Section A-Research paper
ISSN 2063-5346

COMPARATIVE ANALYSIS OF LIVE BACTERIAL CELLS ISOLATED FROM SOIL TO ENHANCE THE DURABILITY OF CONCRETE STRUCTURES

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***Abstract*– Cracks in concrete are the main cause to structural failure. The remediation of such building cracks can be done by incorporating an autonomous self – healing mechanism in concrete. A novel technique using live microbial cells bearing the capacity to precipitate calcite mineral is applied for healing the repair in concrete. In the present study two different microorganisms such as *Bacillus* sp and *Pseudomonas aeruginosa* were isolated and their ability to heal the micro cracks were compared. The results showed that the compressive strengths of the cubes containing live *Bacillus* sp increased than with *Pseudomonas aeruginosa* comparatively. It was observed that the cracks filled with bacteria and sand demonstrated a significant increase in compressive strength when compared with those without live cells. *Bacillus* sp showed increase in resistance towards water penetration than *Pseudomonas aeruginosa* and thus demonstrating that *Bacillus* sp being a spore former can enhance the durability of building materials**.

Key Words: *Bacillus* sp, *Pseudomonas aeruginosa*, Bacterial concrete, compressive strength, resistance to water absorption.

INTRODUCTION

Concrete the largest consumed man-made material despite its wonderful properties has few draw backs in which the main one is vulnerability to cracking. There are several factors responsible for cracking like natural, physical, chemical and biological factors. The crack in the concrete tends to expand further if proper and immediate treatments are not made which will require costly repair. Several modern technologies have been developed for remediation of cracks in concrete and it has been subjected to further research also (Beenakumari, 2015). Synthetic materials like epoxies are used for remediation (MayurShantilal and JayeshkumarPitroda, 2013). But this application is not cost effective and they require constant maintenance. We are in need of techniques which has the potential to contribute better environment sustainability as well as enhanced mechanical and durability concrete properties. A bio natural technique using live microbial cells in concrete has emerged in recent years which can be used for various purposes, amongst which remediationof cracks in concrete is the primary one (MayurShantilal and JayeshkumarPitroda, 2013). Biomineralization, a microbiologically induced calcite/ calcium carbonate ($CaCo_3$) precipitation (MICP) is highly desirable because the calcite precipitate induced as a result of microbial activities is pollution free and natural. The compressive strength and the stiffness of the cracked concrete specimens can be improved. Several studies with aerobic microorganism such as *Bacillus pasteurii*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *B. subtilis* showed significant improvement in compressive strength of cement mortar (Ramakrishnan *et al.*, 1998; Ramachandran *et al.*, 2001). In our present study, two different microorganisms such as *Bacillus* sp and *Pseudomonas aeruginosa* were isolated and their ability to heal the microcracks were compared.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF BACTERIA

Bacterial species such as *Bacillus*sp and *Pseudomonas aeruginosa* were isolated from soil by serial dilution technique. The organisms were identified based on morphological and biochemical characteristics (Kandler and Weiss, 1986; Sharpe *et al*., 1979). Purified cultures were maintained at -20°C in nutrient broth with 10% glycerol.

PREPARATION OF MICROBIAL CEMENT MORTAR

The mortar was prepared using sand: cement ratio 3:1 by weight. On inch cubic molds were prepared. Sand and cement were thoroughly mixed, adding along with grown culture, at a W/C ratio of 0.46, of *Bacillus* sp *and Psuedomonasaeuruginosa* correspondence to OD at 600 nm of 0.5, 1.0 and 1.5 OD; bacterial cells concentration was measured by spectrophotometer at wave length 600 nm. The fresh mortar pastes were cast into the mold and compacted on a vibration machine then cured in humidity chamber with relative humidity 100% for 24 h. After de-molding the control specimens were cured under tap water and the specimens with bacteria were cured under solution of 20 g/l urea and 25 mM $CaCl_2$ at room temperature until the times of testing at the intervals of 3, 7, 14 and 28 days.

WATER ABSORPTION

Water absorption measurements were done by weighing the saturated specimens (W_1) and dried specimens in oven at 80 °C for 24 h (W_2) at curing times of 3, 7, 14 and 28 days.

The water absorption is calculated from the following equation:

Water absorption,%=[(W2-W1)/W1]×100.

COMPRESSIVE STRENGTH TEST

To study the compressive strength test of cement mortar, *Bacillus* sp *and Psuedomonasaeuruginosa*were grown in NBUmedia. The cement to sand ratio was 1:3 (by weight), and the bacterial culture/water to cement ratio was 0.47. A cube mould of 70.6 mm was used, as per IS 4031-1988. Sand and cement were thoroughly mixed, adding along with grown culture of bacteria correspondence to the optical density (600nm) of 1.0. Cubes were cast and compacted in a vibration machine. After de-molding, all specimens were cured in NBU medium at room temperature until compression testing at the intervals of 3, 7 and 28 days. Control specimens were also prepared in similar way where water and NBU medium replaced bacterial culture. Compression testing was performed using automatic compression testing machine

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.

RESULTS AND DISCUSSION

IDENTIFICATION OF BACTERIAL ISOLATES

The bacteria to enhance the durability of concrete is isolated from the soil and were identified as *Bacillus* and *Pseudomonas aeruginosa* based on several biochemical characteristics and microbial characteristics (Table 1& Plate A).

