

# Microstructural Characterization of Calcite Mineral Precipitation in Bacteria Incorporated Concrete

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**Abstract**— Metabolic activity of alkali-philic calcite ( $\text{CaCO}_3$ ) mineral precipitating bacteria when introduced into concrete heals the cracks and improves the microstructure of the concrete. This process of bio-mineralization by mineral producing bacteria can be characterized and quantified by using microstructure characterization techniques. The present paper is focused on characterizing the mineral precipitation in concrete by *Sporosarcina pasteurii* as calcite using SEM, XRD and TGA nano-characterization procedures to validate that cracks and pores in bacteria incorporated concrete were closed with the mineral precipitates produced due to ureolytic activity of bacteria by hydrolyzing the urea based nutrients.

**Keywords** — bacterial concrete, *Bacillus subtilis*, SEM, XRD, TGA

## 1. INTRODUCTION

The objective of this research was to observe the microstructure of bacteria induced cementitious specimens and to confirm the presence of calcite crystals using TGA, quantified by X-Ray Powder Diffraction (XRD) analysis and visualized by SEM. The above characterization studies establish the fact that the  $\text{CaCO}_3$  is precipitated in the concrete by *Sporosarcina pasteurii*. Bacterial mineral precipitates were visualized by SEM, quantified by XRD investigations and was also confirmed by TG/DTG Analysis. The samples for the tests were collected, from the bacteria treated cement mortar samples and from control specimens i.e., samples without bacteria, in the form of powders and/or broken pieces.

Aerobic alkaliphilic calcite mineral precipitating microorganism *Sporosarcina pasteurii* strain was isolated and *Sporosarcina pasteurii* is known for its high ureolytic activity and calcium carbonate mineral precipitation. Different cell counts of bacteria were derived from the bacterial growth culture by serial dilution method. The nutrients used to cultivate bacterial culture are – Urea, Peptone: 5 g/lit., NaCl: 5 g/lit., Yeast extract: 3 g/lit.

## 2. RESEARCH FINDINGS & RESULTS

### 1. Influence of cell count on the strength

Influence of bacterial strain count of *Sporosarcina pasteurii* on the strength of concrete is studied by preparing mortar samples with various counts of cells and tested for their compressive strengths. At cell count of  $10^5$  cells per 1

ml of mixing water the strength of concrete was found to be more so for further investigations on bacteria induced concrete this count is considered for optimal production of calcite mineral precipitation in the cracks and pores in the concrete. This process will enhance the pore structure of the bacteria induced concrete. Results are presented in Table 1 shows the bacteria cell count effect on the amount of calcite mineral precipitation in the concrete. More than the optimum bacteria cell count may affect the physical and chemical properties of the concrete matrix. Mortar cube samples were prepared with various cell counts of *Sporosarcina pasteurii* bacterial strain. The optimal bacterial cell count is chosen based on the sample which yields highest compressive strength. Different cell counts were derived from the bacterial growth culture by serial dilution method.

**Table 1 - Compressive Strength of mortar specimens made with various *B. subtilis* cell counts**

| Cells/ml of mixing water | Avg. Compressive Strength (MPa) $\pm$ Standard Deviation |                                |
|--------------------------|--|--------------------------------|
|                          | 28 days  | % Increase relative to control |
| Nil (Without Bacteria)   | 55.81 $\pm$ 0.10   | -                              |
| $10^3$                   | 57.23 $\pm$ 0.11   | 2.54                           |
| $10^4$                   | 59.52 $\pm$ 0.72   | 6.65                           |
| $10^5$                   | 65.79 $\pm$ 0.68   | 17.88                          |
| $10^6$                   | 62.21 $\pm$ 0.49   | 11.47                          |
| $10^7$                   | 54.66 $\pm$ 0.89   | -2.06                          |

### 2. Scanning Electron Microscopic (SEM) Investigations

SEM investigations on the broken pieces of mortar samples confirmed the presence of calcite mineral precipitates, Figure 1 shows SEM images of mortar sample induced with and without bacterial strain. It can be viewed that the concrete sample made with optimal count of bacterial strain has dense distribution of calcite mineral throughout its matrix.

