

Effect of Bacillus Sphaericus Bacteria on the Calcined Clay Incorporated Cement Concrete

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ABSTRACT

The partial substitution of Ordinary Portland Cement (OPC) with Supplementary Cementitious Materials is the most efficient way to reduce CO₂ emissions in the worldwide cement production business (SCMs). Calcined clay can be considered one of the successful CO₂ emission reduction techniques to achieve this. Calcined clay is used as an SCM in this study to replace cement to varied degrees in the proportions of 10%, 15%, and 20%. The self-healing capabilities of concrete are also investigated using calcined clay and Bacillus Sphaericus bacteria with a bacterial population of 10⁸ cells/ml. Samples were evaluated for water absorption and compressive strength at 7, 14, and 28 days, respectively. When bacteria-embedded concrete is used in place of some calcined clay, the compressive strength of the concrete is raised by 21 percent, 24 percent, and 25 percent at 28 days. The bacteria in the calcined clay reduce the concrete's porosity and boost its compressive strength as a result, making concrete building more efficient and cost-effective.

Keywords: *Bacterial concrete; Cement concrete; Autogenous healing; Compressive strength.*

1.0 Introduction

The development of any nation in the modern world is based on concrete. The majority of concrete's parts are used in a variety of construction fields and are frequently required for creating constructions [1]. Because it continuously emits greenhouse gases into the atmosphere, cement-based concrete has a detrimental effect on the environment and jeopardises the ozone layer [2,3]. Due to its widespread availability, durability, and usability, it is also the most extensively used building material in the world. Regularly used cement concrete loses structural strength as it becomes older[4]. Such damaged concrete frequently exhibits microcracks, lower resistance, decreased mechanical strength, and dramatically reduced concrete durability [5]. The lesser resistance of the structures to climatic issues, which result in a significant loss of strength, is the reason of the increasing permeability in the structures [5,6]. Several studies employ supplementary cementitious materials, SCMs, or pozzolans, to address these problems. Pozzolans have been employed in earlier investigations, including fly ash, finely ground blast furnace slag, silica fumes, and rice husk ash [7,8]. Additionally, a cutting-edge method for lowering tolerated CO₂ levels and obtaining superior features is emerging: the use of calcined clay and bacterial cultures in cement concrete [9].

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When microorganisms are utilised to prepare the calcined clay for use in concrete, construction becomes cleaner, greener, and more sustainable. A sampling of the several bacteria cultures employed in concrete technology include *Bacillus subtilis* [10], *Bacillus megaterium* [11], and *Bacillus cereus* [5]. Siddique et al. [8] employed alkaliphilic bacteria at a concentration of 105 cfu/ml of water with silica fume as a partial replacement for 5%, 10%, and 15% of the cement to make cement concrete. Reports claim that silica fume inclusion is the most effective way to lessen the permeability of undesirable agents in concrete cells. The author claims that by forcing calcite to precipitate, silica fume and bacterial solution can assist lessen water absorption, capillary water rise, and porosity in concrete. Tziviloglou et al. examined the effects of the self-healing capabilities of concrete and mortar. They found that bacteria cultivated in an alkaline environment were far better able to withstand the elements. But the goal of the tests was to use fewer pore designs to still achieve the requisite lasting properties. However, the results were useful in achieving these goals.

Shashank et al. [13] searched outside the *Bacillus* family to locate novel colonies of calcite-precipitating bacteria that might endure in the alkaline environment of cement concrete. This study discovered that the new calcite bacterium increases the mechanical strength and durability of concrete. Priya et al. demonstrate in their publication [11] the experimental performance of micronized biomass silicon cement concrete with bacterial culture to evaluate the mechanical and durability properties of high strength concrete. The outcome demonstrates that the self-healing properties of concrete, as determined by SEM examination, were used to prevent crack formation in concrete specimens. During the preparation for mixing the concrete, micronized biomass silica and *Bacillus Sphaericus* with an ideal density of one were introduced.

