Strength improvement studies using new type wild strain *Bacillus cereus* on cement mortar

S. Maheswaran[1,*], S. S. Dasuru[1], A. Rama Chandra Murthy[1], B. Bhuvaneshwari[1], V. Ramesh Kumar[1], G. S. Palani[1], Nagesh R. Iyer[1], Sarayu Krishnamoorthy[1] and S. Sandhya[2]

[1]CSIR-Structural Engineering Research Centre, CSIR Campus, Taramani, Chennai 600 113, India
[2]CSIR-National Environmental Engineering Research Institute, CSIR Campus, Taramani, Chennai 600 113, India

This article presents details of the experimental studies carried out on cement mortar using *Bacillus cereus* and *Bacillus pasteurii* in different cell concentrations. Test results showed that the addition of bacterial cultures of both species, enhanced the compressive strength of cement mortar due to the bio-mineralization of calcium carbonate in the cement mortar matrix. The test results revealed 38% increase in compressive strength using *B. cereus* and 29% increase in the case of *B. pasteurii* over the control cement mortar. The chloride ingress capacity of *B. cereus* incorporated concrete found through rapid chloride permeability test confirms the reduction of chloride penetration compared to control sample. Characterization studies have been performed to confirm the calcite precipitation through different experimental techniques, viz. X-ray diffraction, scanning electron microscope, thermo-gravimetric analysis and Fourier transform-infrared spectroscopy.

Keywords: *Bacillus cereus*, *Bacillus pasteurii*, biomineralization, cement mortar, compressive strength.

IN the construction sector, concrete is considered as one of the most important building materials around the world. Advancement in concrete technology is in its strength improvement and its enhancement in durability, using pollution-free and natural methods. This needs to be taken care of at the design stage itself. The general ideas of biomimetics, which can be used for concrete are biodeposition and biomineralization, which are the natural biological processes of certain species of microorganisms such as bacteria. One of the inspiring biomimetics processes that occurs in nature is conversion of sand to sandstone by soil-thriving bacteria. Gollapudi *et al.*[1] introduced the novel technique in fixing cracks employing environment-friendly biological processes. It was found that fracture sites acted as new nucleation sites for capturing bacterial clusters leading to improved selective plugging as well as mineral precipitation. Alvarado[2] found that the bacterium *Sporosarcina pasteurii*, present in the natural soil deposits precipitates calcite ($CaCO_3$), which acts as binding agent to convert sand to sandstone. The idea is to use microorganism for bio-deposition of available minerals inside the concrete[3]. The aim of the present work is to study the biomimetics application in the improvement of strength and durability in the cement mortar/concrete involving *Bacillus pasteurii* and *Bacillus cereus* and related characterization studies.

*For correspondence. (e-mail: smahes@serc.res.in)

Literature review

The process of microbial mineral plugging in porous media was induced by Stocks-Fischer *et al.*[4] by using an alkhalophilic soil microorganism, *B. pasteurii*. It was found that urease activity is high in alkaline pH, where calcite precipitation is favourable. They suggest that microbial calcite precipitation process can be used in remediation of surface and subsurface of porous media. Ramakrishnan *et al.*[5] studied the role of *B. pasteurii* in crack-filling by using artificially cracked cement mortar beams. Microscopic observations confirmed the microbial calcite precipitation in cement. In another study, Ramachandran *et al.*[6] used *B. pasteruii*, for remediating cracks and fissures in concrete utilizing microbiologically induced calcite ($CaCO_3$) precipitation. As a microbial sealant, $CaCO_3$ exhibited its positive potential in selectively consolidating simulated fractures and surface fissures in granites and in the consolidation of sand. The aim of their studies was to know the effect of different concentrations and efficiencies of bacteria when suspended in different media (water, phosphate-buffer and urea-$CaCl_2$) on the durability of concrete. It was concluded that the presence of bacteria in different media increased the resistance of concrete towards alkali, sulphate, freeze–thaw attack and drying shrinkage. Willem *et al.*[7] highlighted the gaps between conventional surface treatments and bacterial carbonate precipitation (bio-deposition). It was reported that the effect of bio-deposition improves the durability of cement mortar/concrete specimens. It was also observed that deposition of $CaCO_3$ crystals decreased the water absorption of the sample depending on the inherent porosity of the specimen leading to a decrease in the carbonation rate by about 25–30%.

It is essential to improve the strength and durability of concrete where similar biomimetic techniques have been used worldwide. Ghosh *et al.*[8] found that the addition of an anaerobic hot spring bacterium (closely related to *Shewanella* species) to mortar/concrete increased the compressive strength by about 25–30% with respect to control mortar. It was observed that the filler material within the pores of the cement–sand mortar increased the compressive strength by about 25% with the addition of anaerobic microorganism (*Shewanella* sp.) of about 10^5 cells/ml of water concentration after 28 days of curing. However, they did not find any improvement in strength in the cement mortar using *Escherichia coli*.

