AN EXPERIMENTAL INVESTIGATION ON BACILLUS SUBTILIS IN HEALING MICRO-CRACKS IN CONCRETE

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ABSTRACT

Concrete is the most widely used material in the entire world, based on the quantity of usage. Concrete also has a certain amount of limitations. They mainly include crack formation over a period of time and further by extension, an adverse effect in its strength, permeability and durability. Cracks may not be visible to the eye but can be accounted for an initiation in the deterioration of reinforcements. Bacteria belonging to the bacilli family that aid in improving the condition of the concrete by precipitation of calcium carbonate which is used to fill the cracks, by the process of M.I.C.P. In this study, M30 grade bio concrete was prepared and was tested for healing cracks successfully. The hardened state characteristics include Compressive Strength, split tensile strengths, durability aspects such as R.C.P.T and Water Absorption. The results show that the replacement of water with bacterial solution gives additional strength to the concrete. The cube compressive strength of concrete at the age of 28 days has been increased by 31.2% .There is a 19.2% increase in the split tensile strength. Water absorption tests reveal that there is a 27.2% decrease in the water absorption of concrete.

Key words: B*acilli, calcium carbonate, M.I.C.P, M 30, Bio concrete, R.C.P.T.*

1. INTRODUCTION

Crack formation in concrete is a common phenomenon mainly due to the relatively low tensile strength. Without immediate and proper perfected treatment, the micro-cracks and major cracks tend to expand further and eventually require costly repair. If micro-cracks grow and reach the reinforcement, not only the concrete itself may be attacked, but also the reinforcement will be corroded when it is exposed to moisture, water, oxygen, possibly carbon dioxide and chlorides. Micro-cracks are therefore precursors to structural failure and are henceforth futile for the life of a concrete structure. They observed that there was increase in compressive strength and tensile strength when compared with controlled specimen. Surface treatment with bacteria resulted in decrease in water absorption and increases the resistance to water and hazard material penetration [1]. This biological surface treatment shows promising prospect for increasing durability aspects of concrete mortar. Cracks in concrete should be redeemed in as many possible ways as possible [2]. The most used methods for the redemption of cracks in concrete are using epoxy based fillers to fill cracks that have arisen in concrete and usage of polyvinyl acetate and various other polymeric complex compounds [3]. Among these remedies of crack control and redemption of cracks that occur in concrete, they account to the following side effects namely different thermal expansion coefficients compared to concrete, weak bonding to the concrete complex, environmental & health hazards, high cost of the polymers/chemicals; and various other drawbacks. Analogous to the bacteria that help in the daily natural processes of natural phenomenon, there is a potential solution to the above mentioned problem of cracking phenomenon in the concrete matrix by the means of some selected innocuous micro-organisms species belonging to the bacilli family that aid in improving the condition of the concrete and assisting in the process of crack redemption [5]. The bacteria

to be used as self- healing agent in concrete should be fit for the job, i.e. they should be able to perform long-term effective crack sealing, preferably during the total constructions life time [4], [5], [6]. The various types of the micro-organisms that belong to the gene of "*bacillus*" are *bacillus pasteurii, bacillus sphaericus, escherichia coli, bacillus subtilis, bacillus cohnii, bacillus balodurans, bacillus pseudofirmus* and others [7], [8], [9].
The prime reason for the short listing these species of bacteria is their ability of precipitation of calcium carbonate which is an integral part of the concrete matrix and allows the complete occupancy within the concrete matrix [10]. The newly produced calcium carbonate-based mineral precipitates should then act as a type of bio-cement what effectively heals newly formed cracks. The pH value of concrete is very high about 12, hence the bacteria will have the ability to maintain pH of concrete and thrive in it [11]. This could be particularly useful in earthquake zones where hundreds of buildings have to be flattened because there is currently no easy way of repairing the cracks and making them structurally sound [12].

2. MATERIALS AND METHODS

2.1. Cement

The cement used in the making of conventional concrete and bio concrete tests samples are Bharathi Cement of 53 grade O.P.C. This cement satisfies the basic requirements as per IS: 12269 – 1987.

Table 1 Properties of cement used

S.No	Property	Result
1	Fineness of Cement	1.33%
2	Standard consistency of Cement	31%
3	Specific Gravity	3.06
4	Initial setting time	38 minutes
5	Compressive strength @ 7 days	36.45 MPa
6	Soundness of cement	Sound

2.2. Fine aggregate

Locally available sand is used. The sand confirms to zone II as per IS: 383-1987. The specific gravity of the fine aggregate is 2.66 and passes through 4.75mm IS sieve.

