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Genetically-enriched microbe-facilitated self-healing concrete - A sustainable material for a new generation of construction technology

Manas Sarkar[1], Dibyendu Adak[2], Abiral Tamang[1], Brajadulal Chattopadhyay[1*], Saroj Mandal[2]

[1] Department of Physics, Jadavpur University, Kolkata-700032, India
[2] Department of Civil engineering, Jadavpur University, Kolkata-700032, India

Abstract:

The fundamentals of engineering and structural properties such as mechanical strength, durability, bond strength, and self-healing behaviour of a genetically-enriched microbe-incorporated construction material have been explored in this present exertion. The alkaliphilic *Bacillus subtilis* bacterium is able to survive inside the concrete/mortar matrices for an extended period due to its spore forming ability. The bioremediase-like gene of a thermophilic anaerobic bacterium BKH2 (GenBank accession No. KP231522) was thus transferred to bacillus strain to develop a true self-healing biological agent. Incorporation of the transformed bacterial cells at different concentrations in bio-concrete/mortar exhibited higher mechanical strengths and improved durability of the samples in comparison to the normal cement-sand mortar/concretes. Microstructural analyses confirmed the formation of a novel Gehlenite ($Ca_2Al_2SiO_7$) phase besides calcite deposition inside the matrices of the transformed Bacillus subtilis-amended cementitious materials. The gradual development of nano rod-shaped Gehlenite composite within the bio-mortar matrices was due to the biochemical activity of the bioremediase-like protein expressed within the incorporated bacterial cells. This development significantly increased the true self-healing property as well as enhanced the mechanical strength of the bio-concrete/mortar material which was sustained for a prolonged period. This study demonstrates a new approach towards the enhancement of structural properties and true self-healing activity by genetically-enriched spore-forming *Bacillus sp*. with advancement towards sustainable and green construction technology.

Key Words: Gene transformation, Bio-concrete, Self-healing, Mechanical Strength, Durability.

*Corresponding Author: Email id: bdc_physics@yahoo.co.in; Phone: +91-9433343917

1. Introduction:

Growing trends in the development of self-healing property of cementitious material in construction technology has given rise to several smart materials with versatile properties and high sustainability.[1-3] Self-healing phenomenon is an important aspect for construction technology which prolongs the service life of infrastructures. The key notion of this concept is that minor damage in concrete structures is not an issue as long as it is counteracted by a subsequent autonomous process of removing or 'healing' the structural damage. Several types of micro cracks are of common occurrence which arises due to the relatively low tensile strength as well as due to lack of suitable instant treatment of the concrete. The potential of mineral precipitating bacteria for crack remediation and durability improvement have been thoroughly investigated in different studies.[1,2, 6] Recently, attempts are being made to establish autonomous-healing activity by incorporating mineral producing microbes in the concrete mix.[1-2] But the efficient self-healing phenomenon through the process of bacteria assisted bio-mineralization occurs at the initial stage of concretization.[1-6] Self-healing on the cracks within the concrete that appear over a prolonged period will be the most desirable and challenging task that yet to be established. Furthermore, the application of the genetically-modified bacteria for eco-friendly engineering and true self-healing process over a prolonged period will create a new hope for sustainable concrete technology.[7]

Earlier reports demonstrate the applications of anaerobic hot spring bacteria in cementitious material executing higher mechanical strength and enhancing the durability due to the formation of a new phase (Gehlenite) inside the cementitious matrices.[7] However, the application of anaerobic bacterium as a self-healing agent depends on its survival inside the concrete matrices for a prolonged period. The short lifetime of the bacterium inside the concrete matrices restricts it from behaving as a true self-healing activator over sustained duration. [9] Genetically improved *E. coli* bacterium by incorporation of the bioremediase-like gene, was seen to increase the mechanical strength and durability of the mortar samples.[7] However, the transformed bacterium could not survive inside the high alkaline cement matrices for an extended period. In contrast, alkaliphilic spore forming bacterium, *Bacillus sp.* can persist in viable form within the concrete for an extended period and is also capable of forming crack plugging minerals (calcium carbonate) in concrete structure for autonomous healing. [2-6]