Van Tittelboom *et al.*[9] studied biological repair technique using ureolytic bacteria such as *Bacillus sphaericus*, which were able to precipitate $CaCO_3$ by conversion of urea into ammonium and carbonate. Park *et al.*[10] studied the microbiological $CaCO_3$ precipitation ability to improve the compressive strength of concrete. Four calcite-forming bacteria (CFB) located from seven environmental concrete structures were studied. They observed that the bacterial species belonging to *Arthrobactor crystallopoietes* improved the compressive strength of concrete cubes. Another strain (*Bacillus subtilis*) was used by Afifudin *et al.*[11] in the formation of calcium silicate hydrated gel by means of adsorbing silicate using chemically modified *B. subtilis* (CMBS). They reported 28% improvement in the compressive strength of CMBS incorporated concrete compared to control concrete with optimum concentration of 10^6 cells/ml. Thus research in biomimetics has been initiated using different species of bacteria, especially *Bacillus* sp., as depicted in Table 1, for both the mechanisms of bio-deposition and bio-mineralization.

The ultimate aim of the studies listed in Table 1, is to use bacteria in the deposition and/or precipitation of calcite minerals in the cement/concrete matrix so that the newly formed calcites may either remediate the cracks or fill up the pores in the concrete. $CaCO_3$ so produced can be useful as a binding agent and also as a pore-filling medium to improve the strength of concrete. The former improves the adhesive property within the concrete matrix, thereby increasing the strength of concrete, while the latter reduces the capillary pores thereby increasing both the durability and strength of concrete.

Table 1. Different types of bacteria used in cement mortar/concrete

Bacterium	Reference
Bacillus pasteruii	1, 2, 4–6
Escherichia coli	8
Bacillus sphaericus	3, 9
Shewanella species	8
Sporosarcina soli, *Bacillus massiliensis*, *Arthrobacter crystallopoietes*, *Lysinibacillus fusiformis*	10
Bacillus subtilis	11
Bacillus cereus	Present study

Experimental procedure

Materials and methods

The cement used for mortar/concrete was 53 grade Ordinary Portland Cement (OPC) conforming to IS: 12269-1987 (ref. 12); the physical and chemical properties are listed in Table 2. The grade-2 sand conforming to IS: 383-1970 (ref. 13) and the locally available coarse aggregate with equal proportion of 12.5 and 20 mm size conforming to IS: 383-1970 were used. Ordinary potable water was used for control mortar and concrete, while the entire volume of water was replaced with phosphate buffered saline (PBS) suspended bacteria for the test specimens, with bacteria incorporation as described in the subsequent sections. The compressive strength of cement mortar cube specimens of 70.6 mm size with a water binder ratio appropriate to standard consistency measurement was determined after 5, 7, 14, 21 and 28 days of curing. Two cylindrical specimens of 100 mm diameter and 150 mm height were cast from each mix for rapid chloride penetration tests (RCPTs). For RCPT, the cylindrical specimens were cut into 100 mm diameter and 50 mm height using a concrete cutter. After casting, all the specimens were left covered in the casting room for 24 h. The specimens were then de-moulded and transferred to a curing tank, containing nutrient broth (NB) curing medium until further testing.

Bacterial strains, culture medium and nutrient broth curing medium

The strain *B. pasteurii*, Microbial Type Culture Collection, MTCC 1761 (equivalent to American Type Culture Collection, ATCC 11859) was procured from CSIR–Institute of Microbial Technology (CSIR–IMT), Chandigarh,

Table 2. Physical and chemical properties of cement

Chemicals	Constituents (%)
Chemical properties	
SiO_2	20.24
Al_2O_3	5.64
Fe_2O_3	4.07
CaO	63.42
SO_3	3.48
Na_2O	0.19
K_2O	0.56
MgO + MnO	0.88
LOI	1.52
Physical properties	
Colour	Dark gray
Specific gravity	3.162
Bulk density	1.561 g/cm^3
Fineness passing 40 μm sieve	85%

India and the new *B. cereus* was isolated from one of the old concrete buildings at the CSIR campus, Chennai, India. These were cultured to check their morphology on nutrient agar (NA), which contained peptic digest of animal tissue 5 g/l, sodium chloride 5 g/l, beef extract 1.5 g/l, yeast extract 1.5 g/l, and agar 15 g/l, and the final pH of the medium was found to be 7.4 ± 0.2 at 25°C. The culturing was done by spreading the stock culture of the bacteria onto the plates and allowing it to be incubated for 24 h at 37°C. Further based on requirement, slants were prepared in NA, in test tubes of 10 ml and then urea–$CaCl_2$ medium was prepared. The composition of the urea agar per litre was NB 8 g, NH_4Cl 10 g, urea 20 g, $NaHCO_3$ 2.12 g (equivalent to 25.2 mM), $CaCl_2$ concentration taken as 25 g, i.e. 25 mm/l. After autoclaving, $CaCl_2$ was used to make the pH level of the medium 8, 8.5 and 9; the volume of $CaCl_2$ used was approximately 10, 25 and 40 ml respectively.

