

An Experimental study on Performance of Bacterial Concrete

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Abstract

Concrete is a major aspect on which strength, durability of structure and economy depends. Development of cracks is common and frequent phenomenon because of freeze-thaw reaction, shrinkage, mechanical compression and tensile force. For sustainable concrete it is necessary to increase strength and durability of concrete. Maintenance and repairing of cracks tends to have deliberate working process, uneconomical and critical solutions. Cracking of the concrete surface may enhance the deterioration of embedded steel bars as ingress rate of corrosive chemicals such as water and chloride ions into the concrete structure increased due to exposure.

This paper presents the experimental investigation for bacterial concrete using *Bacillus Subtilis*. Bacterial concrete is a concept in which bacteria is induced in concrete or mortar while mixing and these bacteria assist concrete to heal cracks by segregating lime. The experiments were carried out on M25 grade of concrete to evaluate the effect of *Bacillus Subtilis* on the compressive strength, Flexural strength test 7, 28 and 84 days. The Plate count test was conducted to determine the presence of viable cells in a bacterial culture by plate count method. The analysis shows that there is presence of self healing compound in cracked concrete. The comparative study of normal concrete, concrete with direct insertion of bacteria and indirect insertion of bacteria has been done. The experimental results show that by insertion of bacteria it not only heals cracks but also increases compressive strength of concrete. Bacterial concrete is more preferable for underwater construction activities, as it heals the cracks by microbial activity.

Keywords— *Bacillus Subtilis*, Bacterial Concrete, Compressive test, Plate count test

I. INTRODUCTION

Concrete plays an important role in construction industry. Strength and durability of concrete are most important parameter to determine the life and serviceability of the structure. Bacterial concrete is the special type of concrete it has the ability to repair itself autonomously. A composite material concrete consists of coarse aggregate and fine aggregate bonded together with fluid cement that hardens over time. When aggregates are mixed together with dry Portland cement and water, the mixture forms fluid slurry that is easily poured and moulded into shape. The cement reacts chemically with the water and other ingredients to form a hard matrix that binds the materials collectively into a durable stone-like material. Concrete has high compressive strength but very low tensile strength due to which it fails in tension. Therefore to overcome the tensile failure various techniques are being used such as providing steel reinforcement, various fibers as reinforcement or prestressing of concrete. Using these techniques overall strength of concrete is increased but due to the various factors like weathering, aging, asymmetric load, etc. degradation of structure takes place which leads to formation of cracks. Cracks are very harmful to structure because of various chemicals can insert through this cracks damaging the concrete and reinforcement from inside which results in loss of strength and failure of the structures. Failure of structure may causes lot of economical as well as life loss. Finding and repair of these cracks is essential but very difficult and time consuming job. So to overcome these problem bacterial concrete is one of the best solution. As self-healing property of bacterial concrete can repair cracks automatically without any human intervention. The bacteria present in these concrete secrets lime when it gets activated which repair the crack automatically. This reduces time and cost of finding and repairing the cracks manually. The corrective Process is Bacterial Concrete or Self-Healing Concrete. Bacterial concrete is a material, which can effectively remediate cracks in concrete. Bacterial concrete proves to be a better alternative to all conventional processes.

The Objective of the study is, to develop a bacterial concrete by introducing the bacteria of bacillus family (*Bacillus subtilis*). To determine better method for insertion of bacteria i.e. Direct insertion or indirect insertion by forming diatomite pellet capsule coated with cement paste.

II. EXPERIMENTAL INVESTIGATION

The Preliminary investigation is required to be carried out for selection and preparation of bacteria as it takes time and experimental work. Cultivation of bacteria is an important procedure after selection of suitable bacteria type for the study. Experimental procedure is to be carried out for cultural growth of bacteria. As per relevant IS Code calculation of mix proportion of cement, sand and aggregate in concrete along with water and bacterial content. Mixing of bacteria with concrete has done by Direct and Indirect insertion methods to identify the best method. To determine total viable cells in a bacterial culture Plate Count test was conducted.

