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Effect of Bacterial Calcite Precipitation on Compressive Strength of Mortar Cubes

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Abstract: In this paper results are presented on an experimental investigation carried out on mortar cubes which were subjected to bacterial precipitation by different bacterial strains and influence of bacterial calcite precipitation on the compressive strength of mortar cube on 7, 14 and 28 days of bacterial treatment. Three bacterial strains Bacillus Flexus, isolated from concrete environment, Bacillus pasturii and Bacillus sphaericus were used. The cubes were immersed in bacterial and culture medium for above mentioned days with control cubes immersed in water and was tested for compressive strength. The result indicates that there was improvement in the compressive strength in the early strength of cubes which were reduced with time. Among the three strains of bacteria, Cubes treated with Bacillus flexus which is not reported as bacteria for calcite precipitation has shown maximum compressive strength than the other two bacterial strains and control cubes. Bacillus flexus which is capable of surviving at high pH, precipitate high calcite, and has less generation time can be used for bacterial calcite precipitation as concrete crack remediation and improvement of compressive strength of both mortar and concrete.

Index Terms— Bacillus flexus, calcite, compressive strength, mortar cubes.

I. INTRODUCTION

Mortar and concrete which forms major component in the construction Industry as it is cheap, easily available and convenient to cast. But drawback of these materials is that it cracks under sustained loading and due to aggressive environmental agents which ultimately reduce the life of the structure which are built using these materials [1, 2]. This process of damage occurs in the early life of the building structure and also during its life time. Synthetic materials like epoxies are used for remediation. But, they are not compatible, costly, reduce aesthetic appearance and need constant maintenance [2, 3, 4]. Therefore bacterial induced Calcium Carbonate (Calcite) precipitation has been proposed as an alternative and environment friendly crack remediation and hence improvement of strength of building materials [5]. The concept was first introduced by Ramakrishanan et. al., [6, 7, 8].

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A novel technique is adopted in re-mediating cracks and fissures in concrete by utilizing Microbiologically Induced Calcite or Calcium Carbonate ($CaCO_3$) Precipitation (MICP) is a technique that comes under a broader category of science called bio-mineralization. MICP is highly desirable because the Calcite precipitation induced as a result of microbial activities is pollution free and natural. The technique can be used to improve the compressive strength and stiffness of cracked concrete specimens [9]. Research leading to microbial Calcium Carbonate precipitation and its ability to heal cracks of construction materials has led to many applications like crack remediation of concrete, sand consolidation, restoration of historical monuments and other such applications [10].

A. Microbially Induced Crack Remediation (MICP)

Microbial mineral precipitation involves various microorganisms. Considerable research on Carbonate precipitation by bacteria has been investigated by many researchers 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 CO_2 and ammonia, resulting in an increase of the pH and Carbonate concentration in the bacterial environment. Precipitation of Calcium Carbonate crystals occurs by heterogeneous nucleation on bacterial cell walls once super saturation is achieved. The fact that hydrolysis of urea is a straight forward common microbial process and that a wide variety of microorganisms produce the urease enzyme makes it ideally suited for crack remediation for building material applications. This precipitation forms a highly impermeable layer which can be used as crack remediation for concrete or any other building material. The precipitated calcite has a coarse crystalline structure that readily adheres to the concrete surface in the form of scales. In addition has the ability to continuously grow upon itself and it is highly insoluble in water [11].

The present work deals with comparative study of compressive strength of mortar cubes which were cured in the solution containing known bacteria capable of crack healing and the isolated bacteria from concrete source with growth nutrients and water.

II. MATERIALS AND METHODS

A. Materials

Ordinary Portland cement of 53 Grade available in local market is used in the investigation.

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The cement used has been tested for various properties as per IS: 4031-1988 and found to be confirming to various specifications of IS: 12269-1987 having specific gravity of 3.0. Natural river sand confirming to IS-383 zone II having specific gravity of 2.60 has been used.

B. Bacterial Sources

Microorganisms *Bacillus pasteurii* (NCL-2477) and *Bacillus sphaericus* (NCL-2478) were obtained from National Chemical Laboratory (NCL), Pune. Pure bacterial culture was Isolated named as *Isolate 1* from curing tank at Civil Engineering PG Research lab, M S Ramaiah Institute of Technology Bangalore. Isolate 1 was maintained constantly on nutrient agar slants. Media composition used for growth of culture was Peptone 5 g/Lt., Beef Extract 3 g/Lt., Sodium Chloride NaCl 5 g/Lt.

