Bioremediation of Concrete

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***Abstract*—** Concrete is a material, which is by far the most used building material in the world. But natural processes like weathering, faults, earthquakes and some human activities create fractures and fissures in concrete structures which act as a source of ingression of water and chemicals, causing deterioration of embedded steel structures and reduce the service life of the structures. The need of a new technology to overcome the shortcomings of concrete has lead to the development of a very special concrete known as Bacterial Concrete. In our present research, Microbiologically Enhanced Crack Remediation (MECR) technique is used for remediation which involves a selective microbial plugging process, in which microbial metabolic activities promote calcium carbonate (calcite) precipitation, thus reducing the pore concentration and increases its compressive strength and durability. "*Bacillus species*" isolated from soil and cement was used to study compressive strength, and water absorption. The aim of the present investigation is to find microbiologically induced special filler which can reduce the pore concentration and hence enhance the performance of the concrete. *Bacillus sp* obtained from soil and cement was maintained on Nutrient agar medium and grown in Nutrient broth-urea (NBU) medium. Three different types of cement mortar cubes specimens were prepared: microbial enriched cubes cured in NBU media, control specimens cured in NBU media and water respectively. Water absorption test and Compressive strength test were performed on these cube specimens in time kinetics. The current study showed that presence of *Bacillus sp.* in concrete caused a substantial increase in the compressive strength of the cement mortar cubes also this bacterial concrete resulted in increased resistance towards water penetration.

***Keywords*—** Bacterial Concrete, MECR, NBU, *Bacillus species*, Calcite Precipitation

I. Introduction

PORTLAND cement concrete has clearly emerged as the material of choice for the construction of large number and variety of structures in the world today. Despite of being thought as a modern material, concrete has been in use for hundreds of years. Its wide use is attributed mainly to low cost of materials as well as low cost of maintenance. Concrete is a relatively durable and widely used building material, but it can be severely weakened by poor manufacture or a very aggressive environment. A number of historic concrete structures exhibit problems that are related to their date of origin. Also, Concrete is very strong in compression but relatively weak in tension, thus leading to crack formation, which act as a source for the ingression of water and other harmful corrosive chemicals.

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Durability of concrete is also impaired by these Cracks, causing deterioration of embedded steel structures. Concrete is porous in nature due to which it easily absorbs and releases water. This absorbed water can freeze within the concrete and creating freeze-thaw conditions that are prone to spalling and cracking. Thus the above problems associated with concrete can be solved by inducing microbiologically special growth/filler in concrete, which reduces the pore concentration by promoting calcite precipitation.

Microbiologically Induced Carbonate Precipitation (MICP), resulting from metabolic activities of some specific microorganisms in concrete, is an alternative technique to overcome some of the shortcomings of conventional concrete such as poor tensile and impact strength, limited resistance to corrosion & acids, poor behaviour under severe conditions and poor adhesion of fresh mortar or concrete to old concrete. The importance of selective cementation has been widely recognized in civil engineering, which is also related to calcite precipitation. It has been known that microorganisms play an important role in promoting calcite precipitation, previous research on it has illustrated a reduction of the capillary permeable porosity and an increased resistance to damage processes such as chloride ingress and carbonation. Therefore bacterial induced calcium carbonate deposition has been proposed as an alternative and environment friendly technique. Also, microorganisms can be used as construction material which enhances the serviceability of infrastructures and protects the environment.

A. Problems Associated With Concrete

Durability and Strength of concrete are characteristics of its pore structure. The major problem associated with the concrete structures is the deterioration of embedded steel structures, which is caused due to the ingression of corrosive materials such as water, ions and other chemical substances through these pores, thus affecting the strength and durability of concrete structures. Hydration process involved during the formation of concrete, results in formation of pores, and these pores then acts as the entry points for water, water vapour, different gases and chemical substances such as chlorides from the surrounding environment, that may have deleterious effect to the concrete possibly leading to damage, which may even be irreversible in nature. Efflorescence is also one of the major problems in porous concrete which is caused due to the formation of crystalline deposits salts on the surface of concrete. Therefore, greater the porosity of concrete structure, the more it is vulnerable to degradation caused by penetrating substances. However, with the increasing demand being made on concrete technology to serve the needs of society, there is a great need of a new technology which can reduce the porosity of concrete which in turn will enhance the strength and durability of the concrete and also would lead to a new way of designing durable concrete structures, which is beneficial for both local and global economy.