The wild strain *B. cereus* was analysed to obtain genetic information (at M/s SciGenom Labs Pvt Ltd, Cochin, India). The nucleotide sequence information has been published by the National Center for Biotechnology Information-GenBank with ID: BankIt1550475 Seq1 JX292107, in the name of 'CSIR-SERC-I' (ref. 14). Hereafter, we will refer to it as CS-I in this article).

The cell concentration of the bacteria was obtained as a result of serial dilution and plating experiment using optical density data. A microbial culture preparation is a method of multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions. Cell concentration of 10^5 to 10^7 cells/ml was prepared along with its culture medium, and then the prepared microorganisms were incorporated in cement mortar and concrete to study the strength characteristics and durability aspects.

The cement mortar/concrete test specimens were allowed to set overnight before curing and were demoulded the next day. Then they were immersed in an NB–urea medium (8 g nutrient broth (Himedia, India), 5 g NaCl, 2% urea and 25 mM $CaCl_2$). The specimens were cured for 28 days, but the medium for curing was changed every 7 days. Fifteen mortar cube specimens were cast for each control mix with cell concentrations of 10^5 to 10^7 cells/ml. To study the strength characteristics of cement mortar, the two already cultured microorganisms, MTCC 1761 and CS-I, which were grown in the NB-urea medium were used. The high quantity of urea supplied will be useful in stimulating the urease activity cycle, to increase pH and further to generate the required CO_3^{2-} ions. The cement-to-sand ratio was 1 : 3 (by weight), the bacterial cultured solution or the *w*/*c* ratio was fixed at 0.42.

Preparation of test specimens

Castainer *et al.*[15] and Hammes *et al.*[16] showed that the bacterial degradation of urea locally increased the pH and promoted the microbial deposition of carbon dioxide as $CaCO_3$ in a calcium-rich environment. But in the present study, the entire volume of water was replaced by 50 mM of PBS with suspended bacteria. This complete replacement was carried out based on the cement consistency test with ordinary potable water given as 29%, whereas the consistency of cement with buffer was found to be 30%.

Characterization studies

The formation of calcite by means of bio-mineralization was analysed using various characterization techniques/methods. These techniques are specialized or involve all modes of microbial analysis like imaging, diffraction and spectroscopy, including light, X-rays, neutron or electron as primary radiation. To conduct the above studies, the samples were collected from the tested mortar in the form of powders and/or broken pieces. Moreover, these techniques also require specially prepared samples for their characterization as discussed in the following sections.

X-ray diffraction (XRD): Bruker's D2 PHASER XRD system, equipped with 1D LYNXEYE detector was used in the present study. It employs Cu-K_α radiation (30 kV, 10 mA) with nickel filters. A continuous scan from 10° to 70° 2 theta in step width of 0.02° and counting time of 0.5 s/step was performed on less than 25 μm size powder samples.

Scanning electron microscopy (SEM): The Hitachi VP-SEM S-3400N instrument was used for morphological studies of cement mortar with and without microbial incorporation.

Thermo-gravimetric analysis (TGA): TA Instruments Q 600 SDT: Simultaneous Thermal Analyser was used with alumina crucibles fitted with pierced lids. Four samples were selected, viz. pure $CaCO_3$ for reference, cement mortars incorporated with MTCC 1761, CS-I and control samples in powder form of sizes less than 25 μm, and analysed from room temperature to 1000°C, at a uniform heating rate of 20°C/min, under static air atmosphere.

Fourier transform-infrared (FT-IR) spectroscopy: Perkin Elmer Spectrum RX 1 FT-IR system was used. The tested cement mortar cubes which contain bacterial culture of 10^6 cells/ml concentration of CS-I, 10^5 cells/ml concentration of MTCC 1761 samples that exhibited high strength improvement, commercial calcite and control samples were selected. Initially, 0.1 mg of the sample was taken and mixed with KBr in the 1 : 8 ratio and then the sample was compressed into a pellet form of fine thickness using a hydraulic pressure pelletizer with approx. 5 tonnes load. The sample was then loaded in the

sample holder and placed in the test chamber of the FT-IR instrument.

Results and discussions

Compressive strength

The compressive strength test results revealed that there is an increase in strength for all the samples in which bacteria type CS-I was incorporated compared to control (Table 3). A significant increase of 38%, 22.2% and 18.6% was observed for cell concentrations of 10^6, 10^7 and 10^5 cells/ml respectively, for 28 days compressive strength.

Similarly, the compressive strength test results revealed that there is an increase in strength of all the samples in which MTCC 1761 was incorporated compared to control (Table 4). It can be observed that the increase in compressive strength is about 29% for a concentration of 10^5 cells/ml in the bacterial cement mortar, which is in accordance with the literature[17].