C. Media composition for Precipitation of Calcium Carbonate

All the Bacterial cultures were urease active organisms, which are essential for Calcium Carbonate precipitation, so while introducing the bacterial culture into the any material, a source of Urea and Calcium is required. In the present investigation, two calcium sources Calcium Chloride Hydrate $CaCl_2 .4H_2O$ and Calcium Nitrate Penta Hydrate $CaNO_{3.}\ 5H_2O$ for precipitation of Calcium Carbonate was used. The media composition was Urea 20g/Lt and Calcium source 49g/Lt.

D. Preparation of specimen samples of Mortar Cubes

Mortar cubes were prepared as per standards specified by IS: 4031 part 6, standard consistency of the cement was determined as per IS: 4031 Part 4 and was found to be 28%, Cement and sand in the ratio of 1:3 by weight was taken and was dry mixed. Water required was obtained by using the relationship, (P/4 + 3) per cent of combined weight of cement and sand, where P is the percentage of water required to produce a paste of standard consistency. Water was added to the mix as calculated and rodded 20 times and vibrated for two minutes at speed of 12000 vibrations per minute. In all 63 cubes were casted, there were 7 sets of 9 cubes, the first set was for control, The other sets were used for three different bacterial stains and two different calcium source. Cubes after casting were de-molded after one day. The first set of cubes which were control cubes were immersed in water. The other sets of cubes was immersed in different bacterial solutions for one day and in respective media containing different calcium source till the day of the test. The compressive strength of three cubes for each bacterial strain and calcium source was conducted at the end of 7, 14 and 28 day of during. The compressive strength was conducted similarly for control cubes.

E. Characterization of Bacteria

To characterize all the bacteria, conventional physiological and biochemical characterization tests were carried out as described in Bergey's Manual of Systematic Bacteriology [12]. The bacteria which are capable of Calcium Carbonate precipitation are Gram Positive, endospore forming and urease positive. All the three strains of bacteria which were used for investigation were gram positive, endospore forming and urease positive. In addition the effect of pH on the growth of the bacteria, generation time, urease activity and amount of Calcium Carbonate precipitation was carried out. The pure cultures of bacteria *Isolate-1* was used for molecular identification. The extraction of DNA from the pure cultures was performed by Cetyl Tri methyl Ammonium Bromide (CTAB) method.

F. Effect of pH on the growth of bacteria

Growth and survival of bacteria is influenced by pH of the environment. Each microbial species possesses a definite pH growth range and a distinct pH growth optimum. The nutrient broth of different pH ranging from 4 to 12 was prepared in a test tube. Bacterial culture was introduced into it and growth pattern was observed. The test was carried out by measuring turbidity (Optical Density) of the sample using Photo calorimeter.

G. Calculation of Generation Time

Generation time is time in minutes a culture of bacteria takes to multiply to double its number. Generation time in minutes was determined by measuring the doubling of turbidity (Optical Density) of the population in a broth culture using Photo calorimeter.

H. Urease Assay

The urease activity was determined for all the bacteria in Urease media by measuring the amount of ammonia released from urea according to the phenol-hypochlorite assay method [13].

I. X-Ray Diffraction (XRD) Analysis

XRD analysis is carried out to determine chemical composition of the precipitation that occurred due to bacterial mineralization. The result obtained from the analysis was compared with standard data obtained from International Center for Diffraction Data.

J. Method of bacterial treatment by Immersion method

The mortar specimens after de-molding were immersed in triplicates in respective bacterial solution grown overnight separately for 24hrs. After 24hrs the mortar cubes were wiped with a blotting paper to remove any surface bacteria and cured in corresponding calcite precipitation media (Calcium source 49gm/Lt + Urea 20g/Lt) at room temperature (Figure: 1) until compression testing at the intervals of 7, 14 and 28 days. Media were replaced at a regular interval of 7 days. Control samples were also prepared in similar manner and cured in water. Compression testing was performed using automatic compression testing machine.