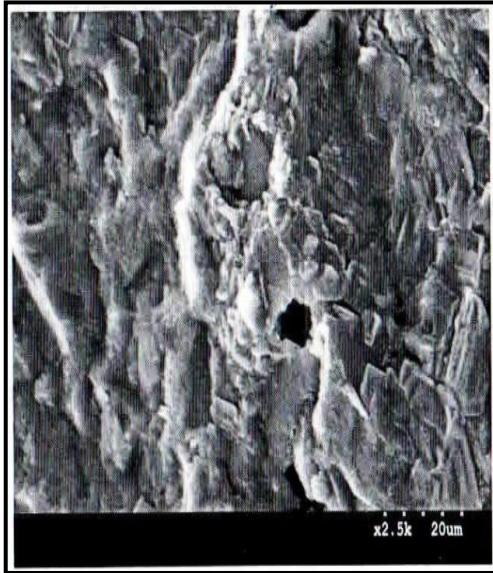
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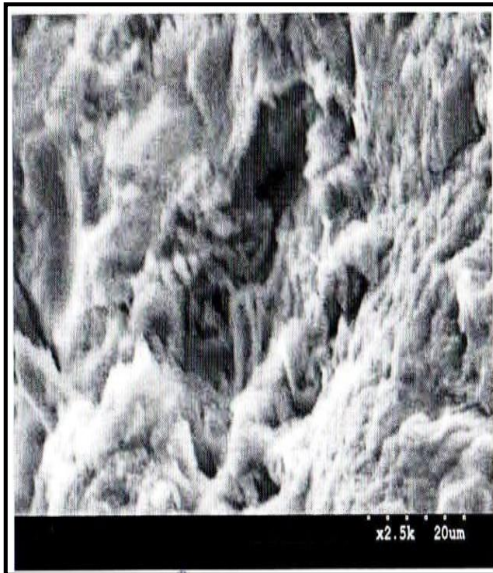
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# MICROSTRUCTURAL CHARACTERIZATION OF CALCITE MINERAL PRECIPITATION IN BACTERIA INCORPORATED CONCRETE



Control Specimen ( Without Bacteria)

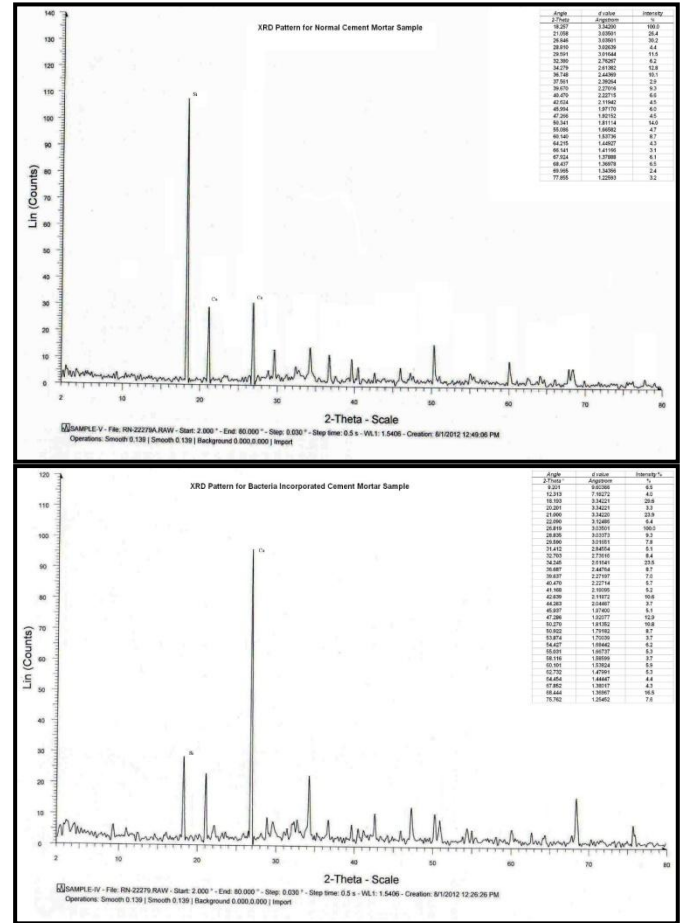


Bacterial Specimen with optimum  $10^5$  cell/ml

**Fig 1: SEM investigations on specimens without and with bacteria induced in different counts**

### 3. X-Ray Powder Diffraction (XRD) Analysis

Figure 2 shows the sketches of XRD plots of bacteria incorporated and normal mortar specimens. The peaks in the XRD plots shows the presence of each and every mineral present in the samples. In bacteria induced specimens, it can be seen that Ca mineral peak is higher than the sample without bacteria. This confirms the precipitation of calcite mineral due to microbiological activity in concrete.