2.3. Coarse aggregate

The coarse aggregate which is used is crushed angular aggregate begotten from a local quarry. In this experiment, the size of coarse aggregate that is used passes through 20mm IS sieve and retained on 6mm IS sieve. The coarse aggregate used is tested as per IS: 2386-1963(I, II, III) specifications.

Table 2 Properties of Coarse Aggregate used

S.No	Property	Result
1	Specific gravity	2.86
2	Maximum Nominal Size of Aggregate	20 mm

2.4. Bacterial culture

The species of bacteria used in this experiment is "*Bacillus Subtilis*" which is a gram positive bacterium and is generally found in rocks and soil strata. This species of bacteria is completely innocuous. The extraction of *Bacillus subtilis* is prepared from a given solution of mixed bacterial colonies, a ten-fold serial dilutions could be 1 M, 0.1 M, 0.01 M, 0.001 M etc; are prepared and are uniformly mixed with nutrient medium agar powder which is put into the autoclave at a temperature of 121^0C for 2-3 hours. After cooling for 20 minutes the solution is transferred to 10 petri dishes and are incubated at 37^0C for a time period not less than 24 hours after properly sealing the plates with paraffin tape. After gram staining, the slides are viewed under a compound microscope and the cultures of micro-organisms are viewed as shown in the below figure.

Figure 7 Serial dilutions of mixed

Figure 8 Nutrient Agar Solution

2.5. Development of B. Subtilis Solution

After the pure culture of the required bacteria is obtained, the target amount of solution is prepared. A Nutrient broth solution is prepared using nutrient broth powder and put into the autoclave at a temperature of 121ºC for 2-3 hours after the conical flasks have been tightly sealed with a cotton plug. The nutrient agar solution henceforth becomes pure and free from any existing contaminants. The solution when cooled for 20 minutes in the laminated air flow chamber, in the presence of U. V. Rays and spirit, is free from surface contamination. Now approximately 1-2ml of the pure culture is added to the solutions present in the conical flasks and is kept in a shaking incubator for a time period of not less than 18-24 hours at speed of 150 - 200 rpm. On careful observation the bacterial clusters can be perfectly viewed with the help of a naked eye. The concentration of the solution on analysis is obtained as 10^5 cells/ml of solution.

2.6. Manufacturing of bio concrete

2.6.1. Mix design of bio concrete

As there is no code of reference in existence for bio concrete, the casting of samples is carried out in a similar fashion to that of conventional concrete mix but with a replacement of 10% of the water used in the conventional mix.

2.6.2. Casting of bio concrete

As calculated in the mix design, the casting of the concrete samples is carried out. The procedure of casting and curing is followed with reference to the specifications as laid down by IS: 516-1959. The types and the number of samples casted for bio concrete are:

- Cubes: (15 x 15 x 15)cm^3 - 24 cubes each – 48 in total
- Diameter of each cube = 100mm, Height = 50mm.
- Cylinders: Diameter = 150mm, Height = 300mm.
- 9 cylinders each – 18 in total
- 9 cylinders each – 18 in total (cells)
- Total amount of concrete for the complete project = 0.2893 m^3
- Mass of cement required: 127kg
- Mass of Fine Aggregate required: 188kg
- Mass of Coarse Aggregate required: 344kg
- Volume of water required: 58litres
- Volume of bacterial solution required: 6.4litres

Table 7 Amount of materials required for a single type of mould

Mould type	Cement	Fine Aggregate	Coarse Aggregate	Water	B.S.solution
BIOCONCRETE					
Cube	1.4787 kg	2.1912 kg	4.0115 kg	0.6655 l	74 ml
Cylinder	2.323 kg	3.442 kg	3.901 kg	1.0452 l	117 ml
Cell	0.775 kg	1.15 kg	2.101 kg	0.35	40 ml

2.6.3. Results of Trial Test samples

Table 8 Compressive Strength of Trail Mix of Bioconcrete

No. of Days	Sample No. 1	Sample No. 2	Sample No. 3	Average (N/mm^2)
7	32.15 N/mm^2	33.55 N/mm^2	35.45 N/mm^2	33.71 N/mm^2
14	40.54 N/mm^2	42.51 N/mm^2	42.25 N/mm^2	41.76 N/mm^2
28	46.59N/mm^2	47.58N/mm^2	46.74N/mm^2	46.97 N/mm^2

On performing the slump cone test for the bio concrete mix, the value of slump was obtained as 100mm.