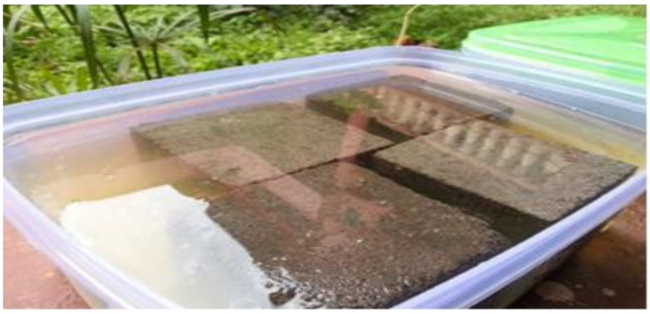

Figure 1: Immersion of samples in precipitation media.

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K. Compressive Strength Test

The cubes were tested in 2000kN capacity compressive testing machine (Figure 2) loaded at constant rate of loading at 200 kg/cm^2/min., as per standard procedure explained in IS: 516-1959(1999).

Figure 2: Compression testing machine

III. EXPERIMENTAL RESULTS

A. Isolation and Identification of Calcite Precipitating Bacteria- Gram staining and Endospore straining

Gram staining and Endo spore straining was conducted to determine the Gram reaction and morphology of the three strains of bacteria used for investigation. All the three strains were found to be gram positive and endospore forming.

B. Urease Test

All the three strains of bacteria were tested for urease activity. The change of the color of the media from yellow to pink indicated that it is urease positive. All the three strains were urease positive.

C. Effect Of pH On the Growth of Bacteria

Growth and survival of microorganisms are greatly influenced by the pH of an environment especially high alkaline environment of the cement mortar. All the three bacteria were tested for optimum pH its growth.

It was observed that *Isolate-1* had optimum growth in pH range 7.5-9.0 However the growth was also observed in the extreme pH upto12. Whereas, *Bacillus pasteurii* had optimum growth in pH range 7-9 and for *Bacillus sphaericus* was 8-9.

D. Generation time

Generation time is the time required for the microbial population to double under standard condition. The generation time of *Isolate1, Bacillus pasteurii* and *Bacillus sphaericus* was 20 minutes, 90 minutes and 120 minutes respectively.

E. Urease Assay

The ability to precipitate Calcium Carbonate (Calcite) is directly related to the amount of the enzyme urease produced by the bacteria. Bacteria species Urease activity after 24hrs of incubation at Room temperate of 28^0 C for *Isolate1, Bacillus pasteurii* and *Bacillus sphaericus* was 14.2, 9.0 and 10µg/ml/minute respectively.

F. Calcium Carbonate precipitation

Ability of all bacterial species to precipitate Calcium Carbonate was studied. It was found that at the end of six days the values for *Isolate1, Bacillus pasteurii* and *Bacillus sphaericus* was 6.6, 5.4 and 4.7gm / lt. respectively.

G. Immersion Method

The mortar specimens were immersed in the respective bacterial solution for 24 hrs. which later was immersed in the different precipitation media at room temperature till they were tested for compressive strength. Also, the control cubes were immersed in water for curing till they were tested for compressive strength.

After 3 days the cubes with *Isolate-1* immersed in Calcite precipitation media started showing white precipitate on the surface [Figure:3,4], whereas *Bacillus sphaericus* and *Bacillus pasteurii* showed white precipitate after 6 days. The amount of precipitate in Calcium Chloride source was comparatively higher than Calcium Nitrate. The white precipitate was due to Calcite formation by bacteria. The precipitation media was replaced every 7 days in order to maintain the pH of the media.

Figure 3: Precipitate on surface of *Isolate-1*.

Figure 4: Precipitate on surface of *Bacillus pasteurii*.

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H. Compressive strengths of the Mortar Cubes

The compressive strength of mortar cubes tested for compressive strength in compressive testing machine at 7 days, 14 days and 28 days for different bacteria in different calcium sources and for control mortar cubes are tabulated in the table 1 and 2.

Table 1: Compressive strength of mortar specimens

Species	**Compressive Strength MPa.**					
	Day		**Day**		**Day**	
	$CaCl_2$	**$CaNO_3$**	**$CaCl_2$**	**$CaNO_3$**	**$CaCl_2$**	**$CaNO_3$**
Isolate-1	**38.60**	**35.70**	**46.50**	**45.20**	**59.8**	**57.2**
B.pasteurii	**37.40**	**34.60**	**45.70**	**42.80**	**58.0**	**55.4**
B.sphaericus	**36.20**	**34.00**	**45.00**	**42.70**	**58.0**	**55.8**

Table 2: Compressive strength of Control specimens

Control specimen	Compressive strength, MPa		
	Day 7	Day 14	Day 28
	32.7	41.5	54.5

It was observed that the compressive strength of cement mortar cubes showed significant increase up to 18%, (Table 3) Compared to control specimens. Compared to other bacterial species *Isolate-1* has shown maximum increase in compressive strength, followed by *Bacillus pasteurii* and *Bacillus sphaericus.* The specimen which showed increase in the compressive strength has exhibited the precipitation even inside the fractured surface (figure 5), which clearly indicates that the Calcite deposition has not only occurred on the surface but also on the interior of the specimen through the microscopic pores. However, the increase of compressive as compared to control specimen was reduced on 14th day and there was a further reduction in the percentage increase in 28th day.