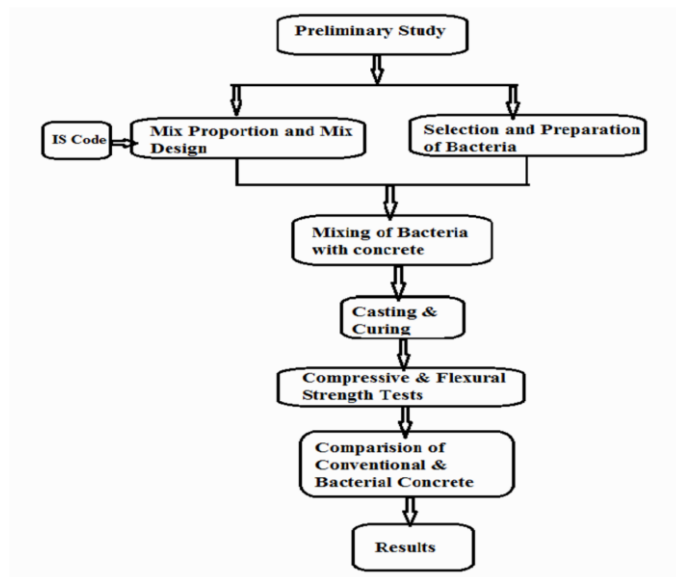


Figure 1: Methodology of the study

A. SELECTION OF BACTERIA

There are various types of bacterias that can be used in the concrete such as *Bacillus Subtilis*, *Bacillus Pasteurii*, *Bacillus Cohnii*, *Bacillus Licheniformis* etc. The selected Bacteria is *Bacillus Subtilis* since this bacteria produce calcium carbonate and due to ease of availability from National Chemical Laboratory (NCL). It is also formally known as Hay bacillus or grass bacillus, is a Gram- positive, catalane-positive bacterium found in soil and gastrointestinal trap of remints and humans. A member of the genus bacillus, *B. subtilis* is rod shaped and can form tough protective endo-spores allowing it to tolerate extreme environmental conditions. *Bacillus Subtilis* has historically been classified as an obligate aerobe, through evidence exist that is a facultative aerobe. *Bacillus Subtilis* is considered the best gram positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. Various studies shows that *Bacillus Subtilis* proves to be an better alternative to achieve greater strength and cracks healing. The selected bacteria is having Ph 12.0 at 30 degree temperature and subculturing period is 3 to 6 months, source of isolation is lonar lake soil sample.

B. CULTIVATION OF BACTERIA

The pure culture of bacteria that is *bacillus subtilis* preserved on nutrient agar slants. It forms irregular dry white colonies on nutrient agar slants. Two colonies of bacteria are inoculated into nutrient broth of 250 ml in 750 ml conical flask and incubated at temp of 37 degree C and 150 rpm orbital shaker incubator. The medium composition used for growth of bacterial culture of peptone, Nacl, beef extract.

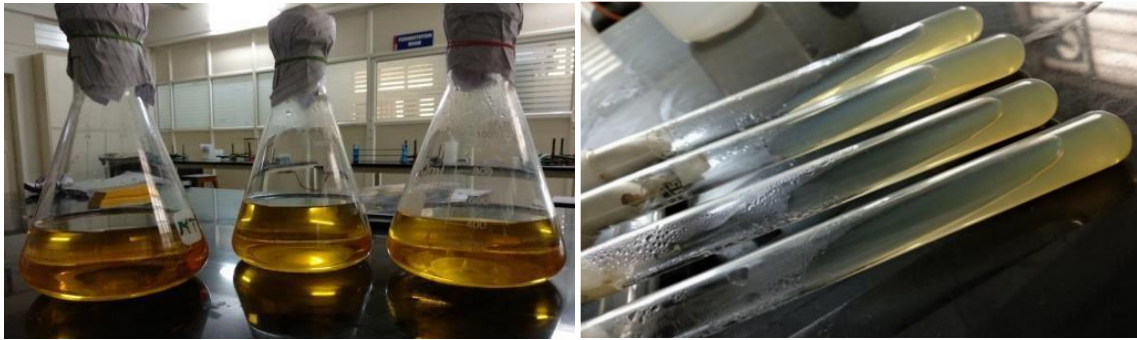


Figure 2: Bacteria Culture Liquid Media and Bacteria Sub Culture

C. EXPERIMENTAL PROCEDURE FOR CULTURAL GROWTH OF BACTERIA

Bacillus Subtilis MCC 2183.B subtilis is a common soil bacterium, which can produce calcite precipitates on suitable media supplemented with a calcium source. The bacteria were cultured in a liquid medium according to the suppliers recommendations. The medium used to grow bacteria consisted of 10gm peptone, 10gm beef extract, 5gm NaCl per lit of distilled water. For solid medium 2% of agar was added to the same. The mixture was first sterilized by autoclaving for 20 mins at 121 degree C, allowed to cool down at room temperature (25 degree C). The whole 9 culturing process was performed under sterile condition. Then the cultures were incubated at 37 degree C on a shaker incubator at 150 rmp for 72 hrs. Bacterial count has been taken by using digital calorimeter. The amount of light absorbed by the sample is displayed on the screen that data can be use to calculate the number of bacterial present in that solution.