Kadapure et al. [14,15] investigated the effects of various dosages of an alkaline bacterial culture coupled with fly ash as an extra cementitious material in the production of cement concrete. They discovered that using more bacterial culture and using it for longer periods of time results in concrete that is stronger and more durable than regular concrete. Karimi and Mostofinejad [16] produced a remedy for conventional concrete and presented their work using bar wire, steel, polypropylene fibre in volumes of 0.75 percent, 1 percent, and 0.3 percent, as well as the strain of *B. Subtilis* at a concentration of 107 cells/ml of water. Following curing in either plain water or a calcite lactate and urea solution, the sample was observed for 28, 56, and 90 days. The results demonstrate that these treatments were very successful in reducing the depth of CO₂ penetration and the intrusion of chloride ions. Achal et al. [17] used *B. Megaterium* and fly ash with mortar concrete to demonstrate the microbial calcite precipitation uptake in order to assess compressive strength, water absorption, and water impermeability. The author found that fly ash pozzolana and *B. megaterium* improved mechanical characterization and durability properties. In this work, cement concrete is made using *Bacillus Sphaericus* bacteria and calcined clay instead of ordinary Portland cement. It is hoped that the bacterially calcined clay concrete will have the appropriate mechanical properties and reduce environmental pressure by minimising the continuous release of CO₂. Additionally, the inclusion of calcined clay containing *B. Sphaericus* bacteria was investigated by contrasting the compressive strength of such concrete to conventional and pozzolanic concrete.

2.0 Material Used

2.1 Cement

In the current experiment, bacteria-embedded cement concrete was prepared in accordance with IS: 8112-2013 [18] using 43 grade OPC with a specific gravity of 3.12 and initial and final setting periods of 62 and 281 minutes, respectively. The fineness of cement was 6.45 percent.

2.2 Calcined clay

The process of turning the raw material into cement requires a number of operational units, which consumes a lot of energy and pollutes the environment. Continuous CO₂ emissions are therefore harmful to the ecosystem. Calcined clay has the potential to be a practical method for reducing greenhouse gas emissions. In the current study, calcined clay was used as pozzolans to produce durable concrete properties, increase mechanical properties, and minimise the amount of cement required in construction. It was finer than cement particles and had a specific gravity of 2.64 and a fineness modulus of 2.21 percent. The chemical makes-up of cement and calcined clay are shown in Table 1.

Table 1: Chemical Composition of OPC and Calcined Clay

| Compounds | OPC (%) | Calcined Clay (%) |
|--------------------------------|---------|-------------------|
| SiO ₂ | 27.31 | 48.13 |
| Al ₂ O ₃ | 8.42 | 33.03 |
| CaO | 44.31 | 0.65 |
| Fe ₂ O ₃ | 4.90 | 1.68 |
| MgO | 1.09 | 0.70 |
| Na ₂ O | 0.15 | 0.15 |
| K ₂ O | 0.04 | 0.12 |
| LOI | 2.60 | 14.37 |

2.3 Fine aggregates

In the current investigation, angular natural sand from a river with a specific gravity of 2.63 in accordance with IS383: 2016 [19] was utilised as fine aggregate in the preparation of concrete.

2.4 Coarse aggregates

In this experiment, volumes of 10 mm and 20 mm in proportions of 40% and 60% of the total coarse aggregate were used. The used coarse particles were subangular in shape and had a clean surface. The specifications for coarse aggregate met with IS 383 [19].

2.5 Super plasticizer

Conmix SP 1030 super-plasticizer was used to provide the same workability and maintain the flowability of fresh concrete. Sulphonated naphthalene formaldehyde, the main ingredient of the super-plasticizer, has a specific gravity of 1.2 at room temperature, which enables it to rapidly reduce the amount of water. Prior to use in the preparation of concrete, the super-quantity plasticizer's and quality should be checked and evaluated.

2.6 *Bacillus Sphaericus* bacteria

The *Bacillus Sphaericus* bacteria (297) strain was used as a microbiological agent to fill the microscopic pores and fissures in cement concrete. The dried, freeze-powder version of the bacterial strain was bought from IMTECH MTCC Chandigarh. In the top layer of soil, where rod-shaped, gram-positive, aerobic, and calcifying bacteria were already present, the *B. Sphaericus* bacteria first appeared. By keeping the culture's pH between 7.2 and 0.2 and incubating it at 37 C in a shaker incubator, this bacteria was created in liquid form. By passing a wavelength of 600 nm between a neutral medium and a bacterial culture using a spectrophotometer, the McFarland method and the

ideal density value were used to calculate the number of bacteria per millilitre. The growth of a bacterial culture on an agar plate is shown in Figure 1.

Figure 1: Growth of B. Sphaericus on Agar Plate



2.7 Bacterial nutrient

Nutrient broth is created in a liquid form using 100 ml of triple-distilled water, 0.5 g of peptone, 0.5 g of sodium chloride, and 0.3 g of beef extract to feed the bacteria. This nutrient broth (CAS number M002-500G) was used to develop bacterial cultures and was added to the concrete mix during casting as a bacterial nutrient.