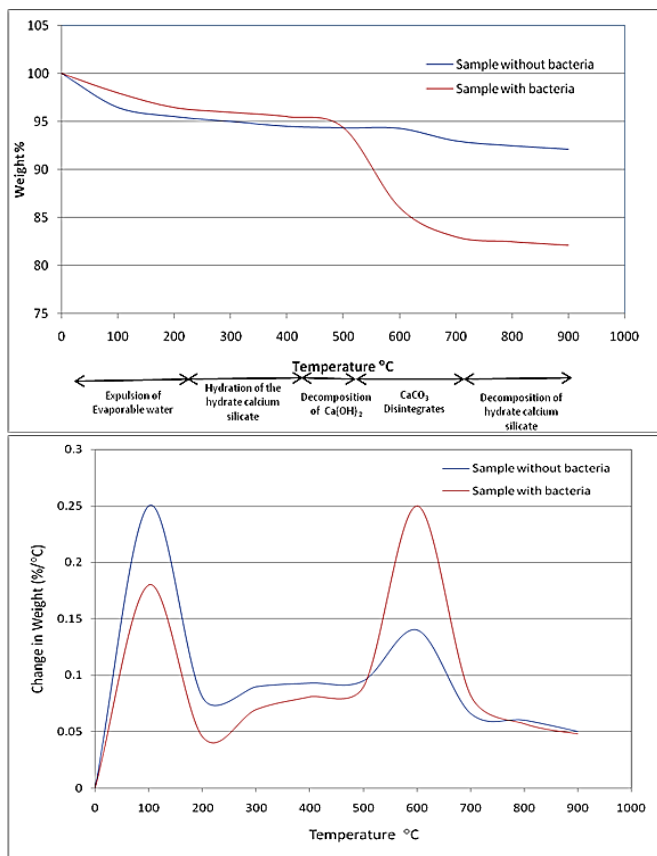


**Fig 2: XRD of bacteria induced mortar and concrete specimens**

### 4. Thermo-gravimetric (TG) Analysis

Thermo-gravimetric Analysis (TGA) is a technique which measures a sample's weight as it is heated or cooled in a furnace. TGA thermal curve is drawn between temperature on x-axis and weight (mg) or weight percent (%) on y-axis. The descending TGA thermal curve indicates the loss of weight. In this investigation, the bacteria induced sample is subjected to 50 to 900°C temperature. The calcite mineral precipitates in bacterial concrete when exposed to 500 to 700°C temperature will decompose and releases carbon dioxide due to which there will sudden loss of weight upto 20% in the bacterial concrete sample. This can be observed in Fig 3.





**Fig 3: Thermo-gravimetric Analysis on specimens with and without bacteria**

### CONCLUSIONS

Based on the above investigations, the following conclusions are drawn:

(1) The improvement in compressive strength is due to deposition on the microorganism cell surfaces and within the pores of cement–sand matrix, which plug the pores within the mortar. TG, SEM and XRD analyses reveal the growth of fibrous filler material within the pores due to the presence of such microorganisms. This growth is beneficial in the modification of the porosity and pore size distribution of cement mortar. Plugging of pores and cracks with calcite mineral precipitate reduces the permeability of concrete and eventually enhances the strength and durability of concrete.

(2) The SEM analysis revealed the dense growth of calcite crystals embedded with bacterial cells in bacteria incorporated specimen.

(3) The XRD scanning image of powdered bacteria incorporated sample endorses the existence of high amount of calcium carbonate precipitate.

(4) In the XRD pattern Figure 5.4 (a), of controlled specimen shows the presence of the relatively high amount of quartz mineral when compared to other minerals present in the sample. It can also be noted that the presence of calcite in control mortar sample is due to formation of hydrated C-S-H gel. In case of bacteria incorporated sample XRD spectra Fig 5.4 (b), confirms the presence of relatively high amount of calcite crystals when compared to other minerals present in the sample. This can be attributed to the copious deposition of  $\text{CaCO}_3$  in bacteria induced samples by *Sporosarcina pasteurii* during its microbial activity. So the presence of  $\text{CaCO}_3$  was substantiated using X-Ray

Diffraction (XRD) analysis. More number of calcite peaks suggests maximum calcite precipitation which would thereby reduce the pores in concrete.

(5) TGA analysis performed on powdered bacteria incorporated cement mortar sample showed an extreme loss of weight at 500 to 700°C confirming the presence of high amount of  $\text{CaCO}_3$ . It authorizes the presence of  $\text{CaCO}_3$  in bacteria incorporated specimens.

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