From the above results, it can be observed that there is an initial early strength gain for the first seven days of curing in both the cases. The improvement in compressive strength by CS-I and MTCC 1761 could be attributed to bio-mineralization of $CaCO_3$ on the cell surfaces and within the pores of the cement–sand matrix, i.e. pore-filling effect within the mortar specimens. From Tables 3 and 4, it can be noted that there is a significant increase in compressive strength for 7 days compared to 28 days in both the cases. This may be due to PBS, which enabled high pH level to provide good nourishment and buffering action to microbial cells within the cement–sand matrix initially. Due to the high pH in the cement mortar, the microbial cells were able to grow fast by precipitating calcite, subsequently filling the pores; thereafter there could be pore-filling with calcite resulting in subsequent reduction in porosity. This could have prevented further flow of nutrients and oxygen to the bacterial cells in the mortar, the post-seven days behaviour, i.e. the strength gain gradients are normal, thus may be due to the stoppage bio-mineralization of the microbial cells, the reason being that resulting is eventual death of micro-organisms.

Table 3. Compressive strength for wild strain CS-I incorporated mortar

	Compressive strength (MPa)			
Days	Control	10^5 cells/ml	10^6 cells/ml	10^7 cells/ml
5th	15.34	24.9	25.54	30.59
7th	17.93	25.65	31.41	34.9
14th	22.77	32.8	38.8	38.32
21st	25.67	40.4	47.8	42.3
28th	36.64	43.47	50.52	44

Table 4. Compressive strength for MTCC 1761 incorporated cement mortar

	Compressive strength (MPa)		
Days	Control	10^5 cells/ml	10^7 cells/ml
5th	15.34	18.65	31.3
7th	17.93	28.65	33
14th	22.77	36.43	40.58
28th	36.34	46.88	41.19

Rapid chloride penetration test

The rapid chloride permeability test was conducted according to ASTM C1202-97 to examine the chloride ion permeability[18]. For this study, the concrete cylinders incorporated with wild type strain CS-I of various cell concentrations were selected in addition to the control concrete, as they showed significant improvement of compressive strength in 28 days. The results of the study are presented in Table 5.

According to the ASTM standards, the rate of chloride ion permeability in various cell concentrations in control concrete showed that the quality of concrete is at moderate level. But in the microbial incorporated samples, the chloride ingress capacity decreased compared to the control and notable reduction was found in the chloride permeability of samples with cell concentration of 10^6 cells/ml. This ability to prevent chloride penetration is a critical parameter to concrete structures, especially in the marine environments.

Characterization studies to confirm calcite precipitation

XRD: From the XRD spectrum shown in Figure 1, for the bacterial incorporated mortar samples CS-I, MTCC 1761 and control mortar, the principal calcite peaks

Table 5. Charge passed for CS-I type concrete at different cell concentrations

Sample ID	Charge passed (C)	Average
Control	3403	3488
	3605	
	3457	
10^5 cells/ml	3209	3052
	3324	
	2623	
10^6 cells/ml	2910	2974
	3119	
	2895	
10^7 cells/ml	2884	2998
	2981	
	3130	

can be observed at 29.41°, 35.97° and 57.4°. The observed values for calcite at the respective principal peaks (counts/s) are given in Table 6. In the intensity mapping of the characteristic peaks of calcite, it is found that the higher calcite intensity peaks with reference to International Crystal Diffraction Database (ICDD) were formed for both the bacterial incorporated specimens, especially more in the CS-I incorporated specimens (Figure 1).

This is an indication of high percentage of calcite in the bacteria incorporated specimens, which could be attributed to an active transformation of the unstable calcium ions into stable $CaCO_3$. The wild strain is able to produce higher amount of calcite, thus resulting in significant higher compressive strength percentage (38% higher than the control). This could be attributed to the fact that the wild strain has a previous exposure to concrete alkaline environment acclimatization.

SEM: To confirm microbial calcite formation in the cement sand matrix, the powder samples from the tested mortar specimens was studied using SEM. CS-I and MTCC 1761 incorporated mortar samples were chosen with cell concentration 10^6 and 10^5 cells/ml respectively, as they showed the maximum compressive strength.

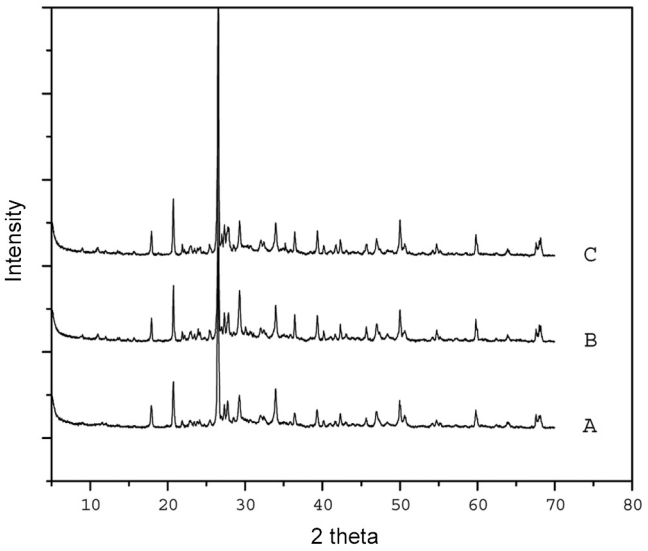

Figure 1. X-ray diffraction spectrum of control sample (A), CS-I with 10^6 cells/ml (B) and MTCC 1761 with 10^5 cells/ml (C).

