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Strength Comparison of Bio-Concrete With Conventional Concrete

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Abstract

This paper describes an innovative biotechnology and civil engineering utilizing Microbiologically induced calcite precipitation MCIP for the improvement in properties of surface concrete. Newly, it is found that microbial mineral precipitation resulting from metabolic activities of favorable microorganisms in concrete enhanced the overall behavior of concrete. Calcite precipitation induced by Bacillus cereus was studied in three different percentage of incorporation of bacterial culture in Portland cement concrete specimens. Bacterial culture was mixed with basic ingredients of concrete to obtain microbial concrete. The addition of bacteria was found out to be very effective in increasing the strength of concrete. About 57% of increase in compressive strength was observed by microbial treatment. Different tests like EDTA and pH have also confirmed presence of calcium carbonate precipitation. Involvement of bacterial culture shows detrimental effect that bacteria can cause on the formation of passive layer that prevents rebar from its corrosion. SEM analysis have also confirmed the presence of bacteria forming spore further the following approach has also found out to be efficient in reducing water permeability in concrete structure.

Keywords: *MCIP, SEM and Bacterial Culture.*

1. Introduction

Portland cement concrete has clearly emerged as the material of choice for the construction in the world today. This is mainly due to low cost of materials and construction for concrete structure as well as low cost of maintenance. Therefore, much advancement of concrete technology has occurred depending on the speed of construction, the strength of concrete, the durability of concrete and the environmental friendliness of industrial material like, fly ash, blast furnace slag, silica fume, metakolin etc. There are number of techniques that can be used to increase strength and durability of a concrete structure.

In recent years, it has been focused out that not only structural safety but also durability is significant when designing building or concrete structures. However, in traditional structural design the deprivation of structures over long periods of time is not regarded as serious problem; the demolition of structures has a much greater impact on people or society. Taking these social factors in to consideration, it is obvious that we should not consider the problem of durability of building materials as a problem of the past. It is essential to develop some new eco-friendly self-remediating techniques to support already designed and future constructing buildings to meet various demands to enhance their durability.

Many techniques have been adopted for the improvement of strength and durability of a concrete. Microbial treatment is latest and found out to be effective among all the techniques. Microbial concrete is a revolution metabolic by product of microbial induced calcite precipitation with the help of urease, which is a hydrolyzing enzyme, gives a hopeful research in biotechnology and civil engineering for the enrichment of strength and

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durability of a building material and reinforced concrete structures. Earlier strength was the main criteria considered during construction.

Microbial mineral precipitation (bio deposition) involves various microorganisms, pathways and environments. Considerable research on carbonate precipitation by bacteria has been done by using ureolytic bacteria. These bacteria are able to influence the precipitation of calcium carbonate by the production of a urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO2 and ammonia, resulting in an increase of the pH and carbonate concentration in the bacterial environment [1]. Precipitation of calcium carbonate crystals occurs by heterogeneous nucleation on bacterial cell walls once super saturation is achieved. Bio deposition technologies have already been used for consolidation of sand columns [1–3], for repair of limestone monuments [4–6] and to a smaller extent for remediation of cracks in concrete [7–9]. Results from life-size experiments on limestone monuments have shown a protective effect of the bacterial deposited calcite layer for several years. It is suggested that a new treatment should be applied after 10 years [10].

Until now, many researchers used cultures in different ways. This paper describes the results of an innovative approach in which use of pure cultures of *Bacillus cereus* have been incorporated in concrete in different percentage. The efficiency of bacterial culture incorporation in different percentage in concrete was important part of this investigation. To gain better insight into the efficiency of the bacterial treatments, results were compared with those of nonbacterial treatment.

2.Function of Bacteria

Research has shown that autogeneous healing happens due to hydration of non-reacted cement particle present in the concrete matrix when comes in contact with ingress water resulting in closure of micro-cracks, studies also stated that only spore forming gram positive bacteria can survive in high pH environment of concrete sustaining various stresses.

Therefore, bacterially induced calcium carbonate precipitation has been proposed as an alternative and environmental friendly crack repair technique. Microbial calcite precipitation is mainly due to urealytic activity and carbonate bio-mineralization of bacteria. The bacteria used in this research produce urease which catalyzes the hydrolysis of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}), First, 1mol of urea is hydrolyzed intracellular to 1mol of carbonate and 1mol of ammonia (Eq. (1)).

$CO(NH_2)_2 + H_2O$ ---$NH_2COOH + NH_3$ (1)

Carbonate spontaneously hydrolyses to form additionally 1mol of ammonia and carbonic acid (Eq. (2)).

$NH_2COOH+H_2O$----$NH_3+H_2CO_3$ (2)

These products subsequently form 1mol of bicarbonate and 2mol of ammonium and hydroxide ions (Eq. (3) and (4)).

