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Development of bioconcrete material using an enrichment culture of novel thermophilic anaerobic bacteria

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In the biosphere, bacteria can function as geo-chemical agents, promoting the dispersion, fractionation and/or concentration of materials. Microbial mineral precipitation is resulted from metabolic activities of microorganisms. Based on this biomineralogy concept, an attempt has been made to develop bioconcrete material incorporating of an enrichment culture of thermophilic and anaerobic bacteria within cement-sand mortar/concrete. The results showed a significant increase in compressive strength of both cement-sand mortar and concrete due to the development of filler material within the pores of cement sand matrix. Maximum strength was observed at concentration 10^5cell/ml of water used in mortar/concrete. Addition of *Escherichia coli* or media composition on mortar showed no such improvement in strength.

Keywords: Aanaerobic bacteria, Compressive strength, Concrete, Microbial precipitation, Mortar, Thermophilic bacteria

Bacteria are the most abundant and metabolically diverse forms of life on earth. They grow under a wide range of geochemical conditions in an unparalleled variety of habitats. Basically, microbial life exits wherever there is liquid water at temperatures from –7°C to about 120°C (ref.1). In the biosphere, bacteria can act as geo-chemical agents, resulting in the concentration of materials. This induces the formation of special minerals, which constitute an area of research of growing interest known as biomineralization[2]. Prokaryotic microorganisms, or bacteria, are remarkably potent agents of biomineralization too[1]. Use of this biomineralogy concept leads to the potential invention of a new material, Bacterial Concrete, an inherent and self-repairing biomaterial that can remediate the cracks and fissures in concrete[3]. Though concrete is quite strong mechanically, it suffers from several drawbacks, such as low tensile strength, permeability to liquid and consequent corrosion of reinforcement, susceptibility to chemical attack and low durability[4]. Modifications have been made from time to time to overcome such difficulties of concrete but all those processes are not easy and good[5]. Recently, microbial remediation of concrete has been started to solve these difficulties. Some bacterial species e.g. *Bacillus pasteruii* or *Pseudomonas aeruginosa* along with cement-sand produce minute particles within the matrix that can be used as a filling material to remediate cracks in structures[3,6]. This method was already showing positive results in the field of enhanced oil recovery, prevention of acid mine drainage, prevention of leaching in channels and other such areas[1].

In the present study, the aim is to develop an environmentally accepted, useful bio-concrete material using an enrichment culture of thermophilic and anaerobic bacteria to increase the strength in concrete structures. This bacterium was isolated and characterized by Prof. S. Pal and his colleagues of Jadavpur University from the mud of hot springs of Bakreswar, West Bengal[7] and the bacterium was found to belong to Shewanella-related thermophiles. This bacterium is environmentally innocuous and handling the bacterium creates no problem.

Materials and Methods

Fine chemicals were purchased from Sigma Chemical Co, USA and Jain Biological Pvt. Ltd,

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India. Bacterial strain was obtained from Prof. S. Pal's Laboratory, Department of Life Science and Biotechnology, Jadavpur University. *Escherichia coli* strain *DH-5α* was obtained from Institute of Microbial Technology, Chandigarh, India. Cement used was of grade 53 ordinary Portland cement and natural sand passing through 4.75 mm sieve and retaining on 0.75mm sieve was used. Graded coarse aggregate of maximum size 10 mm was used for the concrete specimens.

Preparation of media—The bacterium used is water grown hot spring bacterium that has some unique requirement of oxidizing agent[8] and in the Semi synthetic media, iron (0.1 M) was used in +III state, which accepts electron and itself goes to +II state. Final media was prepared by mixing media 1 and media 2 in 1:9 ratios and *p*H was kept at 7.5. Media 1 contains iron in +III state as FeOOH and media 2 contained sodium dihydrogen phosphate (0.6 g/1000 ml), potassium chloride (0.33 g/1000 ml), sodium carbonate (2.5g/1000 ml), yeast extract (0.02%) and peptone (0.5%).

Media 1 composition:

$FeCl_3 + 3\ NaOH \rightarrow Fe(OH)_3 + 3\ NaCl$

$FeCl_3 + 3\ NaOH \rightarrow FeO(OH) + H_2O + NaOH$

Growth condition—The bacteria being anaerobic, was grown in sealed gas-pressure vials (100 ml of the vial contains 30 ml growth medium). Air content in the sealed vial was replaced totally by carbon dioxide using syringe-needle system before inoculation of bacterial culture as described earlier[9]. The inoculated cultures were kept in incubation at 65°C for 6 to 8 days.

