Research Article

Bioconcrete Enhancement from Biofilm Producing Marine Bacterium

Krishna Priya Kota*, Rohini Krishna Kota, John Babu Dulla, Abraham Peele Karlapudi
School of Biotechnology, Vignan University, Vadlamudi, Guntur, Andhra Pradesh, India.
***Corresponding author's E-mail:** youngscholar2013@gmail.com

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ABSTRACT

Concrete is one of the widely used construction materials. It has a variety of disadvantages that should be considered before use. One main disadvantage of concrete is that all structures made from it will crack at some point. Concrete can also crack as a result of shrinkage, which happens when it dries out. These cracks develop within a few days of laying the structure. Crack prevention is often employed by steel reinforcement which is highly expensive and requires large amounts of steel which will never be acceptable. In case of larger constructions such as big dams or projects, if there we observed any crack or corrosion, it becomes deadly difficult to go there and repair it. In such cases, the only way to get rid of all these disadvantages is the use of bio-concrete. Bio-concrete is said to be the new formulation of the concrete with bacteria. In the biosphere, bacteria can function as geo-chemical agents promoting the dispersion, fractionation and concentration of materials. Microbial mineral precipitation is resulted from metabolic activities of micro organisms. Based on this biominerology concept, an attempt has been made to develop bio-concrete material incorporating of an enrichment culture of thermophilic and alkaliphilic bacteria within cement sand mortar/concrete. The results showed a significant increase in compressive strength. Bioconcrete works well with its autonomous self healing capacity. These many applications and advantages of bio-concrete drawn our attention and made us to focus our interest on producing the bio-concrete from a thermophilic and alkaliphilic bacteria which is a new science, that serves a lot for mankind as well as for the environment.

Keywords: Acid fizz test, Bioconcrete, Biofilm, Compressive strength, Polysaccharide matrix.

INTRODUCTION

The current demand for the concrete is massive. For centuries, one of the most common materials used in construction has been concrete. Formed from hardened cement, concrete has been used for everything from driveways to home foundations. However, as technology advances concrete has not remained the end-all tool for building. It has a variety of disadvantages that should be considered before use. One main disadvantage of concrete is that all structures made from it will crack at some point. Concrete can also crack as a result of shrinkage, which happens when it dries out. These cracks develop within a few days of laying the structure. This will generally not limit the durability of a structure. Another disadvantage of concrete is its low-thermal conductivity. While concrete is normally used as a layer of fireproofing between walls, it can be badly damaged when exposed to intense heat. The ongoing research in the field of concrete technology has lead to the development of special concrete considering the speed of construction.[1] The concrete will help to contain the spread of a fire but will become unusable in the process. Concrete also easily corrodes when exposed to seawater. The effects are quick if the concrete is completely submerged for extended periods of time. Concrete can be worn away by waves and by the sand and other materials carried the ocean. Although concrete is the world's most used building material, it has a serious flaw. It can easily crack under tension. The researchers said, if these cracks become too large, they will lead to corrosion of the steel reinforcement, which not only results in an unattractive appearance but also jeopardizes the structure's mechanical qualities. Crack prevention is often employed by steel reinforcement which is highly expensive and requires large amounts of steel which will never be acceptable. Another disadvantage is, in case of larger constructions such as big dams or projects, if there we observed any crack or corrosion, it becomes deadly difficult to go there and repair it. In such cases, the only way to get rid of all these disadvantages is the use of bio-concrete. Bio-concrete is said to be the new formulation of the concrete with bacteria. In the biosphere, bacteria can function as geo-chemical agents promoting the dispersion, fractionation and concentration of materials. Microbial mineral precipitation is resulted from metabolic activities of micro organisms. Based on this, bio-minerology concept, an attempt has been made to develop bio-concrete material incorporating of an enrichment culture of thermophilic and alkaliphilic bacteria within cement sand mortar/concrete. The results showed a significant increase in compressive strength. The astonishing advantage of this bio-concrete is its self healing capacity. Self-healing concrete is a product that will biologically produce limestone to heal itself the cracks that appear on the surface of concrete structures. However it is as durable and strong as conventional concrete and can be used as a better replacement for the normal concrete. So it is more beneficial if we used the bio-concrete in such cases where the repair is tedious such as large dams. Bioconcrete works better with its autonomous self healing capacity.[2] Bio mineralization is a biologically induced process which occurs in some bacteria, has been investigated due to its wide range of scientific and technological implications in which an organism creates a local micro-environment with conditions that allow optimal extracellular chemical precipitation of mineral phases.[3] Bacterial spores are can with stand extreme mechanical and chemical stresses are remain dormant for years but viable bacterial spores immobilized in the concrete matrix will become metabolically active when they get contact with water entering freshly into the concrete. So by the metabolic action of the bacterial spores these cracks will subsequently sealed by a mechanism known as calcium carbonate precipitation. The alkaline environment of concrete with pH around 12 is the major hindering factor for the growth of bacteria, so that more research has been focused on alkaliphic microorganisms.[4] Bacterial surfaces play an important role in calcium precipitation. Due to the presence of several negatively charged groups, at a neutral pH, positively charged metal ions could be bound on bacterial surfaces, favouring heterogenous nucleation. Commonly, carbonate precipitates develop on the external surface of bacterial cells by successive stratification and bacteria can be embedded in growing carbonate crystals and this embedded bacteria in the form of crystals live longer.[5,6] The size of bacterial inoculum and survival of bacteria potentially influences bacterial calcification. Recent studies suggest that survival of bacteria inside carbonate crystals for up to 330 days to 3 years. Significant reduction in the viable cells was noticed after 330 days, no cells are viable. So the task is that survival of bacteria inside cracks can be evaluated. Some bacteria have the ability to produce endospores to endure an extreme environment there by increasing the compressive strength and stiffness of cracked concrete specimens.[7] In this paper, compressive strength of the bacterial concrete are carried out. The goal is to create a concrete mixture that contains bacteria which has ability to form polysaccharide matrix, biofilm[8,9] that will germinate if the water enters through a crack. The bacteria and calcium lactate are both embedded in ca psules, to prevent interaction before the crack appears. The bacteria added to the concrete mixture in the form of spores gets activated when they get contact with water when ever any crack occurs and the bacteria grow active to make the limestone out of calcium lactate there by it grows and forms polysaccharide matrix called biofilm. This biofilm matrix entraps the water to not to enter in to cracks thereby preventing corrosion. When the living conditions become unfavorable again, the bacteria will form spores, the added capsules open when cracks appear and the water leak inside which the bacteria gets activated and get in contact with calcium lactate and will produce limestone by forming extracellular matrix through cell to cell communication by a mechanism known as quorum sensing. Lime stone will fill the cracks and there is no possibility for water to leak in to the concrete anymore. The biofilm producing alkaliphilic bacteria are able to heal the cracks. Cubes with three different cell concentrations are prepared and the compressive strength for each 7days, 14days and 28days is carried out. The results are compared with the control specimens to see the variation in the strengths. And the results are compared to find the optimum amount of bacteria to be added to the concrete to get the maximum compressive strength.