Table 6. Calcite peaks for different samples CS-I, MTCC 1761 and control

	29.41°	35.97°	57.4°
Sample/2θ		Intensity	
CS-I incorporated	3566	1249	812
MTCC 1761 incorporated	2587	1011	765
Control mortar	2487	958	762

Figure 2 *a* shows the SEM image of MTCC 1761 incorporated with cell concentration of 10^5 cells/ml. It indicates clearly the presence of lamellar rhombohedral crystals of calcite and needle-shaped aragonite crystals of $CaCO_3$ that act as precursors for the formation of calcite crystals of $CaCO_3$, which shows that the system supports the continuous formation of calcite[19]. Moreover, the bacterial impressions on the mortar surface and the precipitation of calcite can be clearly noticed around the edges of the impressions as shown in Figure 2 *b*. Figure 2 *c* shows rhombohedral calcite precipitation for the samples of CS-I with 10^6 cells/ml concentration.

TGA: For the thermal analysis study, three samples were selected, viz. pure $CaCO_3$ for reference, the tested cement mortar cubes which contain bacterial culture of 10^6 cells/ml concentration of CS-I, 10^5 cells/ml concentration of MTCC 1761 samples and control samples. From Figure 3 *a*, it can be inferred that there is a weight loss of 39.26%, between the temperature range 650–750°C, which corresponds to the loss of carbon dioxide for the case of pure $CaCO_3$ sample. This study was carried out for reference. At the same temperature range, the TGA graph for MTCC 1761 incorporated mortar sample shows a weight loss of 2.54% (Figure 3 *b*). Similarly, in CS-I incorporated mortar sample, there is a weight loss of 2.30% (Figure 3 *c*). Significant weight losses were observed in both the samples incorporated with microorganisms MTCC 1761 and CS-I, which indicates the calcite decomposition and precipitation. The above TGA results confirm that an appreciable amount of calcite is detected in both the bacteria incorporated samples. Figure 3 *d* depicts the TGA graph for control sample, wherein there is no notable decomposition of calcite for the same temperature range. It can also be noted that in all the above cases, except for commercial calcite, decomposition peaks are found due to the decomposition of $Ca(OH)_2$ in the 400–450°C temperature range.

FT-IR spectroscopy: The C=O bonds (of the carbonate group) would exhibit in-plane bending and out-of-plane bending at about 713 and 875 cm^{-1} (ref. 20). It has been reported[21] that the quantitative detection of $CaCO_3$ mixtures was lower for powder XRD analysis than for IR spectroscopic analysis. It can also be noted that the accuracy of XRD analysis depends on the crystalline phases of the carbonate, whereas vibration spectroscopy is less sensitive to this parameter. FT-IR spectroscopy is a simple and accurate technique to identify and quantify $CaCO_3$ polymorphs. The FT-IR spectra of $CaCO_3$ polymorphs are reported extensively in the literature[17,22]. Because of their different crystal structures, they can be discriminated using FT-IR; a different spectrum is observed for each of the structural forms. Thus, the major vibration bands for calcite are identified as: C–O asymmetric, stretching vibration ($\nu 3$) of carbonates, C–O

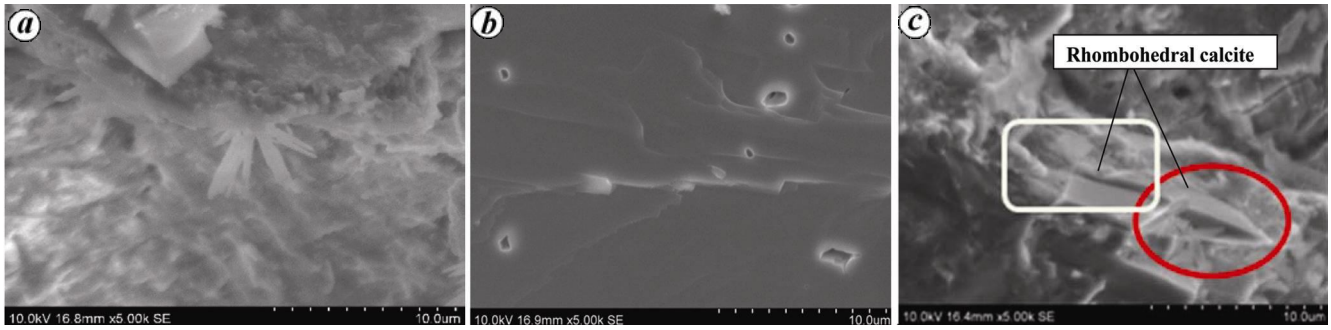

Figure 2. SEM images of bacteria incorporated mortar samples. ***a***, MTCC 1761 incorporated mortar with cell concentration of 10^5 cells/ml. ***b***, Bacterial impressions on the mortar surface of MTCC type. ***c***, Rhombohedral calcite precipitation for the samples of CS-I incorporated mortar with cell concentration of 10^6 cells/ml.

