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Bioconcrete Build Buildings with Quorum Sensing Molecules of Biofilm Bacteria

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Abstract

Quorum sensing mechanisms open the lock of the unexplored bacterial world with its molecular key by cracking the codes of gene programs in bacteria. Bacteria uses quorum sensing AHL molecules to build an Exopolysaccharaide matrix coat which heals the cracks in concrete. Specimens were prepared at 10^5 cells/ml concentration for concrete grade of M_{30} and put for curing. Compressive strength for 28, 50, 75, 90 days is carried out. The addition of biofilm producing *bacterium* at 5 X 10^5 cell/ml concentration gives the maximum improvement in compressive strength. It appeared almost 20 % stronger than control specimens at all tested times for 3 months. The addition of biofilm producing bacteria to the concrete not only increases the comprehensive strength greatly but also self healing capacity of the concrete.

1. INTRODUCTION

Bacteria use a variety of signaling molecules to communicate between the cell communities. Gram-negative bacteria utilize acylated homoserine lactones (AHLs) as signaling molecules and gram-positive bacteria make use of small peptides (lipopeptides) as signaling molecules. These signal molecules enable specific intra species communication[1]. Recently however, a new auto inducer known as AI-2 has been proposed to function as a universal signal for interspecies communication. This type of bacterial cell-cell communication is referred to as quorum sensing[2]. Bacterial cells produce a gene product such as an enzyme or a virulence protein because the concentration of protein would be too low to be effective. Quorum sensing in bacteria involves a different type of signal molecules[3]. A precursor lipopeptides is cleaved into functional signal molecules of 10 to 20 amino acids. These molecules are actively transported out of the cell through a special transporter protein. AHL molecules regulates these signals in gram negative bacteria. When this auto inducer signal reaches a threshold concentration on the outside of the cell, they are detected by a sensor protein located on the surface of the cell[4]. Quorum dependent proteins such as specific virulence factors are produced[5]. Though concrete is an important construction material, It has a disadvantage of cracks appearing in its structure at some point of time. Crack prevention is often employed by steel reinforcement which is highly expensive and requires large amounts of steel which will never be acceptable[6]. In case of larger dams, cracks are highly difficult to repair it, use of bio-concrete is the new formulation of the concrete mixture with Exopolysaccharaide producing bacteria. Microbial mineral precipitation is resulted from metabolic activities of micro organisms. Bio-concrete material incorporating of an enrichment culture of thermophilic and alkaliphilic bacteria within cement sand mortar/concrete provides a good increase in compressive strength as well as self healing capacity[6]. Cracks appeared in the constructions lead to corrosion, water ingress and decrease in durability. Self healing concrete utilizes bacteria that is incorporated into the concrete and the calcium lactate as bacterial feed, As it acts on feed and heal the crack by producing raw material as a byproduct. It can even reduce the greater damage by the concrete structures[7].

2. MATERIALS AND METHODS:

2.1 Biofilm assay and Enhancement of EPS ability

The biofilm producing *Acinetobacter sp. strain M6* was collected from Vignan's University, Guntur (Andhra Pradesh). The biofilm producing ability was tested by crystal violet tube staining method[8]. The alkali-resistant and thermophilic ability of the Acinetobacter strain was tested by growing the bacteria at 20˚C to 60˚C and 7 to 12 pH range. The strain was cultivated in an alkaline medium.

2.2 Acid fizz test for Lime stone production

The overnight culture of *Acinetobacter* was inoculated in Mineral Salt medium (MSM) with 5% calcium lactate pentahydrate incubate for 48 hrs at 37˚C. Supernatant was collected by centrifugation at 16800g x 20 min. Add few drops of 10% HCL and observe the effervescence[10].

2.3 Specimen Preparation

Control Specimens of 50 mm were prepared by mixing Portland cement with tap water using a water/cement weight ratio of 0.48. Bioconcrete specimens were prepared by centrifugation of bacterial cells during stationary phase followed by washing the cells with distilled water and diluted to 10^7 cells/ml concentration[11]. Cement, sand and aggregate were mixed in 1:1.5:3 proportion by weight. When the mixture was set they were subjected for curing and the compressive strength for 28 , 50, 75, 90 days were recorded.

2.4 Self healing capacity of Bioconcrete:

A fine lyophilized mixture of *Acinetobacter* M6 and calcium lactate pellets were added to the concrete mixture with and without addition of dried ginger powder in 0.1% concentration. A minor crack of 0.2 mm deep and 0.5 mm wide was created on the surface of the specimen. The goal

is to create a concrete mix that contains bacteria in lyophilized pellets, which has ability to form polysaccharide matrix that will germinate if water enters through a crack. The bacteria become active when contact with water and make limestone out of calcium lactate[12]. Formation of Calcite layer by the Acinetobacter was dried at room temperature subjected to SEM analysis.