3. EXPERIMENTAL STUDIES

Among the numerous tests that can be performed on hardened concrete, the tests performed on the hardened concrete mixes are cube compressive strength of concrete, water absorption test, splitting tensile strength test, rapid chloride permeability test, self healing analysis.

3.1. Cube compressive strength test

Compression test is the most common because most of the desirable characteristic property of concrete is qualitatively related to its compressive strength. The cube specimen is of the size 15 x 15 x 15 cm and casting is carried out as per specifications laid down by IS: 516- 1959. The concrete is left alone to set itself for a period of not less than 10 hours and is taken out after the setting is done. The moulds are to be cured for the planned time of study and are taken out, sun dried for a time that indicates no amount of visible surface moisture.
The moulds are to be tested in a Compression testing machine at a speed of loading of 5.2kN/s.

Table 11 Results of compression test on Bio concrete Cubes

Days of curing	Sample 1	Sample 2	Sample 3	Average
3 days	27.86 N/mm^2	26.75 N/mm^2	26.94 N/mm^2	27.19 N/mm^2
7 days	32.72 N/mm^2	33.95 N/mm^2	34.45 N/mm^2	33.71 N/mm^2
14 days	40.49 N/mm^2	41.21 N/mm^2	40.27 N/mm^2	40.66 N/mm^2
28 days	49.39N/mm^2	49.89N/mm^2	52.34N/mm^2	50.54 N/mm^2

3.2. Water absorption test

This test on hardened concrete is done as per the specifications laid down by code ASTM C 642.
Three full size blocks shall be completely immersed in clean water at room temperature for required curing period are removed from the water and allowed to drain for one minute by placing them on a 10 mm or coarser wire mesh, visible surface water being removed with a damp cloth, the saturated and surface dry blocks immediately weighed and shall be dried in a ventilated oven at 100^0C to 105^0C for not less than 24 hours and until two successive weighing at intervals of 2 hours show an increment of loss not greater than 0.2 percent of the last previously determined mass of the specimen. The water absorption is calculated as given below:

$$\text{Absorption (\%)} = \frac{(A-B)}{B} \times 100$$

where,

A = wet mass of unit in kg.
B = dry mass of unit in kg.

Table 13 Results of Water Absorption Test on Bioconcrete Cubes

Days of curing	Sample 1	Sample 2	Sample 3	Average
7 days	4.584 %	4.542 %	4.356%	4.494 %
14 days	3.497 %	3.518 %	3.584 %	3.533 %
28 days	2.598 %	2.846 %	2.558 %	2.670 %

3.3. Splitting tensile strength test

The Splitting Tensile Strength test on hardened concrete is done as per the code IS: 5816-1999 specifications. The cylindrical specimen shall not be less than the diameter and not more than twice the diameter. For routine testing and comparison of results, unless otherwise specified the specimens shall be cylinder 150 mm in diameter and 300 mm long. The casting and curing of the moulds is done as per the specifications laid down by IS: 516-1959. The moulds are to be tested in a Compression testing machine at a speed of loading of (1.2 to 2.4) x π/2 x l x d N/min i.e.0.1kN/s.

$$\text{Splitting tensile strength, } f_{ck} = \frac{(2P)}{(\pi \times l \times d)}$$

where;

P= Maximum Load in Newton
l, d = Length and Diameter of the specimen in mm.

Table 15 Results of Split Tensile Test on Bio concrete Samples

Days of curing	Sample 1	Sample 2	Sample 3	Average
7 days	2.947 N/mm^2	3.291 N/mm^2	2.937 N/mm^2	3.061 N/mm^2
14 days	3.284 N/mm^2	3.679 N/mm^2	3.255 N/mm^2	3.406 N/mm^2
28 days	3.659 N/mm^2	3.691N/mm^2	3.595 N/mm^2	3.650 N/mm^2

