

STUDY OF STRENGTH PARAMETER OF CONCRETE BY REPLACING CEMENT BY FLYASH ENRICHED WITH MICROBIAL AGENTS

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Abstract - In construction industry, Concrete is a most widely used construction material and cement is the only manufactured material and other ingredients like coarse aggregate, fine aggregate and water are natural resources. In manufacturing of cement 800 to 900 kg of CO₂ is emitted per ton ordinary Portland cement production and it amounts to 5% of the annual anthropogenic global CO₂ production, which results in global warming. To reduce the harmful effects of CO₂ and to save the power, in this project it is decided to use bacteria in concrete and to enhance its properties of hardened concrete. For this purpose *Bacillus sphaericus* bacteria is chosen based on previous works done. The design mix is made to produce M₄₀ grade concrete. The ingredients for concrete are tested. The bacterial culture is made and growth curve is prepared. Calcium carbonate precipitation is identified by scanning electron microscopy results and elements present in the bacterial culture is analyzed by energy dispersive X-ray spectroscopy results. It is proposed to partial replacement of cement by fly ash enriched by bacteria and to test the strength of concrete at different ages.

Key words: *Bacillus sphaericus*, CaCO₃, Compressive Strength, Fly ash, SEM, EDS.

1. INTRODUCTION

Concrete is weak in tension and brittle, yet it is used in construction of bridges, tall buildings, off shore structures, runways, pavements, railway sleepers, tunnel lining etc. because of its versatility. The estimated quantity of concrete usage is 10 km³ per year. Many concrete structures face premature degradation problems like

carbonation, chloride attack which results in deterioration problems and leads to repair and retrofitting of the structures. Due to the high emission of CO₂ in manufacturing of cement there is an urgent need to minimize the usage of cement by using the recommended cementitious material like fly ash, silica fume, blast furnace slag, metakaoline in concrete as partial replacement to cement. The usage of the above materials in single or combinations of the above is being researched for its beneficial characteristics. In addition to them for enhancing performance of concrete, the induction of bacteria into concrete system is also found in recent era. In this study, *Bacillus sphaericus* that is abundant in soil has been used to induce CaCO₃ precipitation. The "Bacterial Concrete" is a concrete in which bacterial culture is mixed to prepare concrete. Deposition of calcium carbonate in concrete enhances the properties of concrete.

2. MATERIALS AND METHODS

2.1 Selection of Bacteria

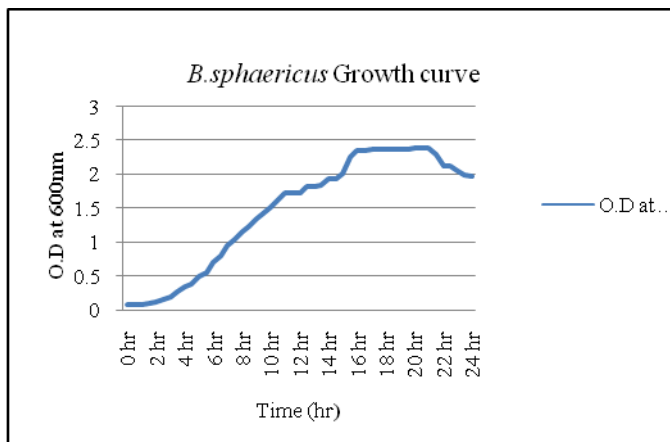
In previous works *Bacillus pasteurii* was the only well-known species used to precipitate the calcium carbonate. *Bacillus sphaericus* was yet another partially characterized species with similar entity, having the capability of precipitating calcium carbonate. Earlier researchers have shown very less implementation of the organism in remediation aspect. *B.sphaericus* was used for this study. Selection of this strain was based upon earlier work by our research group. This strain showed a high urease activity, a very negative ζ -potential and a continuous formation of dense calcium carbonate crystals in liquid medium.

2.2 Culture Collection

B.sphaericus, a universally known ureolytic bacteria was obtained from Microbial Type Culture Collection Centre (MTCC), Delhi. The optimum temperature specified for its growth was 30°C. The culture was maintained on nutrient agar slants at 4°C and sub cultured at every four-week interval on the filter-sterilized medium.

2.3 Growth curve for *Bacillus sphaericus*

The maximum OD was seen between 5 to 15 hours and referred as log phase. Spectrophotometer reading showed that the cultures reached the stationary phase between 16 to 21 hours. But after 21 hours bacterial growth was inhibited, due to the depletion of media components and may be due to release of secondary metabolites.



Graph 1: indicates different growth phases of *Bacillus Sphaericus*

Optimization

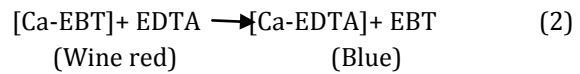
The media optimization was done including the optimization of pH and Temperature.