Table 3: Percentage Increase in Compressive Strength

Bacteria	Percentage Increase In Compressive Strength					
	7 Day		14 day		28 day	
	$CaCl_2$	$CaNO_3$	$CaCl_2$	$CaNO_3$	$CaCl_2$	$CaNO_3$
Isolate-1	18.00	9.20	12.00	8.90	9.72	4.95
B.pasteurii	14.40	6.00	10.10	3.14	6.42	1.65
B.sphaericus	10.70	4.00	8.50	2.90	6.42	2.39

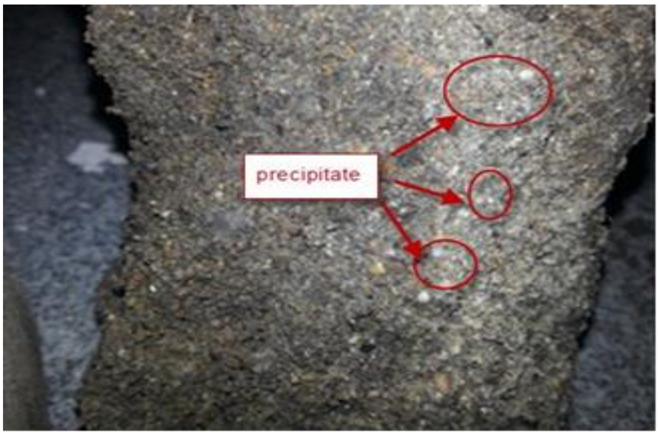

Figure 5: Precipitate on fractured surface treated with *Isolate-1*

I. Molecular Charcterization

ITS (Internal Transcribed Spacer) Sequence isolated from the pure culture of *Isolate-1* (hence forth termed as *Bacillus flexus*) showed 99% similarity with *Bacillus flexus*, the sequence of DNA was submitted to National Center for Biotechnology (NCBI) which is indexed with an NCBI Accession number JX627772. The Phylogenetic position confirms that our *Isolate 1* corresponds to *Bacillus flexus.*

J. X-Ray Diffraction (XRD) Analysis

The precipitate was analyzed for its chemical characteristics by X-ray Diffraction and result indicated positively as Calcium Carbonate figure 6.

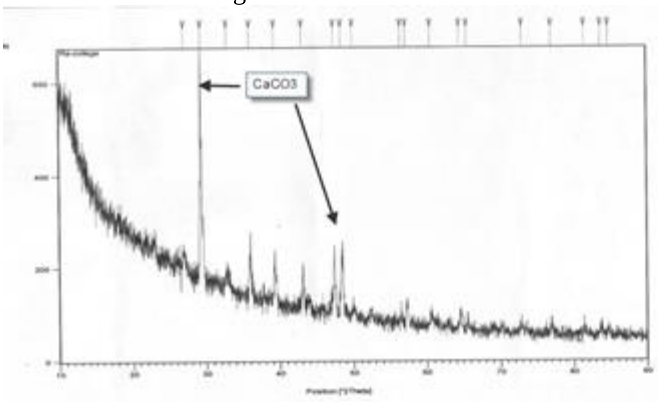

Figure 6: Diffraction spectra of XRD Test.

IV. DISCUSSION

Bacteria inhabit an extraordinary array of habitats, from those that offer ideal conditions for most living creatures to those too extreme to support most life forms. They inhabit the relatively benign and nutrient-rich environments of soils, lakes, oceans and other organisms, but they are also found in extreme environments [14]. The limited diversity of the bacterial community in extreme environments like cement or calcareous sludge, alkaline soil or limestone areas is not surprising; because of its extreme alkaline condition and organisms capable of growing in these conditions can only survive in this environment. Urease produced by bacteria is widely known to precipitate Calcium Carbonate. Typically, to remediate building materials, urease needs to be active and stable in alkaline environments (pH 9–11) that also include high temperature [9]. Isolation and screening of bacteria from these natural environments can be useful for obtaining bacterial strain with the potential of yielding urease enzymes. Also, these areas were selected to isolate indigenous bacteria which can sustain high alkalinity as the aim of the present work was to use these isolate in the remediation of building material and to compare its performance with other reported species across the world.