Procedure: • Prepare standard nutrient broth for setting of the blank reading in digital colorimeter. • Take 1 ml of the nutrient broth using micropipette and set the blank reading on digital colorimeter. • After setting the blank reading, take the respective bacterial sample for bacterial count of different concentrations. • From the bacterial sample, take 1 ml in the test tube and keep it in the digital colorimeter. • The reading which is displayed is multiplied by a standard value to get the actual bacterial count. For bacillus subtilis $600\text{nm} = 1 = 4 \times 10^8 \text{ cells/ml}$.



Figure 3: Digital Calorimeter

D. DIRECT METHOD

From study direct method is adopted in which, firstly the measuring jars were sterilized in oven for a temperature of about 1000 C for 5 min. After 5 min once it gets considerably cooled, the bacterial solution is poured from the flask in the measuring jar. The flask is firstly heated under the candle before pouring it into the jar, so that the bacterium doesn't get contaminated by the other bacteria's present in the environment. 45ml of bacterial solution for each concrete block is to be adopted. Once the bacterial solution is mixed with water, the water is properly stirred and then used for immersion in the concrete.

E. INDIRECT METHOD

Bacterial solution were prepared from urea, CaCl₂, and *Bacillus subtilis* HU58 (109 cfu/g) with proportional mixture. The resource of natural diatomaceous earth Lam Dong was also used in this study. Such diatomite had a moderate grade with silica content about 70 wt. % in the form of silica gels and

20 wt% alumino-silicate compounds. Homogeneous mixture of bacterial solution and dry diatomite powder could be obtained after 10 minutes in laboratory planetary mixer. Taking into account the presence of clay content, plastic forming technique to granulate the mixture in pellet capsule 10 mm diameter, 10 mm height is adopted. The ratio of bacterial solution/solid powder was fixed at 1.26 wt these pellet capsules were then coated with cement paste (w/c 0.5) and placed for drying in dry air before using as normal aggregate component of concrete.

III. RESULT AND DISCUSSION

For this study M25 grade of concrete has been used. The cement used for this study is 53 grade ordinary Portland cement. Well graded sand with bulk density of 1.75 kg/cum was used. 10 mm and 20 mm aggregates with bulk density 1.56 and 1.57 were used. 200 gm of fly ash with fineness 18% was used. The mix proportion adopted was 1:3.12:3.25. Admixtures i.e. super plasticizer 13gm was used. Cubes of 150x150x150mm and beams of 700x150x150mm were used for comparison purpose. Bacteria used are Bacillus subtilis and method of insertion is direct and indirect methods. Compressive strength test has been conducted on Compressive testing machine for 7, 28 and 84 days. Compressive strength comparison between conventional and bacterial concrete is shown in figure 4. Table 1 shows the results obtained for compressive strength for 7, 28 and 84 days for the concrete specimens with bacterial cell concentrations of 10^4 cells/ml, 10^5 cells/ml, and 10^6 cells/ml. Flexural Strength test was conducted on universal testing machine for 28 days and 84 days. The flexural Strength comparison between conventional and bacterial concrete is as shown in figure 6. Table 2 shows the results obtained for flexural strength for 28 and 84 days for the concrete specimens with bacterial cell concentrations of 10^4 cells/ml, 10^5 cells/ml, and 10^6 cells/ml. The process of self healing of cracks in 28 days is shown in figure 5. The study gives emphasis on comparison of compressive strength and flexural strength for conventional and bacterial concrete.

Table 1: Compressive Strength Test Results

Days	Conventional	10^6 cells/ml	10^5 cells/ml	10^4 cells/ml
7 days	22.31	18.8	21.7	19.96
28 days	31.5	30.4	30.33	29.03
84 Days	37.5	34.22	41.9	25.55

