was removed and left to cure in water for self-healing observation. All specimens were separated into two halves, and these values are shown in Figure 7. *Bacillus subtilis* bacteria have a higher split tensile strength than conventional concrete and lightweight aggregate concrete containing *Bacillus subtilis*, as indicated in Table 5. On day 28, split tensile strength for *Bacillus subtilis* with lightweight aggregate (LECA) and standard concrete improved by 7.94% and 3.460%, respectively, whereas for *Bacillus subtilis* with standard concrete was increased by 3.46%.

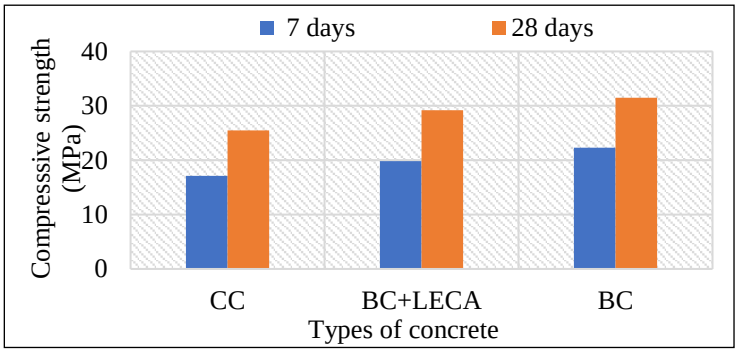


Figure 6: Compressive strength - types of concrete

Table 5: Different types of concrete's split tensile strength

Control Mix (M20)	Conventional concrete (CC)	Bacterial concrete with *Bacillus subtilis* and Lightweight aggregate (BC+LECA)	Bacterial concrete with *Bacillus subtilis* (BC)
	7 days Split Tensile Strength in MPa		
	1.83	1.94	2.03
	28 days Split Tensile Strength in MPa		
	2.77	2.89	2.99

Flexural Strength

A concrete beam's flexural strength was measured after seven and twenty-eight days of curing. Vertical fractures were generated by placing specimens horizontally. An initial failure was seen, and then the specimen was taken out of service and allowed to cure in water for self-healing analysis. Figure 8 shows the averages for the specimens. Based on the data in Table 6, *Bacillus subtilis*-infused bacteria had flexural strength superior to that of ordinary concrete and lightweight aggregate concrete. Flexural strength of *Bacillus subtilis* bacterium concrete is 7.10% higher at 28 days than regular concrete and *Bacillus subtilis* with lightweight aggregate (LECA) concrete.

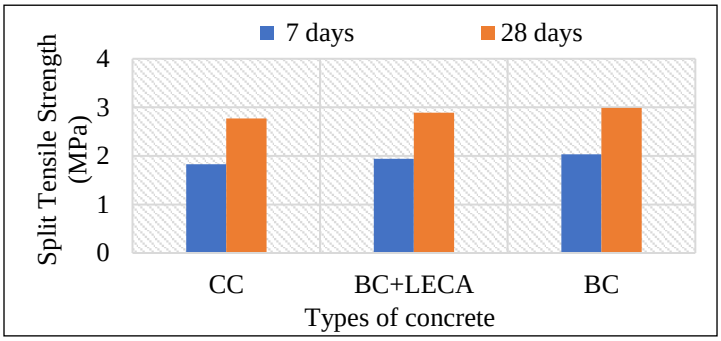


Figure 7: Split tensile strength - types of concrete

Table 6: Different types of concrete's flexural strength

Control Mix (M20)	Conventional concrete (CC)	Bacterial concrete with *Bacillus subtilis* and Lightweight aggregate (BC+LECA)	Bacterial concrete with *Bacillus subtilis* (BC)
	7 days Flexural Strength in MPa		
	2.14	2.23	2.38
	28 days Flexural Strength in MPa		
	3.24	3.32	3.47

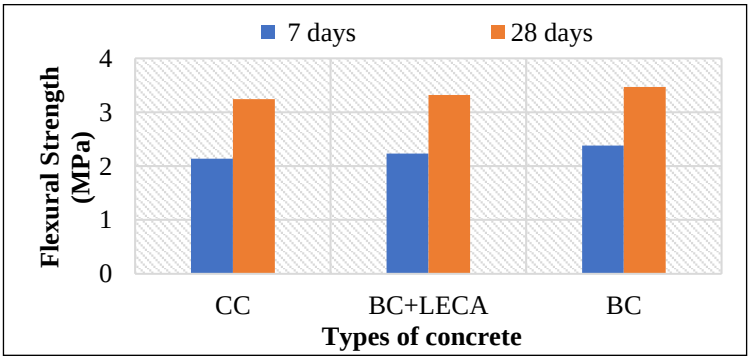


Figure 8: Flexural strength - types of concrete

CONCLUSION

The following conclusion has been obtained from the experiments; *Bacillus subtilis* preparation in the laboratory is safe and cost-efficient, and microbial concrete technology has shown to be more successful than regular concrete in terms of performance. When LECA is used as an aggregate, lightweight aggregate concrete (LWAC's) workability significantly improves. According to the supported research, *Bacillus subtilis* bacteria gathered for laboratory study may be gram-positive and capable of precipitating carbonate. In terms of compressive, split tensile, and flexural strength, the Bacterial concrete exhibited an increase in strength. There are promising results from both cultured bacteria and spore powder bacteria regarding concrete's self-healing.

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Conflict of interest

There is no conflict of interest between us.

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