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Microbial activity on the microstructure of bacteria modified mortar

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ABSTRACT

Microbial modified mortar or concrete has become an important area of research for high-performance construction materials. This study investigates the effects of incorporating a facultative anaerobic hot spring bacterium on the microstructure of a cement–sand mortar. Environmental scanning electron microscopic (ESEM) views and image analysis (IA) of the bacteria modified mortar (thin-section) showed significant textural differences with respect to the control (without bacteria) samples. X-ray diffraction (XRD) study confirmed the formation of new phases of silicates (Gehlenite) within the matrix of such mortar material, which causes an improvement in the strength of the material. Electron probe microstructure analysis (EPMA) suggested that the bacterial treatment promoted uniform distribution of silicate phases and increased the calcium/silicon ratio within CSH gel of the matrices. The bacterium is found to leach a novel protein, which is capable of isolating silica from its source. The addition of such isolated protein, instead of the bacteria, into mortar also improves the strength of mortar.

1. Introduction

Biomineralization is a widespread complex phenomenon by which organisms form minerals, occurring in various geothermal systems. The process creates heterogeneous materials composed of biologic (or organic) and inorganic compounds like carbonate, phosphate, oxalate, silica, iron, or sulfur-containing minerals, with inhomogeneous distributions that reflect the environment in which they form [1]. Biologically induced mineralization is also an important geological process that helps in the formation of microfossil, hot spring deposition and transfer of chemical elements [2–4]. Bacteria are very small, but have the largest surface to volume ratio of any life form. Therefore, they provide a large contact area that can interact with the surrounding environments and are responsible for the transformation of at least one third of the elements in the periodic table [5]. The unique properties and functions of biomineralization have inspired innovative high-performance composites for construction applications, as well as other new materials [6–9].

Use of microorganisms within mortar/concrete leading to the process of biomineralization is now a potential field of research in concrete technology [10]. Recently, an inherent cement based biomaterial has been developed to remediate the cracks and fissures in concrete structures. Previous studies have shown that the addition of specific microorganisms to cement–sand mortar or concrete deposit inorganic substances inside the pores of the matrices, which can be used as a filling material to remediate cracks within the structures [11,12]. It was also noted that the addition of an anaerobic hot spring bacterium (closely related to *Shewanella* species) to the mortar/concrete could increase the compressive strength (25–30%) of the material with respect to control [12,13]. The biologically induced cement based material thus also exhibited better durability and crack repairing performance compared to normal concrete materials [14].

This paper studies in detail the role of such bacterium on the microstructure of the bacteria modified mortar. The microstructural analyses includes environmental scanning electron microscopy (ESEM), image analysis (IA), electron probe micro-analysis (EPMA) and X-ray diffraction (XRD) analysis. It is also noted that this bacterium releases a silica-leaching enzyme (protein) in its growth medium. This isolated protein also improves the strength of the mortar sample when added separately. Thus the silica-leaching protein identified within the bacterium may be used for the development of bacteria modified mortar in the future.

2. Experimental program

2.1. Bacteria and its growth condition

The bacterium was isolated from the crude soil samples of a hot spring at Bakreshwar, West Bengal, India. This is a facultative anaerobic and iron reducing bacterium and closely related with the *Shewanella* species [15]. It can be cultured anaerobically (in presence of CO_2 atmosphere) in sealed glass pressure vials in a growth medium containing $Fe(OH)_3$ – 0.1 M, Na_2HPO_4 – 0.6 g/L,

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KCl – 0.33 g/L, Na_2CO_3 – 2.5 g/L, yeast extract – 0.02% and peptone – 0.5% at pH 8.0 and 65 °C temperature. This bacterium can survive up to a pH 12.0 of the growth medium though its growth rate is retarded at this high pH. During its growth, the bacterium is found to leach few proteins in the medium and one of the proteins (molecular weight 26 kDa approximately) exhibits the catalytic property of silicatein as observed in the marine sponge [16].