3.0 Methodology

3.1 Preparation of bacterial culture

Bacillus Sphaericus bacteria are used in the current investigation to lower the porosity of concrete by precipitating calcite into the concrete void. This bacterium was purchased from IMTECH MTCC Chandigarh in a freeze-dried state for usage in concrete to transform dry freeze bacteria into liquid form concentration of bacteria. Subsequently, nutritious broth was used to inoculate bacillus sphaericus bacteria into conical flasks, which were then kept at 4 C to prevent contamination from the environment. After 24 hours, bacterial sticking will be performed on the agar plate and petri dish in order to use them for additional bacterial culture growth. This bacterial culture in a conical flask was kept at 37 C in a shaker incubator for the ensuing 24 hours, which allowed the bacterial growth to be finished. Then, using a method developed by McFarland, take a sample of 5 ml of bacterial media in culture tubes to count the number of bacterial cells per ml. Then, using spectrophotometer equipment at a wavelength of 600 nm passed between the bacterial media and neutral media of interest, calculate the optimum density (OD). The bacteria at this concentration were prepared to be diluted in water at the time the concrete was being cast, so after that, inject broth media into a culture tube.

3.2 Testing methods

Samples of size (150 mm x 150 mm x 150 mm) were evaluated for compressive strength at 7, 14, and 28 days by taking an average of three samples at a loading rate of 3 kN/s in a compression testing machine. The specimens' compressive strength was assessed in accordance with IS 456: 2000 [20]. A specimen measuring 150 mm 150 mm was tested for water absorption 14 and 28 days after demolding. The following day, these cubes were dried in an oven for 24 hours at 105°C. After being immersed in water for the following 24 hours at a temperature of 21 C, the weight of these oven-dried cubes was now recorded as W1, and the weight of the identical cubes was again noted as W2. The water absorption value was calculated using the empirical Eq. (1) [19].

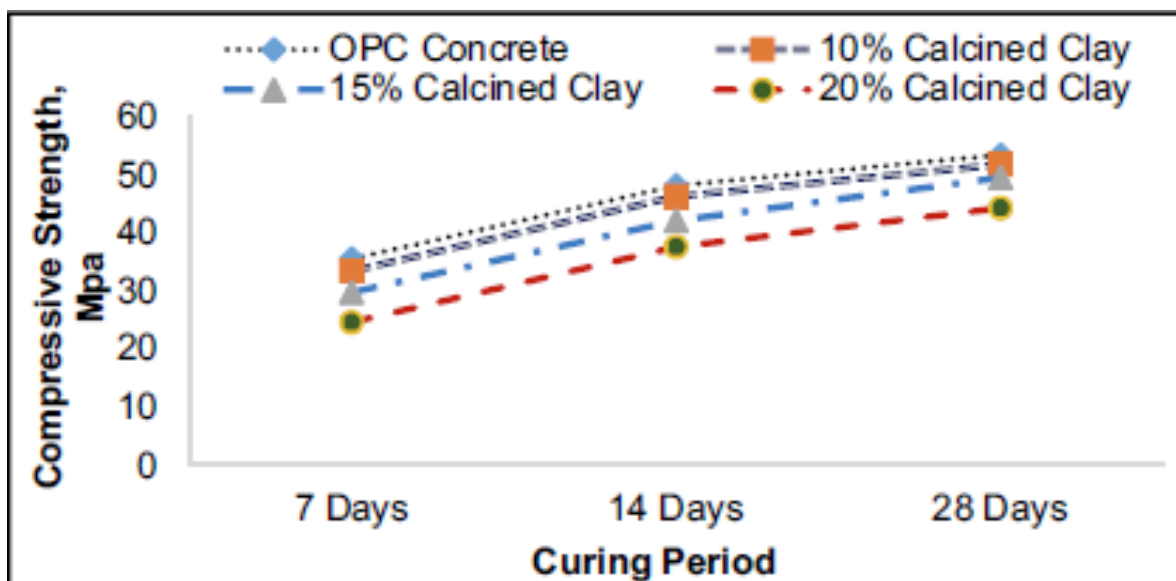
$$\text{Water Absorption} = \frac{(W_2 - W_1)}{W_1} * 100(\%) \quad \dots (1)$$