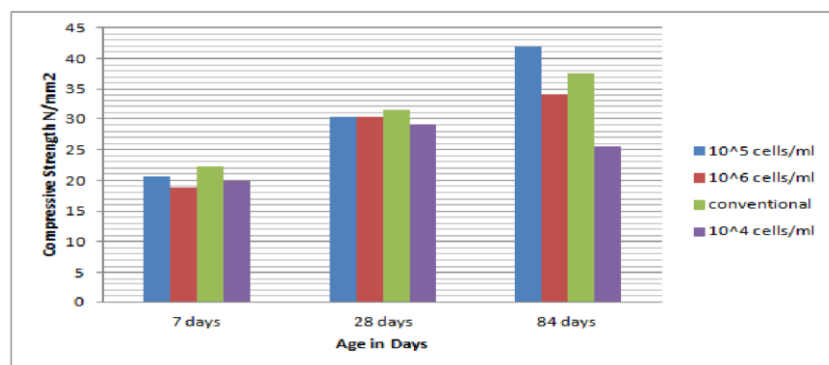


Figure 4: Compressive strength comparison between conventional and bacterial concrete

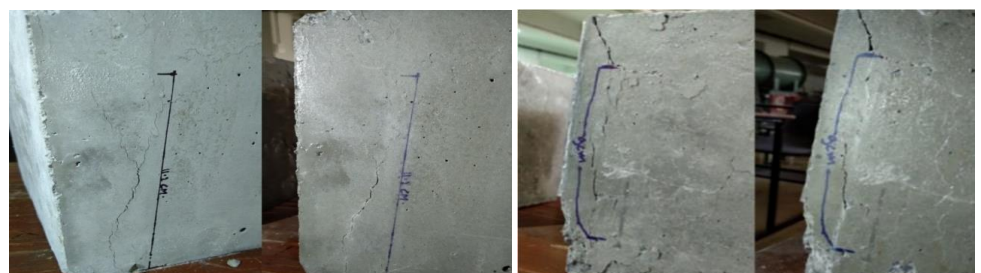


Figure 5: Healing Process in 28 days

Table 2: Flexural Strength test Results

Sr No	Batch	28 days	84 days
1	B1	29.86	37.6
2	B2	30.24	37.6
3	B3	33.70	40.00
4	Conventional	28.8	-

B1= cell concentration 10^4 cell/ml

B2= cell concentration 10^5 cell/ml

B3= cell concentration 10^6 cell/ml

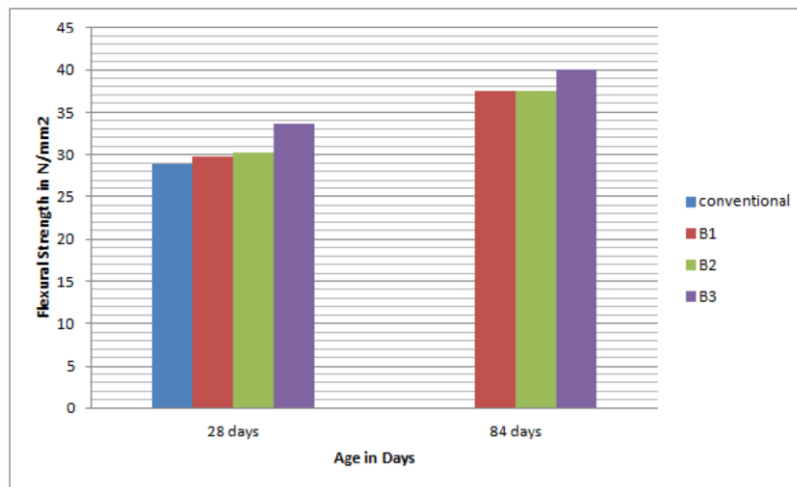


Figure 6: Flexural Strength comparison between conventional and bacterial concrete

PLATE COUNT TEST

The plate count test was conducted to determine total viable cells in a bacterial culture. This method are used for determination of the number of cells. It requires solid culture of bacillus subtilis. Further the media taken is 20ml nutrient broth, pipettes, petri plates, glass marking pencil and spreader. The plate count method is most commonly used for enumeration of viable cells in water, milk, food, and many other pharmaceutical substances. All organisms cultivate, reproducing a visible mass of microorganism called colony. After testing the bacterial concrete cubes in CTM machine, small part of all the samples were tested result shows the formation of visible mass. The development of one colony from one microorganism can occur when the bacterial suspension is homogenous. If microorganism have a affinity to aggregate (e.g-Staphylococci, streptococci, diplococcic) that resulting counts will be lower than the actual no. of individual cells. Therefore, counts of microorganism are often reported as colony forming units/ml rather than no. of bacteria/ml. the original sample is usually diluted so that the no of colonies developing on the plate will be in the range of 30-300. Within this range the count can be accurate and the possibility of mixing of the growth of one organism with other is minimized. The total count of microbial suspension is obtained by multiplying the no. of cells per plate by the dilution factor. 1 g of concrete material from concrete block which was kept for curing for 14 days, from different concrete block collected to study number of viable bacteria by serial dilution method.