2.2. Mortar sample preparation using bacteria

Ordinary Portland Cement 43 grade [17] and standard Ennor sand [18] were used for the study. Standard mortar cubes (70.6 mm × 70.6 mm × 70.6 mm) by mixing with bacteria were cast as described by Ghosh et al. [12]. Bacteria cell concentrations were used as 0–10^7 cells/ml water. Bacteria cells were collected from mature cultures (8–10 days) by centrifugation and washed with deionized sterile water. The precipitate of the bacteria cells was suspended in a small volume (1 ml) of deionized sterile water and its optical density (OD) was measured at 640 nm. The cell concentration was then determined from the standard curve (OD_{640} vs. cell number) prepared in advance. The dilutions of the bacteria cells were done accordingly with the deionized water and directly used for the preparation of mortar samples. No additional food material, except that present in the diluted cultures, was supplemented in the mortar cubes during mixing. For mortar preparation, the cement to sand ratio was taken as 1:3 and water to cement ratio was fixed at 0.4. All the specimens were cured under water after 24 h of casting. The compressive strengths of the mortar cubes were determined at 3, 7, 14 and 28 days of curing.

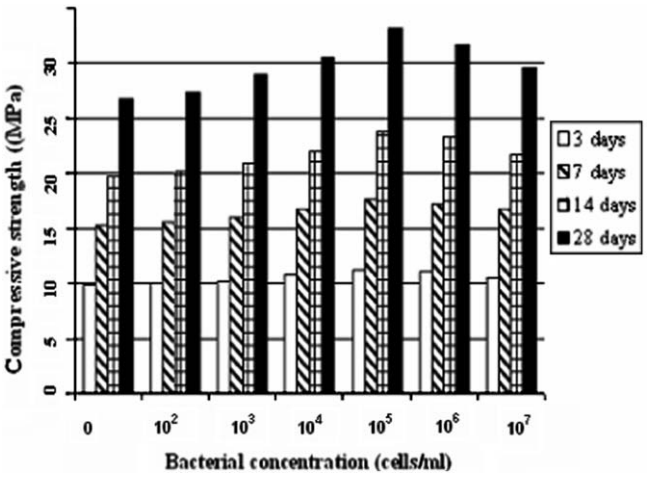

Fig. 1. Compressive strength of mortar vs. cell concentration at different ages.

2.3. ESEM analysis

The broken mortar samples (with/without bacteria) collected after compressive strength testing were dried for 24 h at 40 °C before being impregnated with epoxy resin (Epo-Fix Resin 150 g + Epo-Fix Hardener 18 g) in a plastic container. The samples with resin were kept in vacuum chamber for over night for solidification. The solidified samples were then cut into small pieces by diamond cutter. The samples were then polished for ESEM examination. Polishing of the samples was done first by SiC paper grinding (grade 120, 220, 320, 1200, 4000 successively of Struers, Denmark made) and then by DP (Diamond particle) spray grinding (6, 3, 1, 0.25 μm diamond crystals successively). After polishing, the samples were kept in the ultrasonic bath (a water bath fitted with ultrasonic wave generator) for few minutes to remove the diamond crystals if any remained on the polished surfaces. The polished samples were examined in ESEM (Philips-XL30-ESEM).

2.4. Image analysis

Thin sections were prepared by grinding the solid and sound mortar chips collected from the samples after the compressive