H_2CO_3 -------- $HCO_3^- + H^+$ (3)

$2NH_3+2H_2O$-----$2NH_4^+ +2OH^-$ (4)

The last 2 reactions give rise to a pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of carbonate ions (Eq. (5)).

$HCO_3^- + H^+ + 2NH_4^+ + 2OH^-$ ------$CO_3^{2-} + 2NH_4^+ +2H_2O$ (5)

Since the cell wall of the bacteria is negatively charged, the bacteria draw cat ions from the environment, including Ca^{2+}, to deposit on their cell surface. The Ca^{2+} ions subsequently react with the CO_3^{2-} ions, leading to the precipitation of $CaCO_3$ at the cell surface that serves as a nucleation site (Eq. (6) and (7))

Ca^{2+}+ Cell → Cell Ca^{2+} (6)

Cell $Ca^{2+} + CO_3^{2-}$→ Cell $CaCO_3$↓ (7)

Several bacteria have the ability to precipitate calcium carbonate. These bacteria can be found in soil, sand, natural minerals. This strain showed a high urease activity, a continuous formation of dense calcium carbonate crystals and a very negative zeta-potential. Self-healing concrete is a product that will biologically produce limestone to heal cracks that appear on the surface of concrete structures. Specially selected types of the bacteria genus Bacillus, along with a calcium-based nutrient known as calcium lactate, and nitrogen and phosphorus, are added to the ingredients of the concrete when it is being mixed. These self-healing agents can lie dormant within the concrete for up to 200 years.

3. Materials

3.1 Concrete and concrete specimens

Pozzolana Portland cement of M25 was used for all mixtures. Depending on the type of experiment, concrete samples were chosen for reasons of practical convenience. Standardized concrete cubes (150×150×150mm) prepared, were grounded in powder form for pH, EDTA and SEM experiments. Cubes were made with water-cement ratios (w/c) of

0.45, and cured for 7,14 and 28 days in water as well as bacterial culture prior to treatment.

3.2 Micro-organism and growth conditions

A thermophilic, anaerobic microorganism *Bacillus* Cereus isolated from CT_5 strain was used in this study. This microorganism was cultured anaerobically in a modified medium (pH 11) before adding to the cement sand and aggregate concrete. Bacillus microorganism grown in Nutrient Broth (NB) medium having pH of 11 was used to study their effect on concrete.

3.3 Mix proportion

Microorganism in different ways was added to concrete. The cement to sand to aggregate ratio was used as 1:1.26:2.56 (by weight), and water to cement ratio was fixed at 0.45. A cube mould of 150 mm was used, as per IS 4031-1988 [11]. Three different cases including non-bacterial treated and bacterial treated were studied.

3.4 Culture media

Bacillus Cereus was cultivated under aerobic batch conditions in a culture media.100 ml culture media was prepared.0.65 gms of Nutrient broth was added in 50 ml of water and kept for autoclaving. 3.7045 gms of calcium hydroxide was added in 50 ml of water. Both the above solutions were mixed together and a complete 100 mL solution were obtained. 2.5 mL of calcium acetate and 5 mL of urea were added in 100 mL of solution.0.5 % of bacterial strain were added in 100 mL of solution to obtain compete bacterial culture. The culture so obtained was kept in shaker for 24 hrs. Serial dilution of 10^{-9}/ml and 10^{-10} /ml concentration were studied throughout the complete work, which gave maximum colonies of bacteria forming unit.

4 Method of investigation

4.1 Compressive strength

This test was carried out on three specimens following the procedure described by ASTM [1]. Compressive strength measurements were carried out using five tones German Bruf Pressing Machine with a loading rate of 100 kg/min.

4.2 Scanning electron microscopy (SEM)

The scanning electron micrographs of freshly fractured specimens were taken with Inspect S (FEI Company, Holland) equipped with an energy dispersive X-ray analyzer (EDAX) at the accelerating voltage of 200 V to 30 kV. SEM micrographs were obtained using a Jeol JSM5600LV apparatus. Samples were gold coated with a JFC-1200 fine coater prior to examination.

4.3 pH

pH test was conducted on powder specimen. Water and powder sample were taken in 1:9 ratios and kept for a overnight. Then the pH of each individual case was determined by using pH meter.

4.4 EDTA

Powder samples, 0.5 gms of each individual case in addition with 3 ml HCl, 4 ml NaOH and 43 ml water were titrated against EDTA. Hydroxy napthol blue indicator was used. Variation in solution colour from pink to blue confirms the presence of calcium carbonate in the solution.

4.5 Slump Value Test

Mixed bacterial concrete was poured into the frustum of cone in three layers with 25 blows on each layer. The size of frustum is Upper diameter-100mm Lower diameter-200mm and Height-300mm After pouring the concrete full into the frustum of raise the mould from the concrete slowly in vertical direction and measure the fall from the top of the concrete. Slump as the difference between the height of the mould and that of height point of the specimen being tested.