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B. Need of New Technology: MICP

Microbiologically induced calcium carbonate precipitation (MICP) is a novel technique which to some extent has contributed to the decrease in porosity of concrete structures, as well as has offered higher strength, improved durability, good resistance to corrosion and has reduced water permeability. MICP is a bio-geochemical process that induces calcium carbonate precipitation within the concrete matrix. It comes under a broader category of science called bio mineralization. Bio mineralization is defined as a biologically induced precipitation in which an organism creates a local micro-environment, with conditions that allow optimal extracellular chemical precipitation of mineral [Hamilton, 2003]. Use of this bio mineralogy concept leads to the potential invention of new material "bacterial concrete" an inherent and self repairing biomaterial that can remediate the cracks and fissures in concrete.

Microbiologically-induced $CaCO_3$ precipitation results from a series of complex biochemical reactions described below. The microbial urease enzyme catalyzes the hydrolysis of urea into ammonium and carbonate (1), Due to presence of ammonia the pH of the surrounding increases.

$$CO(NH_2)_2 + H_2O \dashrightarrow NH_2COOH + NH_3 \quad (1)$$

The compound formed then spontaneously hydrolyses into ammonia and carbonic acid that leads to the formation of bicarbonate and hydroxyl ions (2)(3)(4).

$$NH_2COOH + H_2O \dashrightarrow NH_3 + H_2CO_3 \quad (2)$$

$$2NH_3 + 2H_2O \longleftrightarrow 2NH^+_4 + 2OH^- \quad (3)$$

$$H_2CO_3 \longleftrightarrow HCO^-_3 + H^+$$

The production of hydroxide ions results in the increase of pH, which in turn can shift the bicarbonate equilibrium, resulting in the formation of carbonate ions (5)

$$HCO^-_3 + H^+ + 2NH^+_4 + 2OH^- \longleftrightarrow CO_3^{-2} + 2NH^+_4 + 2H_2O \quad (5)$$

The produced carbonate ions precipitate in the presence of calcium ions as calcium carbonate crystals (6).

$$Ca^{+2} + CO_3^{-2} \longleftrightarrow CaCO_{3\downarrow} \quad (6)$$

The formation of a monolayer of calcite further increases the affinity of the bacteria, resulting in the production of multiple layers of calcite.

II. Materials and Methods

A. Culture Media

- LB Broth and Agar Media: LB broth media was prepared by adding 5g/l Yeast Extract, 10g/l NaCl, 10g/l Tryptone in 1000 ml deionized water and additional 1.5% agar was added in the above preparation to make LB agar media.

- Urea-$CaCl_2$ broth and agar Media : Urea-$CaCl_2$ broth Media was prepared by adding 3 g/l Nutrient broth, 20 g/l Urea, 2.12 g/l $NaHCO_3$, 10 g/l NH_4Cl, 25mM $CaCl_2 \cdot 2H_2$ into 1000 ml distilled water and 15g/l agar was added in the above preparation to make agar media.

B. Bacterial Strains

Bacillus Strains were isolated from two different sources: Garden Soil and Ordinary Portland Cement. Liquid dilution of both soil and cement was done and the dilutions were plated and incubated at 30°C for 3 days. The liquid dilution of soil and cement resulted in the isolation of different colonies on the agar plates. The gram staining of these colonies showed that the colonies were of gram positive Bacillus species. The isolated colonies were then streaked on fresh Urea-$CaCl_2$ plates for the future use.

C. Estimation of microbiologically Induced CaCO3 Precipitation

The isolated bacterial strains were cultured in LB broth medium and left for a period of 24 hr in shaking incubator at 130 rpm and 30°C temperature. The culture then obtained was centrifuged and washed in saline water (0.85% NaCl). The pellet obtained was resuspended in sterile saline solution and its absorbance was measured at 600nm. Then 500µl of this bacterial solution was inoculated in 100ml urea-$CaCl_2$ medium and was subjected to constant shaking for 48hrs at 180 rpm and 30°C temperature. 15ml of sample were harvested at regular intervals of 3hrs initially and then for longer intervals till 72hrs. Of this 15ml sample 2ml was used to measure the absorbance at 600nm and the rest was centrifuged at 5200 rpm for 15 min. The supernatant was collected from the centrifuged sample and 10ml of it was placed in Erlenmeyer flask for titration. EDTA titration was performed on these samples to measure the amount of soluble Ca^{2+} and from it amount of precipitated Ca^{2+} was obtained.