4.0 Result and Discussion

4.1 Effect on compressive strength

In this investigation, it was discovered that concrete with embedded bacteria and concrete specimens with some of the cement replaced with calcined clay had increased strength. Cement was replaced with calcined clay in percentages of 10%, 15%, and 20%, respectively. As replaced calcined clay in fixed proportions, the compressive strength fell. Calcined clay was used in place of 10% of the cement in regular concrete, which resulted in a 5.9%, 3.88%, and 3.17% drop in compressive strength at 7, 14, and 28 days, respectively. In line with cement concrete, calcined clay concrete achieved reductions of 16.5%, 12.5%, and 7.38% for a 15 percent replacement of calcined clay while attaining reductions of 30.75%, 21.88%, and 17.17% for a 20 percent replacement at 7, 14, and 28 days, respectively. The compressive strength of OPC concrete and calcined clay concrete are shown in Fig. 2. It is clear from this that increasing the amount of calcined clay in cement reduces its compressive strength.

Figure 2: Compressive Strength of OPC and Calcined Clay Concrete



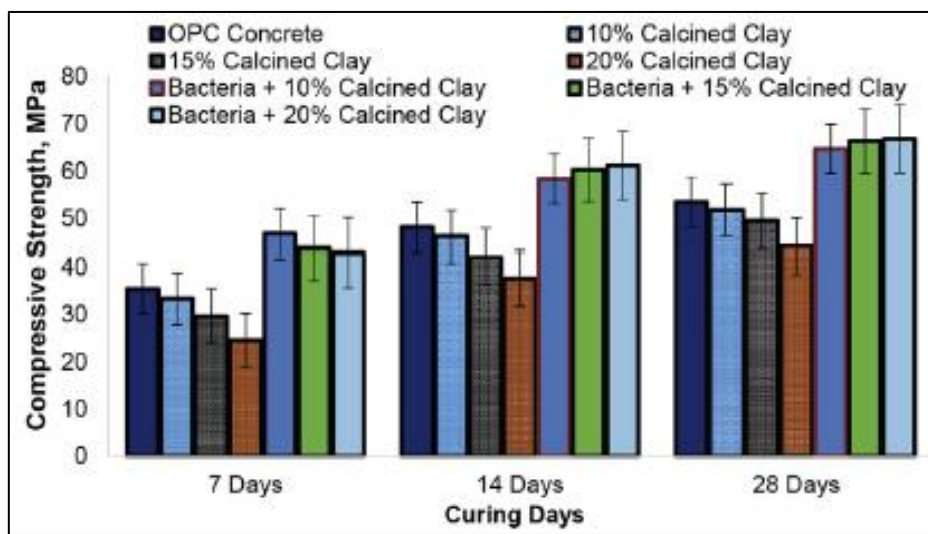
Additionally, bacterial culture was added to the concrete made using pozzolanic cement. It has been demonstrated that adding liquid *B. Sphaericus* bacteria, produced by diverse biological processes, can improve concrete's compressive strength. Bacterial development inside the concrete structure causes the vacant pore matrixes to become calcified. Fig. 3 shows the commencement of calcite precipitation over a 28-day period on the outside surface of calcined concrete.

Figure 3: Precipitation of Calcite on the Surface of CC Concrete



After curing for 7, 14, and 28 days, this concrete, when compared to standard cement concrete, assists in achieving compressive strengths of 31.76 percent, 17.52 percent, and 21 percent for 10 percent calcined clay replacement, 27 percent, 25.19 percent, and 24 percent for 15 percent calcined clay replacement, and 20.84 percent, 27.10 percent, and 24 percent for 20 percent calcined clay replacement. Comparing the compressive strengths of OPC, bacterial, and calcined clay concrete is shown in Fig. 4.

Figure 4: Analysis of Compressive Strength of OPC, *B. Sphaericus* & Calcined Clay



4.2 Water absorption

A water absorption test was done to figure out how much water would get into the concrete structure over the duration of 14 and 28 days. All samples were oven dried before being immersed in the water for the necessary 24 hours. It has been proven through experimentation that concrete's capacity to absorb water is reduced by adding additional calcined clay. As a result, reductions in water absorption of 12.34% and 16.84% for 10% replacement, 8.11% and 8.42% for 15% replacement, and 3.19% and 5.71% for 20% calcined clay replacement were achieved for 14 and 28 days, respectively, compared to ordinary OPC concrete.

In bacteria-embedded concrete, 10% substitution of calcined clay results in reductions of water absorption of 14.63% and 30.97%, 15% substitution results in reductions of 15.55% and 14.52%, and 20% substitution of calcined clay results in reductions of 10.10% and 11.14% at 14, and 28 days, respectively, as compared to conventional concrete specimen. Figure 5 shows how bacteria embedded in calcined clay concrete, OPC, and other materials absorb water.