3.4. Rapid Chloride Permeability Test

Standardized testing procedures are in AASHTO T 277 or ASTM C 1202. The RCPT is performed by monitoring the amount of electrical current that passes through a sample 50 mm thick by 100 mm in diameter in 6 hours. This sample is typically cut as a slice of a core or cylinder. A voltage of 60V DC is maintained across the ends of the sample throughout the test. One lead is immersed in a 3.0% salt (NaCl) solution and the other in a 0.3 M sodium hydroxide (NaOH) solution. Based on the charge that passes through the sample, a qualitative rating is made of the concrete's permeability.

$$Q = 900\ (I_0 + 2I_{30} + 2I_{60} + \ldots\ldots\ldots + 2I_{300} + 2I_{330} + I_{360})$$

Where,

Q = Charge passed (Coulombs)
I_0 = Current (amperes) immediately after voltage is applied, and
I_t = Current (amperes) at t min after voltage is applied.

Table 17 Results of R.C.P.T on Bio concrete Samples

Days of curing	Sample 1	Sample 2	Sample 3	Avg. Charge
28 days	325.8 C	331.8 C	318.6 C	325.4 C

3.5. Self Healing Analysis

Cracks can be healed by using calcium carbonate precipitating micro-organisms. These organisms are embedded in the concrete matrix after immobilization on diatomaceous earth, and will start the precipitation of $CaCO_3$ once a crack occurs. Through this process the cube's crack will be coated with a layer of calcium carbonate, but in the

meantime the crack faces bond together. This test is carried out by the initiation of small cracks on the cube and exposing them to the atmosphere for a period of 2 – 3 weeks.

Figure .22 a) Cube with initiated crack b) Healing of crack due to B. Subtilis by M.I.C.P

4. RESULTS AND DISCUSSIONS

On conducting the experimental investigations that were explained in the previous section, the results are noted and are compared amongst Bio concrete as follows:

4.1. Results for cube compressive strength of bio concrete

Table 18 Average values of Cube compressive Strength for bio Concrete

Curing Period	Bio concrete
3 days	27.19 N/mm^2
7 days	33.71 N/mm^2
14 days	40.66 N/mm^2
28 days	50.54 N/mm^2

Based on the cube compressive strength test results on the samples, the averages of the values obtained for bio concrete are 27.19 N/mm^2, 33.71 N/mm^2, 40.66 N/mm^2 and 50.54 N/mm^2 for 3 days, 7 days, 14 days and 28 days respectively.

4.2. Results of water absorption of bio concrete

Table 19 Average values of Water Absorption of Concretes

Curing Period	Bio concrete
7 days	4.494 %
14 days	3.533 %
28 days	2.670 %

Based on the water absorption test results on the samples, the averages of the values obtained for are 4.49%, 3.85% and 2.67% for 7 days, 14 days and 28 days respectively.

4.3. Results of splitting tensile strengths of bio concrete

Table 20 Average values of Splitting Tensile Strength of Concrete

Curing Period	Bio concrete
7 days	3.061 N/mm^2
14 days	3.406 N/mm^2
28 days	3.650 N/mm^2

Based on the splitting tensile strength test results on the samples, the averages of the values obtained for bio concrete, samples, the averages of the values are 3.061 N/mm^2, 3.406 N/mm^2 and 3.65 N/mm^2 for 7 days, 14 days and 28 days respectively.

4.4. Comparison between Results of R.C.P.T of bio Concrete

Table 21 Trial-wise Results of R.C.P.T of Concretes

Days of curing	Sample 1	Sample 2	Sample 3	Avg. Charge
28 days	1070.8 C	1076.9 C	1068.9 C	1072.2 C
28 days	325.8 C	331.8 C	318.6 C	325.4 C

The average amount of charge that passes through the sample of is 325.4C for bio concrete sample. This indicates that there is a decrease of 69.7% decrease in the permeability value of bio concrete.

5. CONCLUSIONS

Due to the inclusion of bacterial cultures in the concrete matrix the cube compressive strength of concrete at the age of 28 days has been increased by 31.2% i.e. from 38.52 N/mm^2 to 50.54 N/mm^2 by the replacement of water by bacterial solution. The splitting tensile strength of concrete for an age of 28 days is 3.062 N/mm^2. Water Absorption tests reveal that there is a 27.2% decrease in the water absorption of concrete at the end of 28 days due to the presence of bacteria in the concrete matrix, as compared to that of the original one (3.67%). There is a 69.7% decrease in the permeability of concrete of the conventional mix when there is an added amount of bacterial culture solution into the concrete matrix.

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