Preparation of standard curve (OD vs. cell count—Bacterial culture (1 ml; 4-5 days old) was taken in a sealed vial and diluted to 10 times by adding 9 ml of sterilized growth media. From this diluted cell culture 10 times dilution was made again and so on. In this way different cell concentrations were prepared. Optical density of each cell concentration was then measured against the blank media at 620 nm in colorimeter (ERMA INC, AE 11M). Then by counting the cell number in Hemocytometer of each cell concentration, standard graph of cell number vs. optical density was prepared. This graph was used to determine the cell concentration of any culture under study just by observing the O.D at 620 nm of that culture.

Scanning electron micrograph—For scanning electron microscopy, bacterial cells were fixed with 2.5% (v/v) gluteraldehyde in culture medium for about 24 hr at room temperature. Samples were dehydrated by incubation for 15 min in each of a graded aqueous acetone series (20, 40, 60, 80 and 100% acetone). The samples were air dried and transferred onto SEM alumina supports and sputtered with gold by a coater of Blazers (type 07120-A). Photomicrographs of bacterial cells were taken at different magnification in SEM (Model: Jeol JSM 5200).

Testing of mortar/concrete specimens—Bacterial cells at different concentrations (concentration ranges from 0 to 10^7 cells/ml of water used) were mixed with cement and sand mortar/concrete during casting. Before adding to the mortar and concrete mixture, the media containing bacteria was centrifuged and washed several times with deionized double distilled water to remove chloride ions present in the bacterial growth medium. For mortar, cement to sand ratio was fixed at 1: 3 (by weight) and water cement ratio was taken 0.4 (ref.10). For concrete specimens, the designs mix proportion of cement, sand and aggregate was taken as 1: 1.5: 3 (by weight) and water cement ratio was fixed 0.48 (ref.10) for a concrete grade of M_{30}. The specimen size was standard 70.7 mm cubes for mortar and 100 mm cubes for concrete. The cube size of 100 mm is chosen to control of the specimen in better way and to reduce the quantity of microorganism requirements at a particular set. All the specimens were well compacted in the vibration machine. Similarly *DH-5α* was also used for mortar for comparison. For each concentration of bacterial cells, three samples were prepared at a time for both mortar and concrete. After casting all the samples were subjected to water curing for different days (7, 14 and 28 days) before testing the compressive strength in Compressive testing machine. Every set up of experiments was repeated for at least six times to get an average statistical data.

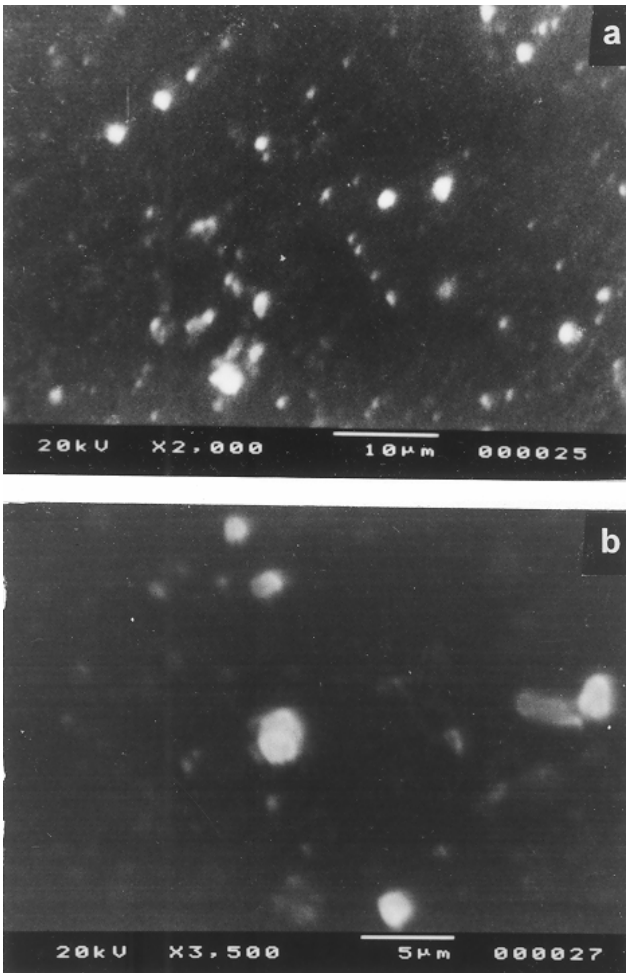
The media (without bacteria) with different concentrations of $FeCl_3$ (0.05, 0.l0 and 0.20 *M*) were also added with cement-sand mortar to check whether the media composition has any active role on strength improvement. Even dead cells at different concentration of the anaerobic bacterium were incorporated in mortar, to study its effect on the strength of the mortar.