In this work, biosilicification gene (bioremediase-like protein gene) of BKH2 has been transformed in a spore forming bacterium *Bacillus subtilis* (*B. subtilis*) and then the

genetically-enriched bacterium has been used in Ordinary Portland Cement (OPC; 43 grade) based concrete material for healing of cracks over long periods to develop a new self-healing material. A comparative investigation has been studied based on structural behaviour (compressive, split tensile, flexural and bond strength) and self-healing attributes of bio-concrete/ mortar materials prepared by incorporating both transformed and non-transformed *Escherichia coli (E. coli)* and *B. subtilis* bacteria respectively to cementitious material. The promising outcomes of this study reveal the true self-healing capability of the transformed *B. subtilis* (T- *B. subtilis*) for future concrete industries.

2. Experimental Details

2.1. Materials

All analytical grade chemicals were purchased from the Sigma-Aldrich, USA; Merck- Germany; and the Spectrochem Pvt. Ltd. of India. Locally available sand (Specific gravity 2.52, water absorption 0.50%, and fineness modulus of 2.38) and 12 mm down aggregates (Specific gravity 2.78, water absorption 0.42%, and fineness modulus of 4.89) were used as fine and coarse aggregates respectively. Ordinary Portland cement (OPC) grade 43 and 20 mm diameter high yield strength deformed rebar and mild steel rebar of grade Fe500 were also used in the study. *E. coli* strain (JMJ107; MTCC 1669) and *B. subtilis* (MTCC 441) strain were procured from IMTECH, Chandigarh, India. The transformed JMJ107 (T-JMJ107) strain, a genetically-modified *E. coli* strain was obtained from the stock culture of Biophysics Laboratory, Department of Physics of Jadavpur University.[7]

2.2. The bacteria and growth conditions:

The T-JMJ107 and JMJ107 strains were cultured in Luria Britani (LB) medium (0.5% Peptone, 1% Yeast extract, 0.5% NaCl, pH 7.0) at 37 ^{o}C. Similarly the alkaliphilic spore forming *B. subtilis* bacterium was cultured in Luria-broth (LB) medium. To enhance the sporulation (spore formation) of the *B. subtilis* bacterial culture, a specific mineral media (pH 10.0) containing 0.2 g NH_4Cl, 0.02 g KH_2PO_4, 0.225 g $CaCl_2$, 0.2 g KCl, 0.2 g $MgCl_2$. $6H_2O$, 0.01 g $MnSO_4$. $2H_2O$, 1 ml trace element solution (SL12B), 0.1 g yeast extract, 5.16 g citric acid tri sodium salt, 4.2 g $NaHCO_3$ and 5.3 g Na_2CO_3 per litter distilled water was used.[10] The cultures containing large number of spores were washed by repeated centrifugation and re-suspension of the cell pellet in sterile ultrapure Milli-Q water to harvest the spores. Suspensions were subsequently heated for 30 min at 80 ^{o}C to inactivate

the present vegetative cells and numbers of viable spores in water suspension were quantified by using haemocytometer.

2.3. Genetic transformation to develop improved *Bacillus subtilis* strain

The bioremediase-like protein (molecular weight about 28 kDa) secreted by BKH2 is capable of leaching silica from silicate substrates.[7] The corresponding gene (~800 bp.) of the bioremediase-like protein was fished out from the whole genome of BKH2 as described earlier.[7] The isolated gene was amplified by Polymerase Chain Reaction (PCR) and cloned into T-vector. The transformation of bioremediase gene through T-vector to *B. subtilis* was carried out by calcium chloride treatment of the bacterial cells as described by Lederberg and Cohen.[11] The transformed *Bacillus subtilis* (T-*B. subtilis*) was grown in ampicillin (0.5 mg/ml) containing agar plate and pure culture of T-*B. subtilis* strain was obtained from a single colony grown on the agar plate.