2.4 Calcium carbonate estimation

1 ml of the media was used for the titration along with Eriochrome Black T (EBT) as the indicator; and the appearance of the wine-red color indicated the end point of the titration. It is a complex metric titration where the media sample is titrated with standard solution of di-sodium salt of EDTA using EBT indicator. When the EBT indicator was added to the media at pH = 10, the calcium present in media forms a wine-red color Ca-EBT complex, which is unstable.



During the titration with EDTA, the calcium first reacts to form Ca-EDTA²⁻ complex releasing the free indicator (blue) and the color changes from wine-red to blue at the endpoint.



2.5 Properties of Ordinary Portland cement

Ordinary Portland Cement 53 grade was used. It was tested as per Indian specifications and its properties are given in table 1.

2.6 Properties of Fly ash

Physical and chemical properties of fly ash from Ennore Thermal Power Plant (Tamil Nadu) was analyzed as per IS 3812-1981. Fly ash is conformed as class - F, results are given in table 2 and 3 [11].

2.7 Properties of Fine and Coarse aggregate

Natural sand with a 4.75 mm maximum size as fine aggregates and coarse aggregate with 12.5 mm nominal size was used. They were tested as per Indian Standard Specifications IS: 383-1970. As per code, sand is conformed as Zone II. Their physical properties are given in Table 4.

2.8 Preparation of Test Specimens

The concrete cube size used for testing is 100 x 100 x 100 mm. The concrete mix design was carried out as per IS 10262-2009 for M40 grade of concrete. Mix ratio is 1:1.23:2.3 with w/c ratio 0.40. Fly ash was added by replacing the amount of cement at the concentrations of 0%, 10%, 20%, 25%, 30%, 35% and 40%. The concrete cubes with and without bacteria were cast and cured for 28 days.

2.9 Scanning Electron Microscopy

The precipitation of calcite in bacterial culture was analyzed under SEM. The samples are taken from medium of bacterial culture was converted into lyophilized powder.

2.10 Energy Dispersive X-Ray Spectroscopy

Elemental and chemical compositions of the deposits on the coverslips were analyzed with an energy dispersive X-ray spectroscopy (EDS) system attached to the SEM from the lyophilized bacterial culture, the chemical characterization was analyzed under EDS results.

Table - 1: Physical properties of ordinary Portland cement

Fineness of Cement	3.4 %
Standard Consistency	31.5 %
Initial Setting Time (min)	36 min
Final Setting Time (min)	215 min
Specific gravity	3.14

Table - 2: Physical properties of fly ash

Specific gravity	2.12
Specific surface area(m ² /g)	1.240
Moisture content (%)	0.200
Wet density (g/cc)	1.75
Turbidity (NTU)	459
pH	5.86

Table - 3: Chemical properties of fly ash

SiO ₂	56.77
Al ₂ O ₃	31.83
Fe ₂ O ₃	2.82
CaO	0.78
K ₂ O	1.96
TiO ₂	2.77
Na ₂ O	0.68
MgO	2.39

Table - 4: Physical properties of fine and coarse aggregates

Specific gravity of Fine aggregate	2.5
Specific gravity of Coarse aggregate	2.79

3. RESULT AND DISCUSSION

3.1 Optimization

Optimum temperature required for growth of *Bacillus sphaericus* 37°C, and optimum pH is 7.4 was determined. *B. sphaericus* was cultured in the optimized medium, nutrient broth with the other components like sodium bicarbonate, ammonium chloride, urea, and calcium chloride for the production of calcium carbonate. Biochemical test for calcium ions within the bacterial solution was done using Calcium oxalate.

3.2 Calcium carbonate estimation

1ml of bacterial culture media was used for titration process along with EBT indicator it forms wine-red color. It was titrated with EDTA, the calcium present in the medium first reacts to form Ca-EDTA⁺² complex releasing the free indicator (blue) and the color changes from wine-red to blue at the endpoint.



Fig-1: Identification of Calcium carbonate by titration process

3.3 Scanning Electron Microscopy

Calcite precipitation in bacterial culture was carried out by SEM analysis. Fig.2(a) shows the SEM picture for 24 hours bacterial culture, whereas calcite crystal formation has easily seen in it. Fig.2(b) shows the high presence of calcite formation in 48 hours culture.