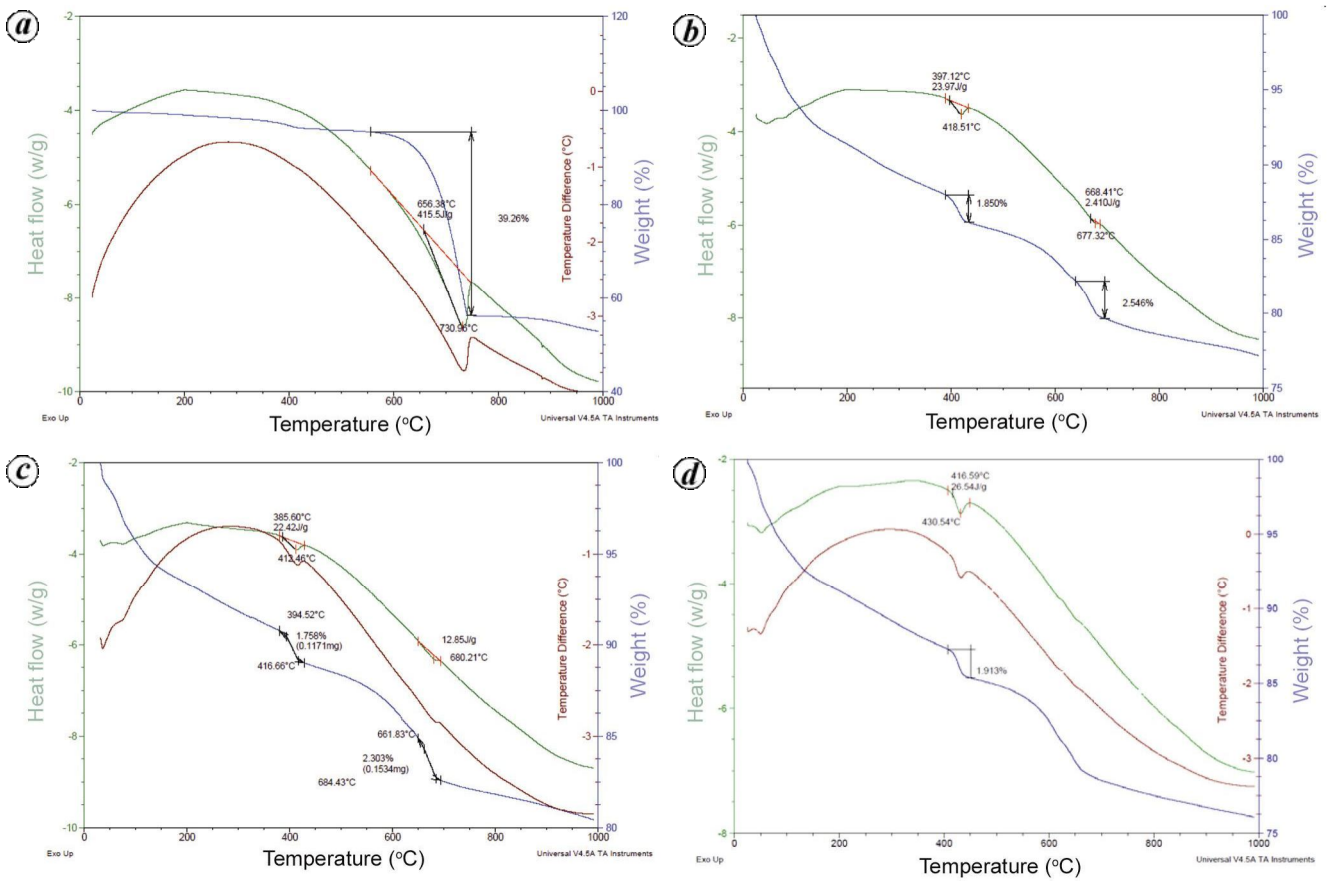

Figure 3. TGA graphs for (***a***) commercial calcite as reference, (***b***) MTCC 1761 sample, (***c***) CS-I sample and (***d***) control sample.

out-of-plane bending vibration ($\nu 2$) of carbonates, C–O planar bending vibration ($\nu 4$) of carbonates, centred at 1390, 871 and 712 cm^{-1} respectively. Victor and Jonkers[23] also observed that the self-healing of concrete is mainly based on the calcite formed in the bacteria.