5. Results and Discussion

5.1 Compressive strength

Control cubes and microbial cubes for three different percentage of addition of bacterial culture such as *C100* – specimens were mixed with 100 % bacterial culture in place of water cement ratio.; *C75*-specimens were mixed with 25 % water and 75% bacterial culture; *C50*-specimens were mixed with 50% water and 50% bacterial culture. After casting, curing of cubes readings were taken on each 3, 7 and 28 days. The results obtained from the testing are tabulated in *Table 1.* From *Fig 2*, it can be seen that increase in strength is maximum at 3 days in all the cases. It shows that till 3 days, bacteria grow properly and has completely adopted the atmosphere inside concrete cube. As a bacterium grows, calcium carbonate precipitation starts and increases the compressive strength of concrete. Since the bacteria grow only in aerobic condition, the maximum precipitation occurs on the surface of concrete. Slowly After 7 days increase in compressive strength were observed due to formation of calcite layer. Layer of calcium carbonates so obtained as shown in *Fig 1* on concrete cube is known as microbial induced calcite precipitation. A significant increase of 39%,41%, and 38.88% were observed for different cases i.e. case1, case2, and case 3 at initial 3 days in comparison to control case respectively. After 28 days it was observed that, the effective increase in compressive strength was less as compared to 3 days strength. Because once all the pores on surface is blocked, anaerobic condition arises in the concrete and the activity of bacteria slows down, with bacteria converting themselves in to spores. A very important point can be seen from *Table1,* that no matter in what percentage w/c ratio were replaced with bacterial culture, it shows same increase in strength for 3,7 and 28 days. This means microbial effect influence concrete in same manner whether it is used in any percentage.

5.2 pH measurement

For determining pH of the given samples, crushed concrete obtained after conducting compressive strength was taken were converted in to powdered form which can pass through 300µ sieve. Powdered sample was mixed with water in ratio of 1:9 in test tube and left for overnight, next day this sample was tested by pH meter to get pH of the mix. From *Table 2* shows value for all the cases on top surface on concrete specimen. It was observed that pH for all the cases was favorable to the bacterial growth condition. *Fig 3* shows the variation of pH value for all the cases.

5.3 EDTA Calculation

By this test we can confirm the presence of calcium carbonate in the powdered sample obtained from broken cubes. Solution was prepared by mixing provided sample of concrete with sodium hydroxide and water. A drop of an indicator, hydroxyl napthol blue was added to the obtained solution which gives light pink color to solution. The pink colored solution so obtained was further titrated against EDTA. During titration, the pink color solution changes its color from pink to violet at a certain point shown in *Fig 4*. The variation of pink color to violet color confirms the presence of calcium carbonate in the concrete sample. Table 3 shows the amount of EDTA added for per gram of sample and the amount of EDTA added can be correlated with the amount of $CaCO_3$ formed [13-14]. Since EDTA

5.4 Slump Test Result

Decrease in height of concrete to that of mould was noted and it was found to be 114mm for conventional concrete and 65,63 and 67 mm for bacterial concrete for C100, C75 and C50 respectively.

5.5 SEM

To validate calcite formation in the concrete, a piece of broken sample from each mix was further investigated under SEM. The SEM was performed at 28 days. Bacteria incorporated samples in each case on 28 days were tested as they showed maximum compressive strength. *Fig 5 a, b, and c* shows the results of all cases. For all other cases with bacterial culture presence of lamellar rhombohedral crystals of calcite and needle-shaped aragonite crystals of $CaCO_3$ were observed, which shows that all cases support continuous formation of calcite. *Fig 5a, b and c* further confirms the presence of bacterial cell embedded between concrete. From all the images acquired from SEM, it was very clear that for all cases except control specimen without bacteria, and density and clarity of crystal in rest of the cases were same. This implies that equal amount of calcium carbonate precipitation was observed in all cases which form a layer of calcite on the surface of cube samples.

6. Tables and Figures

6.1 Tables and Figures

Fig 1-Calcium carbonate precipitation formed.

Fig 2-Compessive strength comaprision

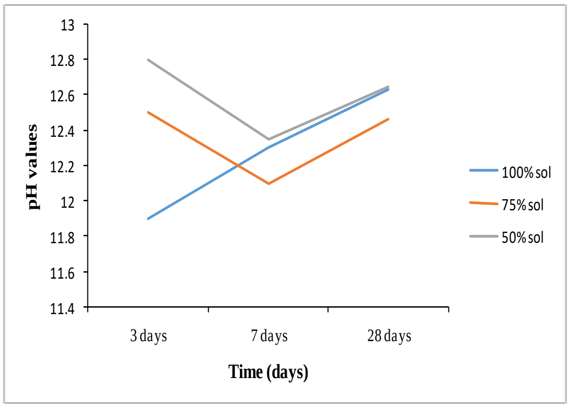

Fig 3-pH value graph.