The expression of the transformed gene in *B. subtilis* was confirmed by observing the protein profile of transformed bacterial cells. The expressed protein (bioremediase-like) was purified by using Sephadex G-100 gel filtration chromatographic technique and detected through sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) along with marker proteins (Sigma-Aldrich, USA). The biosilicification activity of purified proteins was confirmed by the standard biosilicification assay.[7, 12]

2.4. Application of transformed *B. subtilis* (T-*B. subtilis*) in concrete and mortar:

2.4.1. Compressive strength and Ultrasonic-pulse velocity (UPV) test:

Mortar specimens (50 mm x 50 mm x 50 mm dimension) were prepared in which the cement vs. sand ratio was taken as 1: 3 and water vs. cement ratio was 0.4 for this study. The concentrations of the T-*B. subtilis* bacterial cells in the mortar specimens were varied from 10^2-10^6 cells/ml water and the compressive strength of the bacterial cells amended samples was investigated at different periods of water curing.

Concrete specimens (150 mm x 150 mm x 150 mm dimension) were prepared by using the 43 grades OPC, normal river sand and 16 mm down coarse aggregates. The cement, sand and coarse aggregates ratio was maintained as 1: 1.45: 3.25 and the water-cement ratio were fixed at 0.48. To achieve the maximum compressive strength, a bacterial cells concentration of 10^5 cells/ml of water for T-*B. subtilis* strain and 10^8 cells/ml of water for T-JMJ107 strain were used in all further experiments.[7] The compressive strength of

concrete and mortar specimens were tested after 3, 7, 28 and 90 days of water curing.[13] The ultrasonic-pulse velocity of the samples was measured by Pundit plus PC1007 UPV meter as per ASTM C597-02 20.[14]

2.4.2. Flexural Strengths & Split Tensile strengths measurement:

Concrete beam specimens (100 mm x 100 mm x 500 mm dimension) were prepared for the determination of flexural strength as per ASTM C293.[15, 16] The specimens were cured under water for 28 days. Split tensile strength of all the concrete mixtures were carried out by using the cylindrical concrete specimens (100 mm diameter x 200-mm height) after 90 days of water curing.

2.4.3. Rapid chloride ion permeability Test (RCPT):

Rapid chloride ion permeability test is a measure for durability character of concrete in which cylindrical concretes (100 mm diameter x 200 mm height) were prepared by using cement-sand-aggregate mixture along with different bacterial strains (JMJ107, T-JMJ107, *B. subtilis*, T-*B. subtilis* and BKH2). After 90 days of water curing, the RCPT of the samples were tested as per ASTM C1202.[17, 18]

2.4.4. Bond strengths measurement:

The bond test specimens consisted of concrete cubes of similar size (150 mm x 150 mm x 150 mm dimension), with a single reinforcing bar (deformed and mild) embedded vertically (projected down for a distance ~ 10-15 mm from the bottom face of the cube) along a central axis in each specimen. In this experiment, deformed (Fe-500) and plane (Fe-250) bar were used for each category of concrete specimens. The test was performed as per IS2770 after 28 days of water curing.[19]

2.5. Self-healing study:

Different bacterial cells incorporated mortar cubes (50 mm x 50 mm x 50 mm dimension; N=20) were prepared for self-healing study in which the cement-sand ratio was taken as 1: 3 and water-cement ratio was fixed at 0.4. Bacterial cell concentrations were also chosen as 10^8 cells/ ml of water used for JMJ107 and T-JMJ107 whereas for BKH2 amended samples it was taken as 10^5 cells/ ml of water. Mortar samples of similar dimension were also prepared by incorporating the endospore of *B. subtilis* and T-*B. subtilis* at a concentration of 10^5 spores/ml water used. After 120 days of air curing, the average