The FT-IR spectra obtained from the samples of pure commercial calcite indicated the in-plane bending and out-of-plane bending vibrations of the carbonate group with wave numbers 709 and 860 cm^{-1} respectively (Figure 4 *a*). In similar lines, CS-I incorporated bacterial mortar and the peaks are observed at 876 and 712 cm^{-1} (Figure 4 *b*). This would be due to a significant amount of precipitated calcite in the mortar specimen. However, there were no such peaks obtained for the control mortar sample; only a small bulge was observed instead of a peak, which could be attributed to trace amount of calcite in the cement–mortar matrix (Figure 4 *c*). Further, no significant peaks were observed in the specimen with the

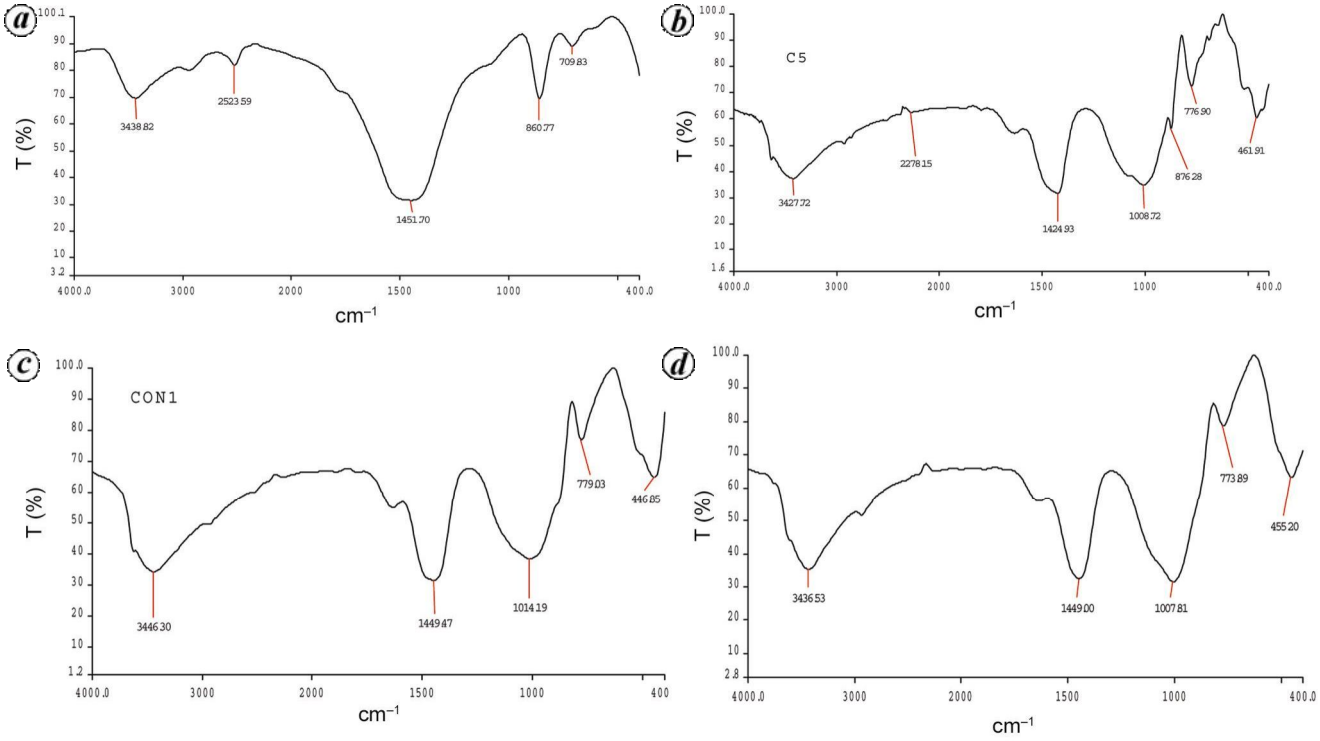

Figure 4. FT-IR spectra of (***a***) pure calcite, (***b***) sample of CS-I, (***c***) control sample and (***d***) sample MTCC 1761.

MTCC strain (Figure 4 *d*), which was unexpected. This could be due to error in the sampling of data and incorrect sample preparation while conducting FT-IR experiment.

Conclusions

Compressive strength studies have been carried out on mortar cubes by incorporating *B. cereus* and *B. pasteurii* strains with various concentrations along with control mix. From the strength studies, it is observed that in the case of MTCC 1761 with 10^5 cells/ml, the compressive strength increased by 29%; this is in accordance with the literature. For the case of the wild strain, it is observed that the strength improvement is significantly higher (38%) for the concentration of 10^6 cells/ml. The increase in strength could be due to the formation of calcite and its precipitation is substantiated with relevant characterization studies. The achievement of 38% strength improvement by using *B. cereus* in cement mortar and its ability to prevent chloride penetration in concrete structure is a critical parameter, especially in the marine environments.