Fig 4- color changes from pink to violet.

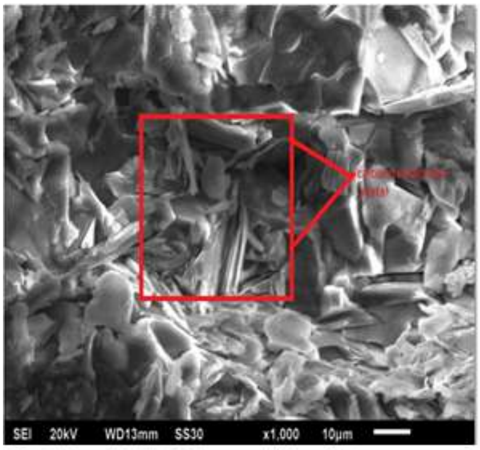

Fig 5 (a) -Presence of Bacterial spore for C100

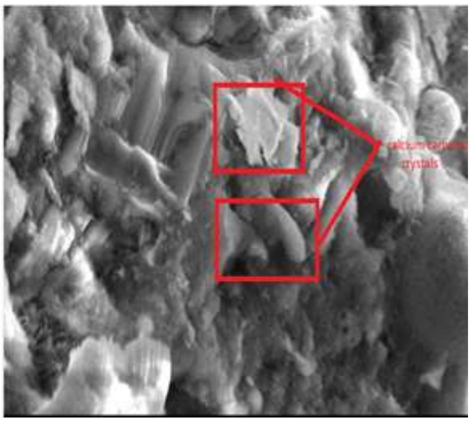

Fig 5 (b) -Presence of Bacterial spore for C75

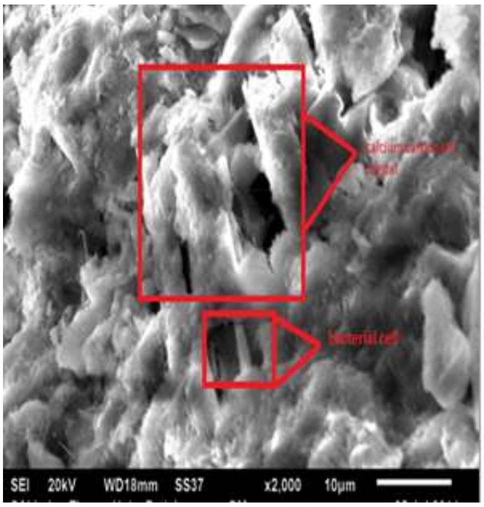

Fig 5 (c) -Presence of Bacterial spore for C50

Days	Strength (N/mm^2) C0	Strength (N/mm^2) C100	Strength (N/mm^2) C75	Strength (N/mm^2) C50
3	12.73	20.88	21.73	19.80
7	19.13	28.60	29.55	27.88
28	30.07	35.22	36.3	38.13

Table 1- Compressive Strength comparison.

Days	C100	C75	C50
3	11.9	11.9	12.8
7	12.3	12.3	12.35
28	12.63	12.63	12.65

Table 2-pH value representation.

	EDTA change (mL)	EDTA used	$CaCO_3$ calculation Grams	
			For 1 gm	For 0.5 gm
C0	41.4 -42.2	1	5.006	2.503
C100	36.3-41.2	4.9	24.52	12.26
C75	45.2-50.4	5.2	26.04	13.01
C50	31.9-36.5	4.6	23.02	11.51

Table 3- EDTA calculation

7. Conclusions

From compressive strength result and discussion, it is clear that participation of bacteria in any form in concrete cubes increases the strength in comparison to control cubes. It is observed from outcome result that, initially after 3 days bacteria activities were on its peak. Radical changes of about 60% were observed. After 7 days increase in compressive strength were significant. After 28 days there were considerable increases in strength but not much effective as after 3 days. Because once all the pores on surface is blocked, anaerobic condition arises in the concrete and the activity of bacteria slows down, with bacteria converting themselves in to spores. It is also concluded from the result that there was no as such difference in strength were observed if bacterial source were used in different forms. That means microbial effect influence concrete in same manner whether it is used in any form. pH of all samples were calculated and it is found out to be constant for all considered days 3, 7 and 28 days respectively. Involvement of bacterial culture shows detrimental effect that bacteria can cause on the formation of passive layer that prevents rebar from its corrosion. EDTA shows the presence of calcium carbonate precipitation by changing pink color solution into violet color. SEM results show rhombohedral crystal structure of calcite in bacterial concrete which confirms the presence of calcium carbonate precipitation.

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