UPV and breaking load of each category mortars (n=10) were measured. After measuring the breaking load, 50% of the corresponding breaking load was applied to the rest bio-mortars (n=10) of each category of samples. The artificial cracks with various widths and depths were generated on every specimen as viewed by Crack detection microscope (VJT6330, VJ Tech, England). The samples were then immersed in a closed bucket containing 10% LB-medium (v/v) in water for next 28 days at ambient temperature. Closed environment was set up to avoid free diffusion of oxygen and carbon dioxide over the water–air interface through the entire curing period. After curing, the UPV and breaking load of the remaining samples (n=10) were measured. Specimens were air dried for stereomicroscopic inspection by Crack detection microscope to investigate the crack-healing attributes.

The development of Gehlenite phases inside the T-*B. subtilis* incorporated mortars under 10% LB curing was examined by using Field Emission Scanning Electron Microscope (FESEM; INSPECT F50 SEM, FEI Europe BV, The Netherlands)

2.6. Microstructure analysis of self-healed bio-material:

The crack-healing materials developed inside the cracks of the *B. subtilis* and T-*B. subtilis* incorporated mortar samples was collected and crushed into fine powder by using pestle-mortar. The powder samples were examined under the FESEM and analysed by Energy dispersive spectra (EDS) for elemental quantification studies. The morphology of the self-healing material (from the crack of T-*B. subtilis* incorporated mortar samples) was evaluated by using Transmission Electron Microscopy (TEM; JEOL, JEM 2100). XRD analysis was also performed (Bruker AXS, Inc., Model D8, WI, USA) with mono-chromatic Cu-K_α radiation of wavelength 1.5406 Å at 40 kV and 40 mA. The samples were examined at 2θ from 10^o to 70^o and identified by referring to data of Joint Committee on Powder Diffraction Standards (JCPDS) files.

Statistical analysis:

There were 10 concrete samples prepared for each category of testing and each experiment was repeated thrice. Data are represented as mean over 30 samples ± SD in the bar diagram.

3. Results:

3.1. Gene transformation & protein isolation

The corresponding gene of the bioremediase-like protein was fished out from the whole genome of BKH2 by using the primers constructed from the sequence of carbonic anhydrase II of *Bos Taurus*.[7, 12] The DNA fragment was amplified by PCR technique and the product was then transformed into *B. subtilis* bacterium through a suitable T-vector. The transformation was confirmed by growing the bacterial cells (colonies) in an ampicillin-containing LB-agar plate, as shown in Figure 1A. Figure 1B shows the whole-cell protein profiles of *B. subtilis* and genetically improved *B. subtilis* (T-*B. subtilis*) when analysed by SDS-PAGE. A new protein band appeared in the protein profile of T-*B. subtilis* bacterium, molecular weight was ~ 28 kDa. This newly expressed protein was purified from the crude mixture of whole cell protein through the column chromatographic technique. The silica leaching activity of the purified protein (28 kDa) was confirmed by biosilicification assay (Table 1).

3.2. Compressive strength and ultrasonic-pulse velocity analysis

T-*B. subtilis* cells showed compressive strengths increasing attributes for the mortars when incorporated at different cell concentrations for different days of incubation (Fig. 2A). The maximum strength increment of the bacteria amended bio-mortars was achieved at a concentration of 10^5 cells/ ml of water used. The T-*B. subtilis* incorporated mortar (TBM) samples also exhibited better compressive strengths and UPV in comparison to the other bacteria incorporated samples at all ages (Figs. 2B & 2C).