1. Gollapudi, U. K., Knutson, C. L., Bang, S. S. and Islam, M. R., A new method for controlling leaching through permeable channels. *Chemosphere*, 1995, **30**, 695–705.
2. Alvarado, D., Bio-mediated soil improvement: cementation of unsaturated sand samples. Thesis, Arizona State University, USA, 1995.
3. Dick, J., De Windt, W., De Graef, B., Saveyn, H., Van der Meeren, P., De Belie, N. and Verstraete, W., Bio-deposition of a calcium carbonate layer on degraded limestone by *Bacillus* species. *Biodegradation*, 2006, **17**(4), 357–367.
4. Stocks-Fischer, S., Galinat, J. K. and Bang, S. S., Microbiological precipitation of $CaCO_3$. *Soil Biol. Biochem.*, 1999, **31(11)**, 1563–1571.
5. Ramakrishnan, V., Deo, K. S., Duke, E. F. and Bang, S. S., SEM investigation of microbial calcite precipitation in cement. In Proceeding of the International Conference on Cement Microscopy, Las Vegas, USA, 1999, pp. 406–414.
6. Ramachandran, S. K., Ramakrishnan, V. and Bang, S. S., Remediation of concrete using micro-organisms. *ACI Mater. J.*, 2001, **98**, 3–9.
7. Willem, D. M., Debrouwer, D., Belie, De, Verstraete, W., Bacterial carbonate precipitation improves the durability of cementitious materials. *Cem. Concr. Res.*, 2008, **38**, 1005–1014.
8. Ghosh, P., Mandal, S., Chattopadhyay, B. D. and Pal, S., Use of microorganism to improve the strength of cement mortar. *Cem. Concr. Res.*, 2005, **35**(10), 1980–1983.
9. Van Tittelboom, K., De Belie, N., De Muynck, W. and Verstraete, W., Use of bacteria to repair cracks in concrete. *Cem. Concr. Res.*, 2010, **40**, 157–166.
10. Park, S. J., Yu-Mi, P., Chun, W. Y., Kim, W. J., Ghim, S.-Y., Calcite-forming bacteria for compressive strength improvement in mortar. *J. Microbiol., Biotechnol.*, 2010, **20**(4), 782–788.
11. Afifudin, H. N., Hamidah, W. and Noor Hana, M. S., Microbial participation in the formation of calcium silicate hydrated (CSH) from *Bacillus subtilis*. *Proc. Eng.*, 2011, **20**, 159–165.
12. IS: 12269, Specification for 53 Grade Ordinary Portland Cement, 1987.
13. IS: 383, Specification for Course and Fine Aggregates from natural sources for concrete, 1970.

14. Murthy, A. R. C. *et al.*, *Bacillus cereus* strain CSIR-SERC-1 16S ribosomal RNA gene. Partial sequence, NCBI (2012). GenBank: JX292107.1.
15. Castanier, S., Le Metayer-Levrel, G. and Perthuisot, J., Ca carbonates precipitation and limestone genesis – the microbiogeologist point of view. *Sediment. Geol.*, 1999, **126**, 9–23.
16. Hammes, F., Boon, N., de Villiers, J., Verstraete, W. and Siciliano, S. D., Strain-specific ureolytic microbial carbonate precipitation. *Appl. Environ. Microbiol.*, 2003, **69**(8), 4901–4909.
17. Ghosh, S. N., IR spectroscopy. In *Handbook of Analytical Techniques in Concrete Science and Technology, Principles, Techniques, and Applications* (eds Ramachandran, V. S., Beaudoin, J. J.), Noyes Publications, William Andrew Publishing, New York, 2001, pp. 174–200.
18. ASTM C1202-97, Standard test method for electrical indication of concrete's ability to resist chloride ion penetration, ASTM, USA, 1994.
19. Rodriguez-Navarro, C., Jroundi, F., Schiro, M., Ruiz-Agudo, E. and González-Muñoz, M. T., Influence of substrate mineralogy on bacterial mineralization of calcium carbonate: implications for stone conservation. *Appl. Environ. Microbiol.*, 2012, **78**(11), 4017–4029.
20. http://webbook.nist.gov/cgi/cbook.cgi?Scan=cob4660&Type=IR
21. Dickinson, S. R. and McGrath, K. M., Quantitative determination of binary and tertiary calcium carbonate mixtures using powder X-ray diffraction. *Analyst*, 2001, **126**, 1118–1121.
22. Gayathri, S., Lakshminarayanan, R., Weaver, J. C., Morse, D. E., Manjunatha, K. R. and Valiyaveettil, S., *In vitro* study of magnesium–calcite biomineralization in the skeletal materials of the sea star *Pisaster giganteus*. *Chem. Eur. J.*, 2007, **13**, 3262–3268.
23. Viktor, V. and Jonkers, H. M., Quantification of crack-healing in novel bacteria-based self-healing concrete. *Cem. Concr. Compos.*, 2011, **33**, 763–770.

ACKNOWLEDGEMENTS. The scientists and staff members of Advanced Materials Laboratory, Advanced Concrete Testing and Evaluation Laboratory of CSIR-Structural Engineering Research Centre, Chennai and Central Instrumentation Facility of Pondicherry University, Puducherry, are acknowledged for providing support. This paper is being published with the permission of Director, CSIR-SERC.

Received 17 May 2013; accepted 22 October 2013

Smile with Science

By – S. K. Sharma
e-mail: santosh_ujj@yahoo.com