D. Materials Used For Concrete

The following are the details of the materials used for concrete making.

Sand: Locally available Yamuna river sand, well graded passing through 4.75mm sieve was used for the formation of cement mortar cubes.

Cement: Ordinary Portland cement of 53 Grade confirming to IS 12269-1987 was used.

Water: Locally available river water confirming to IS 456 is used

E. Preparation of specimen for compressive

Different types of cubes were prepared for concrete mix with and without addition of microorganisms. Bacterial cells at different concentration, those mentioned above were mixed with cement and sand mortar during casting. Before adding to the mortar mixture, the media containing bacteria was centrifuged and washed several times with distilled water to remove chloride ions, as they are known to weaken the integrity of cement matrix, if present in bacterial growth medium [Ghosh et al.2005]. The size of the cube mould was taken as 70.6mm x 70.6mm x70.6mm. The cement to sand ratio was 1:3 (by weight), and the bacterial culture/water to cement ratio was 0.47 [Achal et al., 2010]. All cubes were cast and compacted in a vibration machine. The cubes were prepared according to Indian specifications and were demoulded after 24 hours. Total numbers of 54 mortar cubes were casted out of which 18 cubes were casted using bacteria (Bacillus species) and subsequently cured in NBU medium. The remaining 36 cubes were casted without addition of bacteria, 18 of which were cured in NBU medium and remaining were used as control specimen which were cured in water bath for 28 days. After removing the cubes from the medium, the surface of each cube was completely dried at 25°C prior to a compressive strength test. Compression testing was performed at the intervals of 3, 7 and 28 days respectively. Compression testing was performed using automatic compression testing machine, COMPTEST 3000 [AIML Ltd., New Delhi, India].

III. Results and Discussion

A. *Isolation of Bacteria*

10 different colonies were isolated from soil and cement out of which 5 colonies which were found to be gram positive and 1 gram negative bacillus strains which were used for further studies.

B. *$CaCO_3$ Precipitation*

The following results of Ca^{2+} solubility were obtained when EDTA titration of different bacterial sample was done :

The amount of solubility of Ca^{2+} in medium is inversely proportional to the amount of Ca^{2+} precipitated; hence the above results show that the greater amount of Ca^{2+} was precipitated by Bacillus strain C3, C5 and C6 which were further studies.

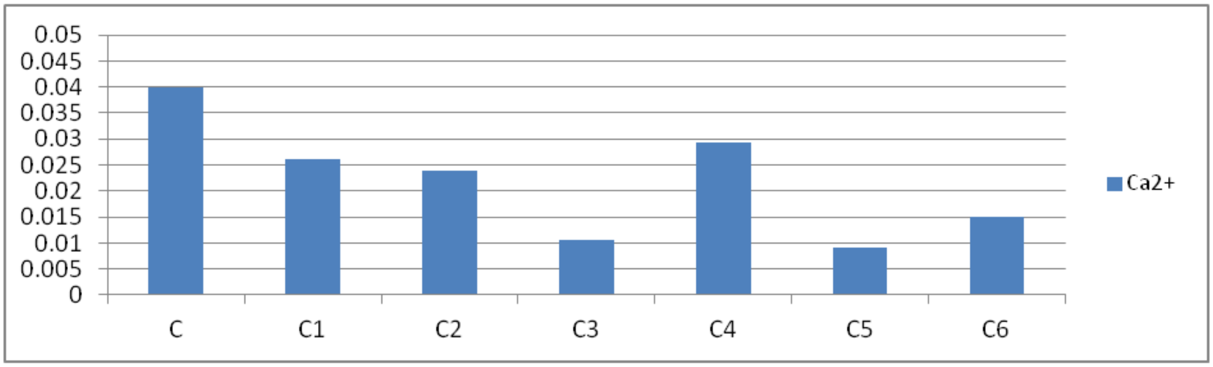

Fig. 1 Calcium solubility of different samples.