$Ca(C_3H_5O_2)_2 + 7O_2$ ---- $-CaCO_3 + 5CO_2 + 5H_2O$

Calcium Lactate+ Oxygen ----Limestone + Carbon Dioxide+ Water[12]

3. Results And Discussion

Our preliminary results provide us *Acinetobacte*r M6 was showing thermophilic and alkaliphilic nature by growing at 55˚C and pH-12. Bacteria with standing in adverse conditions like high temperatures and in high alkaline environment inside the concrete is the most crucial factor.

The addition of *Acinetobacter* M6 at 10^7 cell/ml concentration gives the maximum improvement in compressive strength. The compressive strength for *Acinetobacter* M6 was due to the effect of polysaccharide matrix from the biofilm producing bacterium which fills the matrix. The addition of *bacterium* at 10^7 cell/ml concentration gives the maximum improvement at 0.1% dried ginger powder concentration in compressive strength. It appeared almost 19 % stronger than control specimens. The biofilm producing bacteria has ability to produce Exopolysaccharaides which absorbs water. The addition of calcium lactate has no affect on compressive strength of the concrete cubes.

The calcite precipitation in the crack was clear and visible after 3 days after curing. *Acinetobacter* M6 started to covert calcium lactate to make calcium carbonate to fill the cracks. Almost complete healing of crack was occurred after 24 days, Due to adverse conditions inside the concrete bacteria under stress produce biofilm matrix immediately and it prevents the water leakage into the cracks.

Type Of Additive	%increase (28 days)	%increase (50 days)	%increase (75 days)	%increase (90 days)
Acinetobacter pellets (10^7 cell/ml)	8	12	15	16

Table 1: Percentage Increase In Compressive Strength

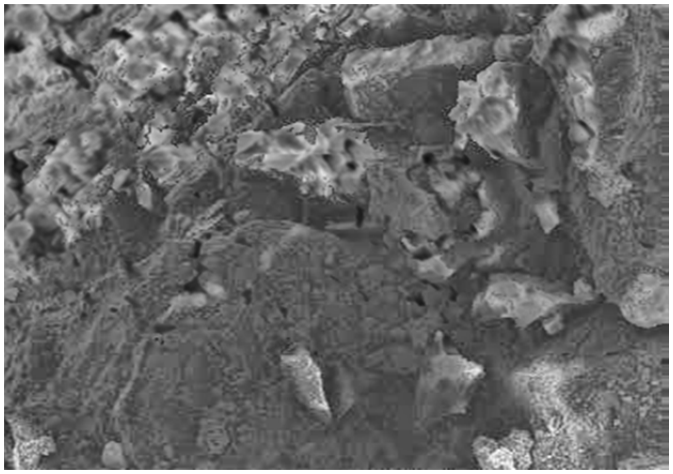

Figure 1: SEM Micrograph of bacterial concrete showing formation of calcite layer at 10^7/ml cell concentration

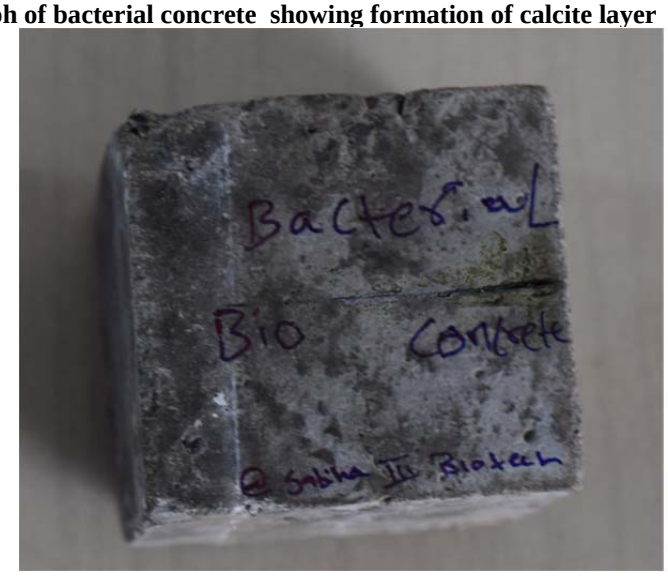

Figure 2: Crack Self-Healing capacity of biofilm producing *Acinetobacter* M6

4. CONCLUSION

The *Acinetobacter* M6 strain which has the capability of forming biofilm matrix helps the concrete in terms of durability and preventing corrosion. *Acinetobacter* showed the ability to tolerate a wide range of pH and temperatures to sustain in concrete for calcite precipitation. The nature of biofilm matrix forming and ability to sustain in adverse environmental conditions is the main advantage for *Acinetobacter* M6 to select as a crack sealing agent in bioconcrete .The maximum amount of calcite was deposited in the upper layer followed by middle and lower layer. Calcite precipitation occurred predominantly in the areas close to the surface of crack in concrete block. *Acinetobacter* M6 Cells grows both aerobic and anaerobic environments and induces active precipitation of calcium lactate present as its first feed and then acts on $CaCO_3$ around its surface. our preliminary study provides the results of Bacterial mediated calcite precipitation. More Research has to be focused on this line of study to materialize the things from laboratory scale to construction level. This is the first report on biofilm producing bacteria *Acinetobacter* M6 used in bioconcrete mixtures.

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