MATERIALS AND METHODS

The marine bacterium

Spore-forming alkali-resistant and thermophilic bacterium which is capable of forming biofilm was isolated from marine environment. To induce copious spore formation strain was cultivated in an alkaline medium containing 20 mM sodium citrate as energy and carbon source for growth. The medium was further composed of 0.2 g NH_4Cl, 0.02 g KH_2PO_4, 0.225 g $CaCl_2$, 0.2 g KCl, 0.2 g $MgCl_2.6H_2O$, 0.375 g KNO_3, 50 mM $NaHCO_3$, 50 mM Na_2CO_3, 1 ml trace elements solution and 0.1 g yeast extract per liter Milli-Q ultra pure water. Before addition to cement mixture for test specimen preparation, bacteria were cleaned from culture residues by repeated centrifugation and resuspension of obtained cell pellet in water.[7]

Acid fizz test for Lime stone production

The overnight culture of marine bacterium[8,10] was inoculated in Mineral Salt medium (MSM) with 5% calcium lactate pentahydrate was used without calcium lactate was kept control and incubate for 3 days at 37°C for the utilization of the calcium lactate by the bacterium to give the by product as limestone. After 3 days collect the supernatant by centrifugation at 16800g x 20 min at room temperature and Observe the effervescence as the gas appears as bubbles by adding 2 drops of 10% HCL

Culturing, Testing and Sample preparation

A series of experiments was conducted by growing marine bacterium from 25 to 55°C and alkali PH range of 7 to 12 to test the thermophilic and alkaliphilic potential of the bacterium. Biofilm ability of the bacterium was screened using crystal violet assay by tube method[11]. Four specimens (50 mm) were prepared by mixing ordinary 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 to remove chloride ions and the pellet was diluted to 10^5 cells/ml concentration. Mix proportion of cement, sand and aggregate (Chips) in 1:1.5:3 (by weight) and cell suspension cement ratio was fixed to 0.48 for a concrete grade of M30. Two mixtures were prepared one with cell suspension and other with water as control. When the mixture was set, it is then taken off from the specimen and it is then put for curing and the compressive strength for each 3days, 7days, 14days and 28days is carried out.