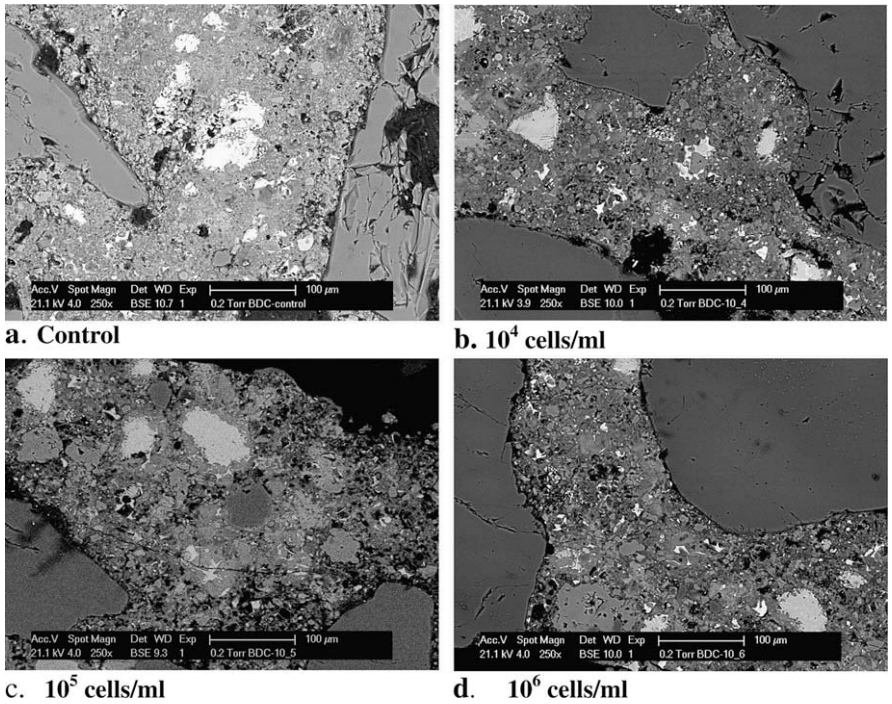

Fig. 2. SEM photomicrographs of mortar samples. (a) Control sample. (b) Bacteria treated (10^4 cell/ml) sample. (c) Bacteria treated (10^5 cell/ml) sample. (d) Bacteria treated (10^6 cell/ml) sample