Salt tolerance, growth temperature range, growth pH range, and extracellular products are important taxonomic criteria which were used to differentiate species in the genus *Bacillus* [12]. The three bacteria used in the present work showed the ability to tolerate a wide range of pH and presented ureolytic activity that lead to Calcite precipitation, which provides the advantage in uses for various remedial processes in construction materials.

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Bacillus flexus which was isolated from concrete environment has shown pH tolerance up to 12, as compared to other two reported species namely *Bacillus pasteurii* and *Bacillus sphaericus.*

The isolated bacteria, *Bacillus flexus* of the present study was identified as *Bacillus* genera and *Bacillus* species which is already known to produce a large amount of urease in soil environments [15, 16, 17]. There was significant difference in urease production between *Bacillus flexus* and standard species when grown in nutrient media containing Urea and Calcium Chloride for different time interval. It was observed that *Bacillus flexus* was found to be having maximum urease activity when compared to standard species.

A. Improvement in the Compressive Strength

Bio mineralization is based on the ability of bacteria to promote the precipitation of Carbonates. *Bacillus flexus* which was selected to study strength characteristics and its efficacy was compared with *Bacillus sphaericus* and *Bacillus pasteurii. Bacillus flexus* showed a positive effect on the compressive strength of mortars. Improvement in compressive strength reaches a maximum at about 18% as compared to control mortar cubes without bacterial treatment.

However it was observed that the pH of the precipitation media would increase up to 12 reducing the growth of the bacteria which in turn reduces the amount of precipitation. If the pH would have been maintained at optimum range, the strength of the mortar blocks could be increased further.

In general, microbial induced Calcite precipitation is effective in surface consolidation in porous media and further remediation of surface cracks. The overall trend of an increase in compressive strength up to 14 days might be attributed to the behavior of microbial cells within the cement mortar matrix. During the initial curing period, microbial cells obtained good nourishment, because the cement mortar was still porous; but growth was not proper as there was completely new environment for microbes. It is also possible that as the pH of the specimen remained high, cells were in inactive condition and as curing period was increased, it started growing slowly. Upon cell growth, Calcite would precipitate on the cell surface as well as within the cement mortar matrix which might be also attributed by the various ions present in media. Thus, the cement mortar became less porous and permeable and increasing the compressive strength of the mortar cubes. This explains the behavior of the increased compressive strength at the age of 14 days in cement mortar cubes treated with microbes. However, the percentage increase after 28 days was not as significant as on 7 day or 14 day. This may be attributed to the fact that as hydration of cement advances, most of the pores which result in reduction in compressive strength in earlier days will gets filled up with hydration products of cement. But there was a marginal increase in compressive strength of mortar cubes which were immersed in *Bacillus flexus* and *Bacillus sphaericus* as compared *Bacillus pasteurii.*

There was improvement in the compressive strength of mortar specimens based on MICP produced by *Bacillus flexus, Bacillus pasteurii* and *Bacillus sphaericus* supported by previous studies [3, 8, 18]. This improvement in compressive strength is likely due to deposition of $CaCO_3$ on the microorganism cell surfaces and within the pores of cement–sand matrix, which plug the pores within the mortar [7,19].

V. CONCLUSION

In conclusion, the positive potential of microbial induced Calcite precipitation that has been demonstrated in present study offers an interesting concept of the enhancement of material properties such as compressive strength and crack remediation in building materials.

It can be concluded that the increase in compressive strengths is mainly due to consolidation of the pores inside the cement mortar cubes with micro biologically induced Calcium Carbonate precipitation.

As *Bacillus flexus* which is not reported so far in any literature which was isolated from concrete environment, has surpassed all other reported organisms in terms of its ability multiply rapidly, tolerance for very high pH of concrete environment, its ability to fill the cracks and increase in compressive strength. It is recommended that for future work and application of bacterial concrete can be concentrated on *Bacillus flexus.*

The use of MICP has thus become a viable solution not only enhancement of compressive strength and durability problems but also as an environmentally responsible course of action.

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