Characteristics	**Isolate A**	**Isolate B**
Gram Stain	+	-
Cell Shape	Rod	Rod
Spore Stain	+	-
Indole	-	-
Methyl Red	+	-
VP	+	-
Citrate	+	+
Fermermentation		
Glucose	+	+
Mannitol	+	-
Xylose	-	+
Sucrose	-	-
Lactose	+	-
Probable Bacteria	Bacillus	Pseudomonas

Table 1: Morphological and Biochemical characterisation of bacterial isolates from soil

Pseudomonas aeruginosa *Bacillus*

Plate A: Gram Reactions of the bacterial isolates from soil

WATER ABSORPTION TEST

An increase in resistant towards water penetration was carried by a sportivity test at 3,7,14 and 28 days. It was observed that among the two isolates *Bacillus* sp absorbed less water when compared to the other isolate *P. aeuruginosa*(Fig 1) and a significant decrease in water uptake resulted in comparison to the control. VarenyamAchal*et al.* (2011) has stated that *Bacillus* sp CT-5 isolated from cement absorbed 6 times less water than control cubes. Absorption of bacteria and precipitation of carbonate crystals resulted in weight increase of mortar specimens (BeenaKumari, 2015).The increase in bacterial concentration precipitates the

calcite content at a higher rate which fills the open spore which decreases the extent of water absorption.

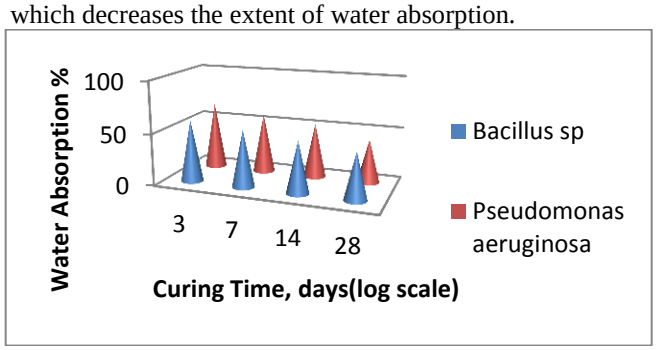

Fig 1. Comparative analysis of water absorption of cement mortar with two different bacterial cells upto 28 days

Compressive strength

The compressive strength of CM specimens cured under tap water and CM specimen mixed with different bacterial isolate are graphically represented in Fig 2. During the initial period as the microbes were exposed to new environment the growth of both the isolates were not proper. The bacterial cells obtained good nourishment because the cement mortar was still porous. As the curing period increased slowly microbial cells started growing slowly. The pH of the cement remained high which made the cells to survive in active conditions (Abo-El- Enein*et al*., 2013). The compressive strength of *Bacillus* sp increased with increase in number of days at a higher rate than *Pseudomonas aeruginosa*. Bacillus sp CT-5 showed 36% increase in compressive strength of cement mortar with the addition of bacterial cells (VarenyamAchal*et al.*, 2010). The improvement of compressive strength is probably due to the deposition of $CaCO_3$ on the microorganism cell surface and within the pores of cement – sand matrix which plug the pores within the mortar.

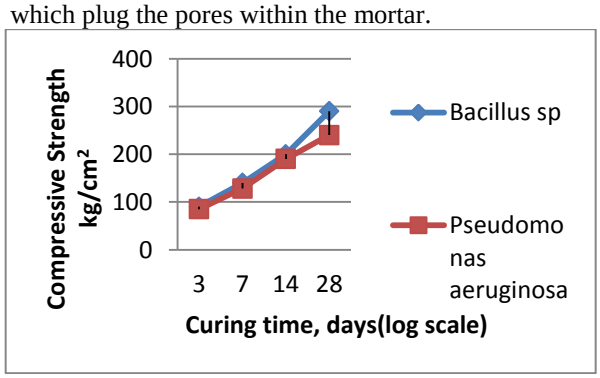

Fig 2:Comparative analysis of compressive strength of cement mortar with two different bacterial cells upto 28 days

SEM Analysis

The formation of microbial calcite precipitation in the mortar samples were examined under SEM. The samples showed calcite crystals frown all over and precipitated with bacterial rod shaped structures (Fig 3) showing distinct sharp edges which indicate a full growth of crystals. Achal*et al.* (2009b) reported that rod shaped structure of *Sporosarcinapasteurii*helped in calcite precipitation in sand column

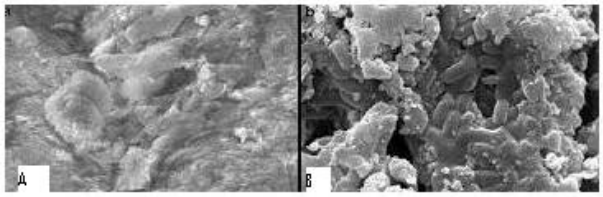

Fig 3:A. Rod shaped impressions housed by the bacterial isolates and B. Precipitation of calcite crystals

CONCLUSION

The significance of this research is to compare the urease producing bacterial isolates like *Bacillus*sp and *Pseudomonas aeruginosa* from the soil. The study has identified that *Bacillus* sp showed a positive effect on compressive strength of cement mortar and increased its strength when compared to *Psuedomonas aeruginosa*. The resistant nature towards water penetration has been made to evaluate the performance of bacterial isolate. The calcite precipitated by *Bacillus* sp is more effective than *Psuedomonas aeruginosa* which are found in common soil. This technology is safe and also persists in the natural environment for an extended period of time.

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