C – control specimen, C1- Soil sample 1 diluted to 10^{-7} times, C2 – Soil sample 1 diluted to 10^{-6} times, C3- Soil sample 2 diluted to 10^{-5} times, C4- Soil sample 2 diluted to 10^{-6} times, C5 – Cement sample diluted to 10^{-4} times, C6- Soil sample 2 diluted to10^{-4}times. C1, C2, C4, C5 and C6 were gram positive Bacillus sp. and C3 was gram negative Bacillus sp.

C. *Compressive Strength Test*

The cubes and cylinders have been tested as per IS specifications .The compressive strength test were carried out on all 3 specimens. The mortar cubes incorporated with calcite-forming bacteria for 3,7 and 28 days in NBU medium were tested for compressive strength using automatic compression testing machine, these specimens were then compared with control cubes incubated in water and NBU medium respectively. From the compressive strength test, it was observed that the cubes consolidated with microbial cell showed a significant increase in compressive strength after 28 days relative to the control specimens cured in water, but the control cubes incubated in NBU medium did not show improved compressive strength even after 28 days. Although the cubes cured in NBU medium were stronger then compared to control, the increase in strength was attributed to Urea-CaCl2 present in the NBU medium, thus enhancing the strength of the concrete. The results of the compressive test on all 3 specimens are in indicated in Table I.

TABLE I
RESULTS OF THE COMPRESSIVE STRENGTH TEST FOR 3 DIFFERENT SPECIMENS

DAYS	Control Water	Control NBU	Bacterial Concrete
3 days	8 MPa	9 MPa	10 MPa
7 days	13 MPa	14 MPa	16 MPa
28 days	23MPa	24 MPa	30 MPa

The improvement in compressive strength by *Bacillus* sp. is probably due to deposition of CaCO3 on the microorganism cell surfaces and within the pores of cement–sand matrix, which plug the pores within the mortar [Ramakrishnan et al. 1998; Ramachandran et al. 2001; Ghosh et al. 2005; Achal et al. 2009a]. There wasn't much increase in the compressive strength of bacterial concrete during the initial curing period for about 7days although it led to a significant increase up to 28 days. The increase in compressive strength was attributed to microbial activity on the cell surfaces as well as within the cement sand matrix. During the initial curing period, there was limited bacterial growth, since this was the bacterial lag period indicating metabolic adaptation. But as the curing period increases there is a significant increase observed in the compressive strength it is due to the deposition of calcite all over the surface of concrete, thus plugging the pores in concrete and preventing the ingression of water and other chemicals which deteriorates the strength of concrete. Thus, the cement mortar became less porous and permeable. And as the pores in the matrix were plugged, the source of media for bacterial growth becomes limiting, leading to decline in cell number and initiation of endospore formation which acts as an organic fiber and increases the compressive strength of the mortar cubes. This explains the behavior of the increased compressive strength at 28 days in cement mortar cubes prepared with microbial cells.

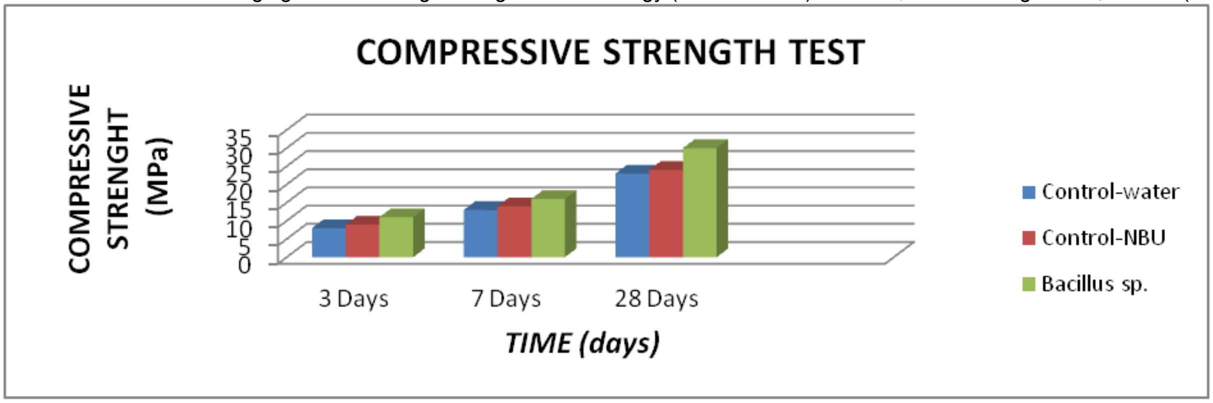

Fig. 2 Change in Compressive Strength of cement mortar cubes at interval of 3, 7 and 28 days respectively. Control- water indicates the conventional concrete cubes cured in water, Control-NBU indicates the conventional concrete cubes cured in NBU Medium and Bacterial Concrete indicates the concrete mixed with microbial cells and cured in NBU medium.