5.0 Conclusions

The investigation backs up the results that are detailed below:

- For all combinations with the exception of concrete containing embedded bacteria, increasing the proportion of calcined clay reduces compressive strength.
- The introduction of bacteria significantly boosts the compressive strength by forcing calcite to precipitate in between aggregate pores.
- Calcined clay concrete absorbs less water than conventional concrete because the pores are filled with fine calcined clay.
- The ability of calcined clay concrete containing *B. sphaericus* bacteria to absorb water was greatly diminished as a result of the usage of smaller particles and the precipitation of calcite at the outer surface.
- The bacterial concrete worked admirably in accomplishing the structure's increased strength and decreased permeability when compared to conventional concrete.

References

- [1] IS 456, Plain and reinforced concrete- Code of practice, Bur. Indian Stand. Dehli, pp. 1–114, 2000.
- [2] T.S. Ng, Y.L. Voo, S.J. Foster, Innovative materials and techniques in concrete construction, *Innov. Mater. Tech. Concr. Constr.* (2012) 81–100.
- [3] M. Rubenstein, Emissions from the Cement Industry, *State of the planet.* pp. 7–11, 2012.
- [4] IS383, Coarse and fine aggregate for concrete 2016 New Delhi, India.
- [5] S. Mondal, P. Das, A. Kumar, Application of bacteria in concrete, *Mater. Today Proc.* 4 (9) (2017) 9833–9836.

- [6] P.A.M. Basheer, S.E. Chidiac, A.E. Long, Predictive models for deterioration of concrete structures, *Constr. Build. Mater.* 10 (1) (1996) 27–37.
- [7] J. Alex, J. Dhanalakshmi, B. Ambedkar, Experimental investigation on rice husk ash as cement replacement on concrete production, *Constr. Build. Mater.* 127 (2016) 353–362.
- [8] R. Siddique, A. Jameel, M. Singh, D. Barnat-hunek, A. Ait-mokhtar, Effect of bacteria on strength, permeation characteristics and micro-structure of silica fume concrete, *Constr. Build. Mater.* 142 (2017) 92–100.
- [9] K. Scrivener, F. Martirena, S. Bishnoi, S. Maity, Calcined clay limestone cements (CALCINED CLAY), *Cem. Concr. Res.*, vol. 114, no. March 2017, pp. 49–56, 2018.
- [10] M.R. Prakash, S.M. Gopinath, Original Research Paper Commerce Engineering Studies on Strength Characteristics of Bacillus Subtilus Induced Self Healing Bacterial Concrete Research Scholar (5VX15PCJ11), Visvesvaraya Technological of Technology , Bangalore 560107, India Professor & H,” no. 11, pp. 509–511, 2016.
- [11] T. Shanmuga Priya, N. Ramesh, A. Agarwal, S. Bhusnur, K. Chaudhary, Strength and durability characteristics of concrete made by micronized biomass silica and Bacteria-Bacillus sphaericus, *Constr. Build. Mater.* 226 (2019) 827–838.
- [12] E. Tziviloglou, K. Van, Tittelboom, D. Palin, J. Wang, Bio-based self-healing concrete: from research to field Application, *Adv Polym Sci* (2016) 345–383.
- [13] B.S. Shashank, B. Dhannur, H.N. Ravishankar, P.S. Nagaraj, Study on development of strength properties of bio-concrete, in: B.B. Das, N. Neithalath (Eds.), *Sustainable Construction and Building Materials*, Springer Singapore, 2019, pp. 423–437.
- [14] [14] S.A. Kadapure, G. Kulkarni, K. Prakash, P.S. Kadapure, Mechanical and durability performance of sustainable bacteria blended fly ash concrete: an experimental study, *Int. J. Sustain. Eng.* 00 (00) (2019) 1–9.
- [15] S.A. Kadapure, G.S. Kulkarni, K.B. Prakash, Study on properties of bacteria embedded fly ash concrete, *Asian J. Civ. Eng.* 20 (5) (2019) 627–636.
- [16] N. Karimi, D. Mostofinejad, Bacillus subtilis bacteria used in fiber reinforced concrete and their effects on concrete penetrability, *Constr. Build. Mater.* 230 (2020) 117051.
- [17] V. Achal, X. Pan, N. Özyurt, Improved strength and durability of fly ash amended concrete by microbial calcite precipitation, *Ecol. Eng.* 37 (4) (2011) 554–559.
- [18] IS:8112, Ordinary Portland Cement, 43 Grade – Specification, New Delhi, India, 2013.