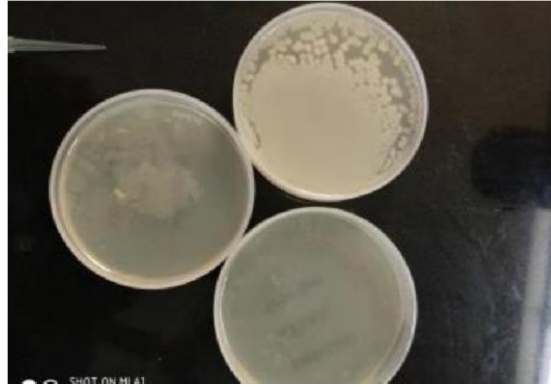


Figure 7: Plate Count Test

Table 3: Bacterial Count in Plate Count Test

	Flask 1	Flask2	Flask3
600nm	0.27	0.22	0.17
540nm	0.59	0.52	0.46

- Cell concentration of Flask 1= 1.08×10^8 cells/ml
- Cell concentration of Flask 2= 8.0×10^7 cells/ml
- Cell concentration of Flask 3= 6.8×10^7 cells/ml

III. CONCLUSIONS

The experimental study shows that the addition of bacteria *Bacillus Subtilis* in concrete shows improvement in various properties of concrete. By evaluating the results it is observed that the strength of bacterial concrete is more than conventional concrete. Bacterial concrete of 10^5 cell/ml shows the maximum strength gain. In bacterial concrete cracks are not only healed but strength also regained after retesting the same specimens. The bacterial concrete is proved to be an economical alternative to achieve more strength, ultimately the repair and maintenance cost of project minimizes. To determine total viable cells in a bacterial culture Plate Count test proves an important tool. Method of direct insertion of bacteria is more economical than indirect insertion of bacteria. It consequently requires appropriate attention in order to make a more sustainable infrastructure to progress towards a more eco friendly construction practices.

ACKNOWLEDGMENT

The authors express their deep gratitude to Department of Civil Engineering RMD Sinhgad School of Engineering Warje Pune for the technical support and valuable guidance for this research.

REFERENCES

- 1 Abhijitsinh Parmar, Ankit Patel, Priyank Shah” Improvement of the Concrete Cracks by using *Bacillus Sphaericus*”, International Journal of Engineering Development and Research 2013, pp-82-84.
- 2 Jonkers, H., 'Self healing concrete: a biological approach', in S. vander Zwaag (ed.) 'Self Healing Materials: An alternative approach to 20 centuries of materials science' (Springer, Dordrecht, 2007) 195-204
- 3 J.Wang, D.Snoeck, S.Van Vlierberghe, W. Verstraete and N.De.Belie, ”X-ray Computed tomography proof of bacterial based selfhealing in concrete”, Cement and Concrete Composites, no. 53, pp289-304, 2014.
- 4 K. Keerthana, A. Ranjani, N. K. Amudhavalli, “Experimental Study on Bacterial Concrete”, International Journal of Scientific Engineering and applied science, Volume 1, Issue 8 pp 456-458, November 2015.

- 5 Manas Sarkar, Nurul Alam, Biswadeep Chaudhuri, Brajadulal Chattopadhyay and Saroj Mandal, “Development of an improved e. Coli bacterial strain for green and sustainable concrete technology” Royal society of chemistry RSC Advances., 2015.
- 6 Medapati Abhinav Reddy, “Temperature Effect on Various Bacteria Used in Microbial Concrete”, International Journal of Innovative Research in Science, Engineering and Technology, Vol. 5, Issue 4, April 2016.
- 7 Meera C. M., Dr. Subha V , “Strength and durability assessment of bacteria based self-healing concrete” IOSR Journal of Mechanical and Civil Engineering (IOSR-JMCE)”,pp 01—07 ICETEM-2016.
- 8 Salmabanu Luhar, Suthar Gourav, “A review paper on self-healing concrete” , Journal of Civil Engineering Research 2015, pp 53-58.
- 9 S.K.Ramachandran,V.Ramakrishnan and S.S.Bang, ”Remediation of Concrete using Microorganisms”, ACI Materials Journal, JanuaryFebruary pp 3-9 2001.
- 10 V srinivasa Reddy, M V seshagiri rao, S sushma, “Feasibility Study on Bacterial Concrete as an innovative self crack healing system” ,International journal of modern trends in engineering and research pp 642-647, 2-4 July, 2015.

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