D. Water Absorption Test

This test determines the rate of absorption (sorptivity) of water by cement concrete cubes by measuring the increase in the mass of a specimen resulting from absorption of water as a function of time when only one surface of the specimen is exposed to water. The mortar specimens were coated at the four edges adjacent to the treated side, to ensure unidirectional absorption through the treated side and were kept in oven at 45°C .The exposed surface of the specimen is immersed in water and water ingress of unsaturated concrete dominated by capillary suction during initial contact with water. For 100 hours at a regular time of 1 hour the specimens were removed from the water and weighed, after drying the surface. Immediately after the measurement the test specimens were submerged again. The sorptivity coefficient, k [cm.s-1/2], was obtained by using the following expression Q/A = k √t (4)

Where Q is the amount of water absorbed [cm³]; A is the cross section of the specimen that was in contact with water [cm²]; t is the time [s], Q/A was plotted against the square root of time, then k was calculated from the slope of the linear relation between the former.[Achal et al. 2009a]

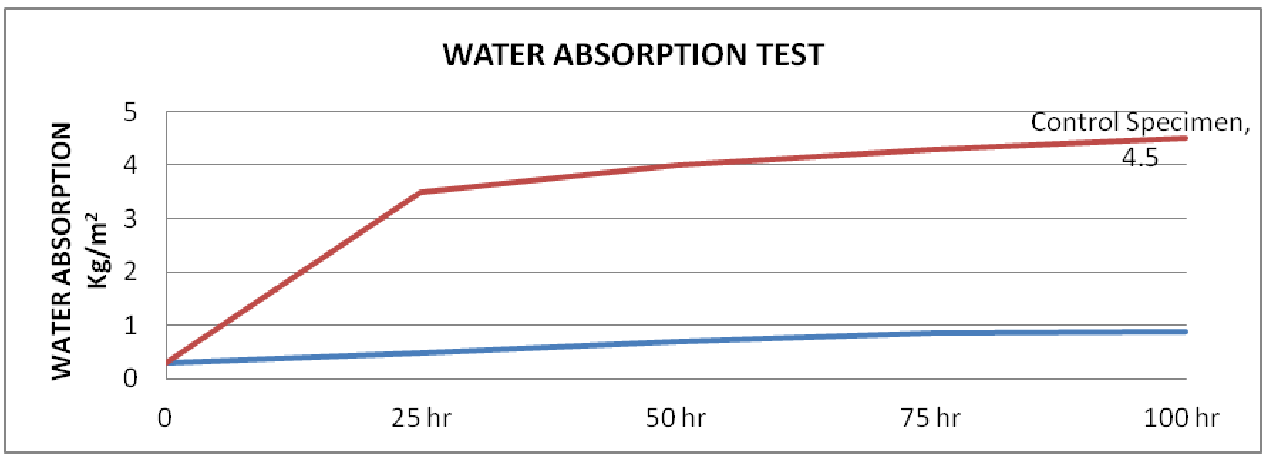

Fig. 3 The Influence of the Bacterial Treatment on the Rate of Water Absorption versus Time for Mortar Cubes. Control specimen were cured in water

IV. Conclusion

This research sheds light on the importance of urease producing bacteria like Bacillus species isolated from soil and ordinary Portland cement in remediation of concrete. Urease is the main enzyme present in the bacteria which is responsible for $CaCO_3$ precipitation which in turn helps in plugging the pores present in concrete. Therefore use of microbiologically induced special growth/filler decreases concentration of pores, thus increasing the compressive strength of concrete. In this study we focused on the use of calcite precipitating bacteria for increase in durability and compressive strength of concrete. Also the microbial concrete produced in this research is resistant towards water permeability which makes it more durable and increases its service life. This bacterial concrete can act as an alternative to the ordinary concrete used today as it is cost effective and environment friendly and will ultimately lead to the enhancement of the durability and strength of the buildings.

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