Table 1: Percentage Increase In Compressive Strength Due To The *Marine Bacterium* With Respect To The Control Cementitious Materials

Type of additive	Percentage increase of 7th day compressive strength	Percentage increase of 14th day compressive strength	Percentage increase of 28th day compressive strength
Marine bacterium	4	11	15

Figure 1: Positive result for Acid fizz test for Lime stone production

Figure 2: Marine bacterium growth at PH 12 (high alkaline) and Temperature 55 ˚C under aerobic and anaerobic condition

Figure 3: Bioconcrete of size 50mm cubes (A) CONCRETE (NO BACTERIA) (B) BIOCONCRETE (BACTERIA)

RESULTS AND DISCUSSION

The addition of *marine bacterium* at 10^5 cell/ml concentration gives the maximum improvement in compressive strength. It can be observed that the optimum concentration of *marine bacterium* concrete is also 10^5 cell/ml. The compressive strength for *marine bacterium* with the highest enhancement of strength is made from those of 10^5 cell/ml concentration. It is expected that the enhancement in strength was due to the effect of polysaccharide matrix from the biofilm producing bacterium that fill up the cement matrix. The addition of additional healing agents to the concrete may decrease the material properties; development of compressive strength of control specimen without additions as well as specimen with bacteria was investigated. Here incorporation of a high number of bacteria appeared to have a good effect on compressive strength development as bacterial test specimen appeared almost 15% stronger then control specimen at all tested times. Effect of bacteria on development of strength appeared however strongly dependent on compound identity. The biofilm producing bacteria has ability to form rich polysaccharide layer which absorbs the water and has the good potential to give the compressive strength to the concrete which was the most peculiar feature of this kind of bacteria. Additions of calcium lactate did not significantly affect strength development as control specimen.

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REFERENCES

1. Stocks-Fischer S, Galinat JK, Bang SS, "Microbiological precipitation of $CaCO_3$", soil biology and Biochemistry, 31, 1999, 1563-1571.

2. Dick J, De Windt W, De Graef B, Saveyn H, Van der Meeren P, De Belie N, Verstraete W, "Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species"Biodegradation, 17(4), 2006, 354-367.

3. Hamilton WA, Microbially influenced corrosion as a model system for the study of metal microbe interactions: a unifying electron transfer hypothesis, Biofoulin, 19, 2003, 65–76.

4. Rivadeneyra MAG, Delgado A, Ramos-Cormenzana, Delgado R, Biomineralization of carbonates by *Halomonas eurihalina* in solid and liquid media with different salinities: crystal formation sequence, Res. Microbiol., 149, 1998, 277–287.

5. Castanier S, Le Metayer-Levrel G, Perthuisot JP, Ca-carbonates precipitation and limestone genesis—the microbiogeologist point of view, Sediment. Geol., 126, 1999, 9–23.

6. Zamarreno DV, Inkpen R, May E, Carbonate crystals precipitated by freshwater bacteria and their use as a limestone consolidant, Applied and Environment Microbiology, 75, 2009, 5981-5990.

7. Jonkers HM, Schlangen E, A two component bacteria-based self-healing concrete. In MG Alexander, H-D Beushausen, F Dhen & P Moyo (Eds.), Concrete repair, rehabilitation and retrofitting II, London: Taylor & Francis group, 2009, 215-220.

8. Abraham PK, Srinivas J, Venkateswarulu TC, Indira M, John babu D, Diwakar T, Vidya PK, Investigation of the Potential Antibiofilm Activities of Plant Extracts, International Journal of Pharmacy and Pharmaceutical Sciences, 4, 2012, 282-285.

9. Vidya Prabhakar Kodali, Abraham Peele Karlapudi, Madhuri Kotam, Rohini Krishna Kota, Tejaswini Punati and Rajendra Babu Byri. Plant Extracts as Antibiofilm Agents, Int. J. Pharm. Sci. Rev. Res., 21(1), 2013, 325-328.

10. Kodali Vidya Prabhakar, Gondhi Nikhil Chakravarthy, Sai Sushmita Tangirala, Madhuri Munukutla, Sravani Pathuru and Karlapudi Abraham Peele. Role of eps and bio surfactant in the biodegradable plastic making based on vegetable waste, Int. J. Pharm. Sci. Rev. Res., 21(1), 2013, 56, 329-332.

11. Kokkiligadda Sujana, Karlapudi P. Abraham, Indira mikkili, Kodali V Prabhakar, Biochemical and Molecular characterization of biofilm producing bacteria, Int J Pharm Bio Sci, 4(1), 2013, (B) 702 – 712.

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