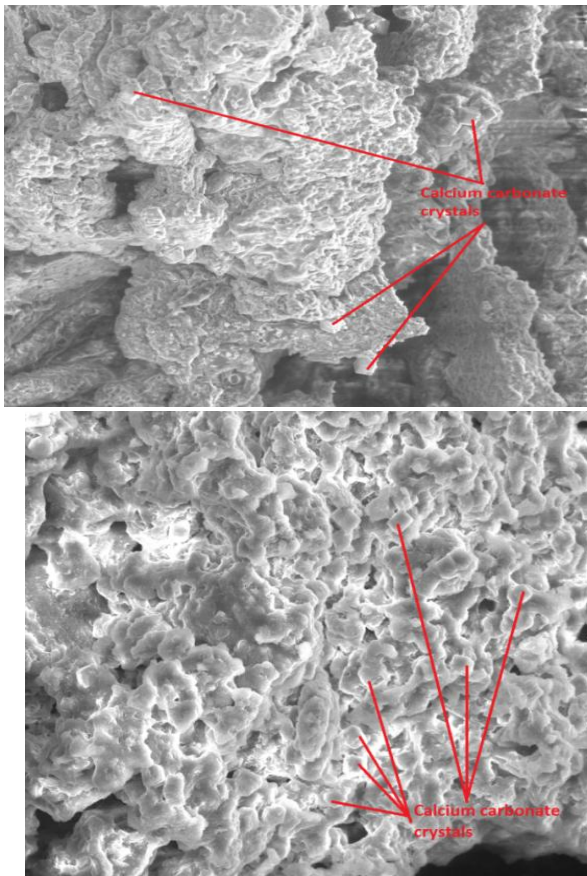
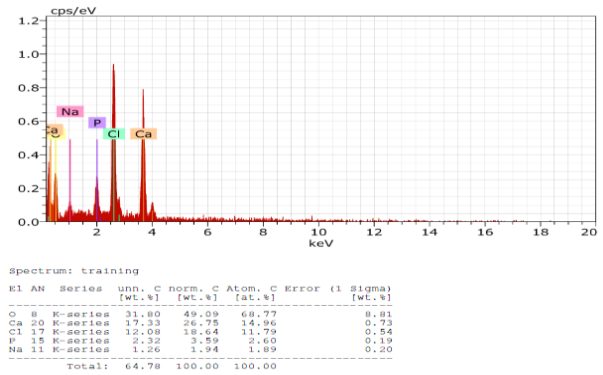


Fig-2: SEM analysis shows the presence of Calcium carbonate crystal in (a) 24 hours bacterial culture, (b) 48 hours bacterial culture.

3.4 Energy Dispersive X-Ray Spectroscopy

EDS is directly attached with SEM, it shows the elements and chemicals present in the samples. In this bacterial culture, the graph revealed that chlorine content is more. The process is going on for removal of chlorine from the optimized medium culture. 50-60% chlorine content is removed by filtration process after adding fly ash with bacterial culture. Fig.3(a) & 3(b) shows EDS analysis for 24 hours and 48 hours culture.

(a)



(b)

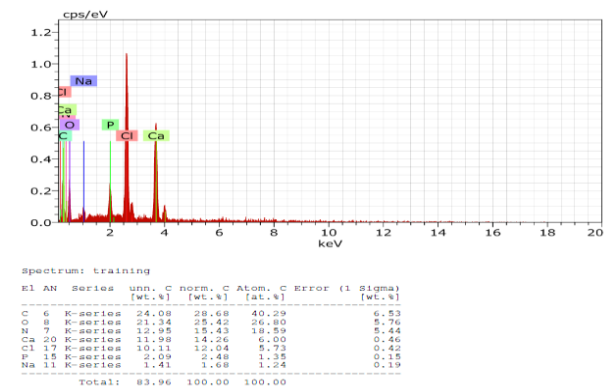


Fig-3: EDS analysis for (a)24 hours culture, (b)48hours culture.

3.5 Compressive Strength Results

In this study, fly ash mixed with bacterial culture before added to the concrete. Effect of *Bacillus sphaericus* bacteria on the 7th day compressive strength of concrete are given in Table 5 and shown in Fig.4.

Table - 5: Compressive strength results with and without bacteria

% of Fly ash	7 th day Compressive Strength (N/mm ²)	
	Without Bacteria	With Bacteria
0 %	33.2	
10%	39.6	20.3
20%	41.5	25.3
25%	39.9	31.76
30%	28.4	28.3

35%	26.6	37.4
40%	24.3	23.5

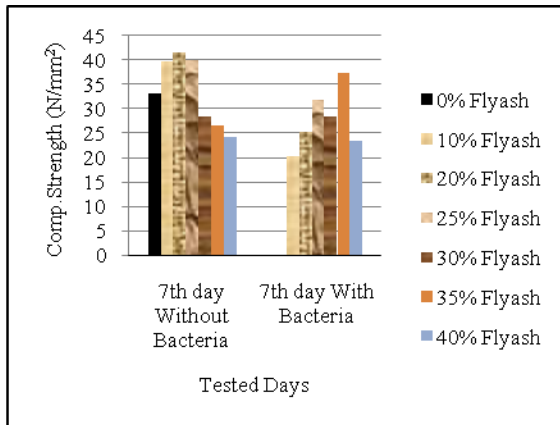


Fig-4: Effect of Bacteria on Compressive strength

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BIOGRAPHIES



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