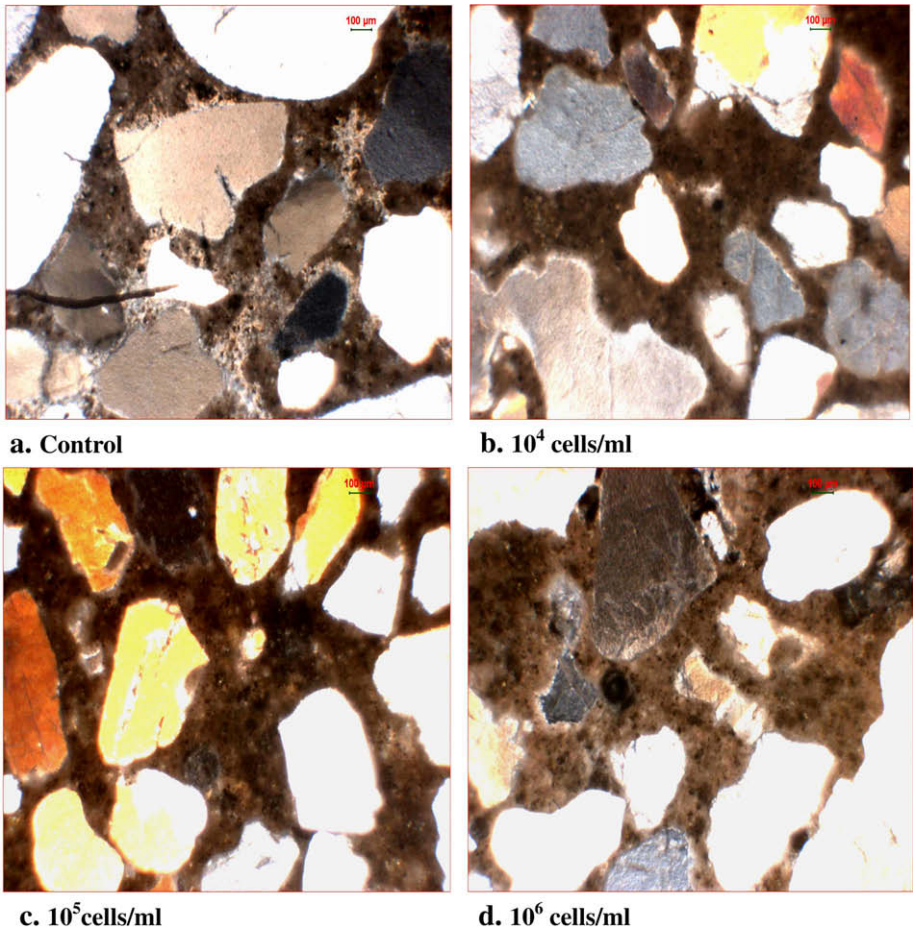

Fig. 3. Thin section image photomicrographs of mortar samples by image analysis. (a) Control sample. (b) Bacteria treated (10^4 cell/ml) sample. (c) Bacteria treated (10^5 cell/ml) sample. (d) Bacteria treated (10^6 cell/ml) sample.

strength tests with Carborundum (silicon carbide manufactured by Carborundum company), washed thoroughly in water and dried at a temperature of about 90–100 °C. The polished surface of the chip was then attached firmly to a glass plate (25.4 cm × 6.35 cm) with Canada balsam such that no air bubbles remained at the interface of the chip and glass plate. The other surface of the samples was then progressively thinned down to a thickness of about 0.03 mm by a second stage of grinding with very fine Carborundum powder over the free surface of the chip and thoroughly cleaned with a soft brush and alcohol to remove the Carborundum powder from its top surface and polished with a Samuel lather sheet and allowed to dry completely. Finally, a cover glass was placed on

Table 1
Comparison of minor peaks from XRD spectra of control and bacterial treated mortar samples with pure Gehlenite XRD spectra.

Gehlenite $2\theta/d/(I/I_0)$	Control $2\theta/d/(I/I_0)$	10^4 bacteria (Cells/ml) $2\theta/d/(I/I_0)$	10^5 bacteria (Cells/ml) $2\theta/d/(I/I_0)$	10^6 bacteria (Cells/ml) $2\theta/d/(I/I_0)$
20.978/4.231/2	20.960/4.235/14	20.960/4.235/14	20.960/4.235/12	20.980/4.231/14
23.130/3.842/1	–	23.120/3.844/1	23.140/3.840/2	23.144/3.842/2
32.930/2.718/5	–	–	32.900/2.716//4	–
35.394/2.534/4	–	35.404/2.531/3	–	–
37.531/2.394/12	–	–	–	37.520/2.395/1
47.268/1.921/8	–	–	47.280/1.921/4	–
48.813/1.864/4	–	–	48.860/1.862/2	–
49.111/1.853/2	–	49.120/1.853/1	49.150/1.852/2	49.080/1.854/3
50.325/1.812/8	50.240/1.814/17	50.240/1.814/27	50.260/1.814/27	50.260/1.814/17
50.780/1.796/1	–	50.780/1.781/2	50.760/1.797/5	50.820/1.795/1
52.092/1.754/25	–	–	–	52.100/1.754/1
61.063/1.516/9	–	61.045/1.509/1	61.080/1.516/4	61.020/1.517/1
61.458/1.507/1	–	61.460/1.507/3	–	–
61.735/1.501/1	–	61.740/1.501/3	61.742/1.500/4	61.740/1.505/1
63.151/1.471/2	–	–	–	63.200/1.470/1
64.931/1.435/3	–	–	–	64.900/1.435/1
65.303/1.428/1	–	–	65.280/1.428/1	–
67.445/1.387/2	–	–	67.440/1.387/1	
68.204/1.374/9	–	68.300/1.372/14	68.260/1.373/14	68.240/1.380/11
69.074/1.359/3	–	69.100/1.358/1	–	–