Results and Discussion

From scanning electron micrographs of the bacterial cells at two different magnifications, it was

seen that the bacteria is regular coccoid type cell (Fig. 1a and 1b). The overall compressive strength of mortar containing live cells was increased substantially compared to control specimens at all ages (Table 1). Similar strength increment was also noticed for concrete with bacteria cells. It was noted that the 7, 14, and 28-day strength of both mortar and concrete showed an initial increase in compressive strength with the increase of cell concentrations and maximum strength was found at about 10^5 cells /ml of water used. The percentage increment of compressive strength was found to 22.62% in concrete and 19% in mortar with respect to control at 10^5 cells /ml of water used. From the observation of scanning electron micrographs of mortar samples, some crystalline structures were found inside the pores of the mortar. It was also observed by mercury instrusion porosimeter experiment that the pores having diameter above 10 μm within the mortar matrix was partially filled up by spatial growth of the bacterial cells[11]. Thus the increment of compressive strength of both mortar and concrete may be due to the deposition of some minute filler material produced by the bacteria that reduces the pore sizes and also modifies the pore size distribution of the mortar and concrete or both. An increase in bacterial cell concentrations, above 10^5 cells/ml of water used, however, reduces the strength of cement mortar and concrete both and this reduction of compressive strength may be due to some disruption of mortar and concrete matrix integrity with higher cell concentrations as suggested by Ramachandran *et al*[3]. Incorporation of *DH-5α* at different cells concentrations did not show any increment of compressive strength, which established the fact that the unique property of strength improvement is not a common property (Table 2). Bacterial growth medium containing $FeCl_3$ also showed no such strength improvement rather it showed slight strength decrement (Table 2). The optimum *p*H of the growth medium for the bacterium was 7.5. Although it was found that the bacterium can grow well at *p*H 11. Above this *p*H, the bacterial growth was inhibited to some extent.

In conclusion it may be inferred that the enrichment culture of the particular anaerobic thermopile grows inside the concrete or mortar matrix and produces minute particles in the form of some crystals resulting in strength improvement by the refinement of pore structure. The major advantage for



Fig, 1—Scanning electron micrographs of Shewanella-related thermophile isolated from hot springs of Bakreswar, West Bengal, at two different magnifications (a & b).

Table 1—Compressive strength values of 7, 14 and 28 days test with Portland cement (A) mortar cubes and (B) concrete cubes mixed with different cell concentration
[Values are mean ± SD]

Bacteria, cell/cm^3			7-days (Mpa)	14-day (Mpa)	28-day (Mpa)
Control	0	A	27.30 ± 0.43	33.20 ± 0.72	40.67 ± 1.08
		B	30.21 ± 0.41	32.53 ± 0.46	39.33 ± 1.15
Live cell	10^1	A	27.54 ± 0.21	34.11 ± 0.21	41.12 ± 0.99
		B	30.88 ± 0.43	33.00 ± 0.27	42.37 ± 0.59
	10^2	A	28.00 ± 0.11	35.00 ± 0.18	42.86 ± 0.81
		B	31.12 ± 0.14	33.11 ± 0.51	44.67 ± 0.58
	10^3	A	28.12 ± 0.33	35.32 ± 0.32	43.73 ± 0.23
		B	32.00 ± 0.61	33.74 ± 0.32	45.00 ± 1.00
	10^4	A	28.45 ± 0.25	38.47 ± 0.50	44.80 ± 0.53
		B	33.11 ± 0.33	35.38 ± 0.63	47.67 ± 2.51
	10^5	A	29.51 ± 0.41	40.53 ± 1.17	48.40 ± 0.80
		B	33.91 ± 0.24	37.49 ± 0.51	48.23 ± 2.63
	10^6	A	28.82 ± 0.23	39.07 ± 0.37	45.40 ± 1.00
		B	33.87 ± 0.31	36.80 ± 0.87	46.67 ± 2.16
	10^7	A	28.52 ± 0.34	37.53 ± 1.05	44.00 ± 1.11
		B	33.12 ± 0.23	35.05 ± 0.53	43.00 ± 2.64

Table—2 Compressive strength values of 28-day test with Portland cement mortar cubes mixed with different type of biomass and media

[Values are mean ± SD]

Control	0	41.20 ± 2.08
DH-5α	10^4	40.60 ±2.27
(cell/cm^3)	10^5	40.53 ±2.97
	10^6	42.53 ±1.27
Media	0.05 *M* $FeCl_3$	37.20 ± 0.60
	0.10 *M* $FeCl_3$	38.00 ± 0.55
	0.20 *M* $FeCl_3$	37.40 ± 0.60

using this anaerobe in concrete technology is that the bacteria being anaerobic and water grown, it grows well inside the concrete or mortar matrix without supply of oxygen or food. The strength improvement was not observed with the addition of heat killed dead bacterium (results not given). This is only a preliminary investigation and needs more detailed study to establish this novel methodology.

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