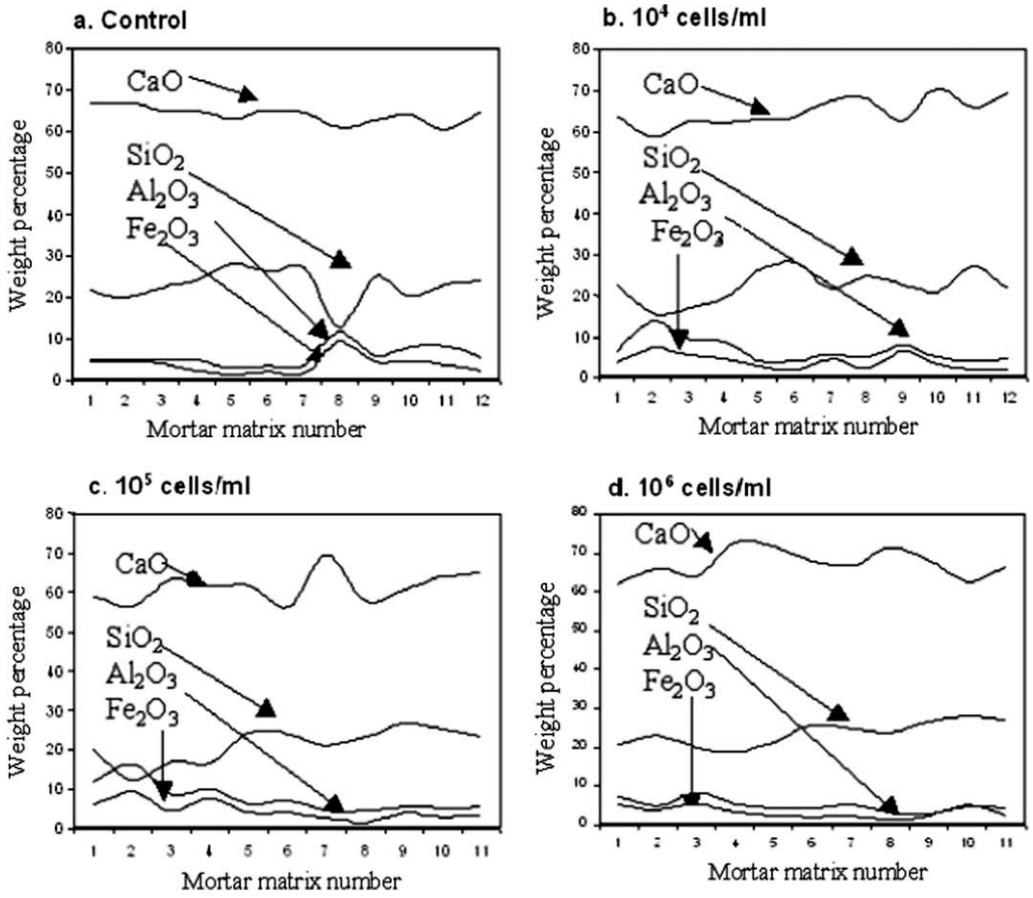

Fig. 4. Distribution of different oxide phases in the matrix of mortar samples. (a) Control sample. (b) Bacteria treated (10^4 cell/ml) sample. (c) Bacteria treated (10^5 cell/ml) sample. (d) Bacteria treated (10^6 cell/ml) sample.

the top surface of the thin section with a veneer of Canada balsam at the interface of the chip and cover glass [19]. The thin section slides were finally examined by LEICA microscope using QWIN software [20] for textural analysis. At least three thin sections were prepared for each mix. A typical micrograph of each mix is presented later.

2.5. XRD analysis

Mortar samples of each mix (after 28 days curing) were taken and crushed into fine powder by pestle-mortar. The powder samples passing a sieve size of 5 μm were analyzed in a powder X-ray diffractometer (Bruker D8 APD). The XRD spectrum were taken from $2\theta = 10^\circ$ to $2\theta = 70^\circ$. The peaks in the new positions of the spectrum were marked, compared and identified from the JCPDS data file [21].

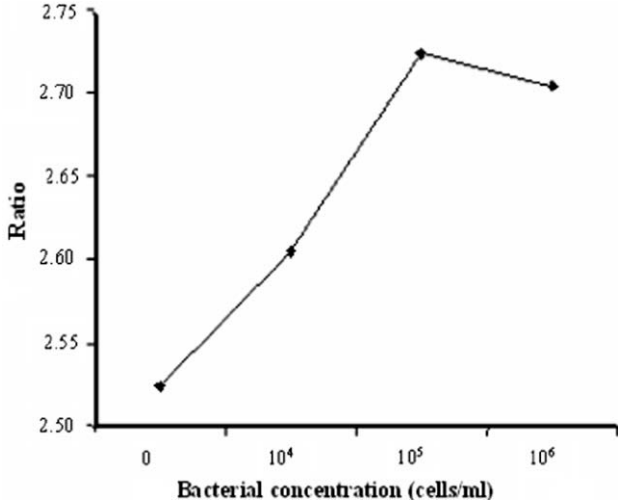

Fig. 5. Variation of Ca/Si ratio of the control and bacteria treated mortar samples.

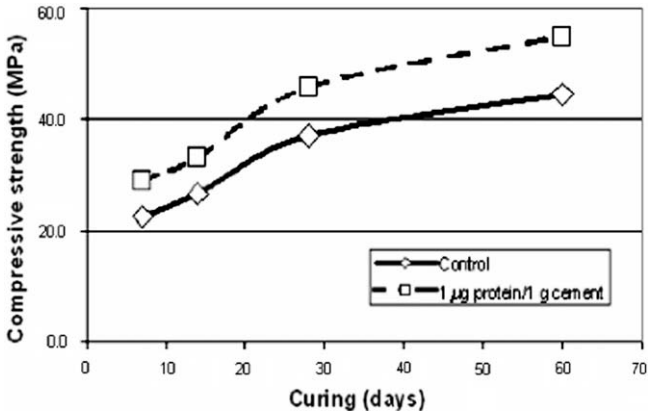

Fig. 6. Compressive strength of bacterial protein added mortar samples

2.6. EPMA

The sample preparation for EPMA was similar to that of the image analysis. The difference was that after final grinding on the glass plate, the thin section was polished successively in two stages. The first one involved polishing of the section with the help of chromic oxide and the final stage involved polishing of the section with diamond paste of specific grade [19]. Thin sections were then coated with carbon in BOC-WARDS vacuum chamber instead of cover glass. Carbon coated slides of mortar samples (with or without bacteria) thus prepared were fixed with holder and analyzed in CAMECA SX 100. Three thin sections were prepared from each mortar mix. From each thin section, four separate matrix areas were identified. Tests were made on about twenty points within each matrix area (mortar matrix number) and the results were averaged for weight percentages of silicon, iron, calcium and aluminum in the form of their oxides. Curves were drawn for studying the distribution of the different elements within the matrices. The ratios of calcium and silicon were estimated from averaging of all data in each mortar mix.

2.7. Mortar samples with protein

A separate set of mortar samples are also prepared by mixing protein (molecular weight 26 kDa approximately) instead of bacteria. The protein has been isolated from this bacterium. Three different grains sizes of sand (125–250 μm:250–500 μm:500–1000 μm = 1:1:1) were used for the mortar. Protein was mixed with the cement paste at ratios of 1:1–1.5:1 μg/g (protein vs. cement). The bar samples were cast with the above mortar and covered with plastic bags to protect from water evaporation and kept for several days for curing. The bars were cut into small cubes ($10 \times 10 \times 10\ mm^3$) at different days of curing. The compressive strength of the small mortar cubes with protein was determined by using a manual pressure bench at different days.

3. Results and discussion

The purpose of this study is to analyze the cause for strength increment of the bacteria modified mortar and also to characterize the microstructures of such mortar for identifying the new phases formed or leached out by the bacterium within it. Fig. 1 shows a bar diagram of the compressive strength of the mortar cubes having different bacterial cell concentrations at different days (3, 7, 14 and 28 days) of water curing. This increment is maximized at the bacterial cell concentration of 10^5 cells/ml, which is in agreement with the earlier results [12]. Microstructures examined by ESEM (Figs. 2a–d) and image analysis (Figs. 3a–d) of control and bacteria treated mortar samples show contrasting textures of their matrices. The matrix of the untreated ones appears to be amorphous, showing no signature of conspicuous crystal growth. On the other hand, mortar samples that were treated with the microorganism shows crystalline matrix, where individual crystals could be recognized. The degree of crystalinity in the matrix of treated samples is somewhat heterogeneous. There occur concentrations of relatively large crystals at the interfaces of sand particles and the matrix. This type of textural setting suggests that the coherence between sand particles and the matrix in micro-scale is probably enhanced due to preferential crystallization at the sand–matrix interfaces.

The powder crystal X-ray analysis of the mortar samples with or without bacteria shows that there were some extra peaks in the XRD spectra of the bacteria treated samples, which are absent in the control samples (Table 1). An elaborate search of the minor peaks [comparing the values of $2\theta/d/(I/I_0)$] from JCPDS data file exclusively shows that these new peaks match with the minor peaks of pure calcium aluminum silicate phase ($Ca_2Al_2SiO_7$ or Gehlenite). This result suggests that the hot spring bacterium is capable of formation new silicate phase within the mortar matrix. This new phase helps in the modification in pore size distribution and thus increases the compressive strength as previously observed [12]. At higher cell concentration, the matrix integrity may disrupt due to excessive bacterial activity and thus the decrease in compressive strength of mortar at higher cell concentration is noted.

The microstructural inhomogeneities can lead to serious effects on strength and other related mechanical properties because these properties are controlled by the microstructural extremes [22,23]. The data obtained from EPMA indicates that the overall distributions of the oxides of Si, Al and Fe within the mortar matrices of control sample (untreated) are non-uniform (Fig. 4a). As the concentration of the bacterial cell is increased to 10^5 cells/ml, uniformity of the concentration of SiO_2 over the matrix is increased (Figs. 4b–c). However, at the concentration of 10^6 cells/ml, such uniformity of SiO_2 is further increased with respect to other cell concentrations, but an abrupt qualitative change is noted as per the periodicity of the trend lines is concerned (Fig. 4d). It is also noted that the distributions of other oxides are quite less uniform in the bacteria treated samples (Figs. 4 b–d). The Ca/Si ratio within the CSH gel of mortar matrix is increased by the bacterium treatment and becomes optimum at 10^5 cells/ml (Fig. 5). This also reveals the optimum compressive strength of mortar at 10^5 cells/ml concentrations. On the basis of molecular ecological techniques and geochemical surveys, Inagaki et al. [24] have shown that thermophilic or hyperthermophilic microorganisms living in geothermal environments are likely to be implicated in the formation of biogenic siliceous deposits, which suggests microbial contribution to silica precipitation.

The bacterium used in this study can survive up to 6–7 days within the mortar when mixed with it. In the growth medium, the bacterium grows well within 3–4 days at 65 °C temperatures and at pH 8.0. The pH tolerance of the bacterium was found to be 12.0. During its growth, the bacterium secretes some proteins in the medium. One of the proteins possesses silicatein like catalytic activity and was isolated and added to the cement–sand mortar separately to observe the change in compressive strength (Fig. 6). It indicates that the protein added mortar samples show greater strength compared to the control mortar at all ages. This protein has been characterized and found to dissociate silica from silica rich substances, which will be communicated in more detail elsewhere.

4. Conclusions

Based on the present study, it is concluded that the strength of the bacteria modified mortar increases due to the deposition of the new material gehlenite by the bacterial activity, which in turn increases mainly the uniformity of SiO_2 concentration of the mortar. The high Ca/Si ratio at 10^5 cells/ml corresponds to the optimum strength of bacteria modified mortar at such cell concentration. Also the protein secreted by the bacterium leaches silica and helps in formation of new silicate phases that fill the micro pores. This protein increases the strength of mortar when it is added separately.

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