3.3. Mechanical strength and durability analysis

Figure 3A exhibits the improvement of compressive strength of different bacterial amended bio-concrete samples. All the bacteria incorporated bio-concrete samples except *E. coli* (JMJ107) amended samples, exhibited higher compressive strength with respect to control samples. The maximum increment of compressive strength of the transformed *Bacillus* bacterial cells incorporated bio-concrete (TBC) cubes was observed with the addition of 10^5 cells/ml of water used at all curing ages. From the split-tensile strength and the flexural strength analysis, it was observed that the T-*B subtilis* bacterium increased the mechanical strengths significantly (Fig. 3B). The experimental bond strength (under tension) of bio-concretes and control concrete with rebar (deformed and mild steel) are shown in

Figures 3C & 3D. The ultimate bond strength and characteristic bond strength of deformed rebar and mild steel rebar were calculated from bond strength vs. slip curve. The bond strength of the TBC with deformed and mild steel rebar were significantly greater compared to other samples. The rapid chloride permeability test revealed that the least amount of charge penetration was realized inside the TBC matrices (Fig. 3E). Figure 3F demonstrated the better workability of TBC in the slump test.

3.4. Self-healing study of biotechnologically modified building material:

Figures 4A & 4B show the variation of compressive strength and UPV respectively of the different bio-mortars after 120 days of air curing. After applying the 50% of their corresponding breaking load, the simultaneously measured compressive strength of each sample were decreased. After 28 days of water (with 10% LB v/v) curing, the compressive strength as well as the UPV of all the samples were increased. The rate of increment was highest in case of the TBM samples.

Images of the cracks and their progressive healings were examined by Crackscope. Partial and complete crack healing abilities were observed in the case of *B. subtilis* and T-*B. subtilis* incorporated mortar samples respectively after 28 days water (with 10% LB v/v) curing period (Fig 5). Gradual formation of nano rod-shaped Gehlenite along with calcite crystals were observed in T-*B. subtilis* incorporated mortar samples as investigated by FESEM (Figure 6).

3.5. Microstructural analysis:

FESEM analysis of the powder samples obtained from the cracked portions of the *B. subtilis* showed irregular crystalline materials (Fig. 7A). The self-healing materials collected from the cracks of the TBM samples showed regular nano needle-like structures (Fig. 7B). The EDS analysis determined that the self-healing materials consisted of calcium, aluminium, oxygen and silicon atoms. The TEM analysis (Fig. 7C (i) & (ii)) of the Gehlenite shows rod shaped morphology (diameter ~ 85 nm) which is in agreement with the FESEM image (Fig. 7B). The XRD analysis of self-healing materials exhibit that additional peaks appear in the TBM matrices (Fig. 7D (ii)) which confirms the formation of Gehlenite (calcium aluminium silicate) phase absent in *B. subtilis* (Fig. 7D (i)) incorporated mortar samples.

4. Discussion:

Cementitious matrices may seem unhealthy for life, as it is very dehydrated and extremely alkaline compared to natural environment of bacterial existence. Nevertheless, some bacteria are found within the earth shell inside rocks in deserts as well as in ultra-basic environments at great depths.[20-22] Under favourable conditions some common aerobic and active alkaliphilic soil bacteria like *Bacillus sp., Pseudomonas sp.* can continuously precipitate/ mineralize impermeable calcite layer over the surface of existing concrete layer which may act as self-healing agent within the concrete structures.[3, 5, 6] A low metabolic activity and extremely long lifetime characterize spores and some *sp.* are known to produce spores which are viable for up to 200 years.[23] Such *Bacillus sp.* spores when immobilized within cementitious materials, produced copious amounts of mineral crystals on exposed surface of the media, therefore able to seal cracks by bio-mineral formation after being revived by water and growth nutrients entering the freshly formed cracks.[2,10] Self-healing processes of bio-concrete need additional nutritious environmental stimuli as triggers, such as calcium lactate and urea along with suitable environment.[6,10, 24-27] Though some anaerobic hot spring bacteria (BKH1 & BKH2) and its secretary protein(s) both are able to increase the compressive strength and durability of the mortar/concrete samples, they fail to survive for a long period in the harsh environment of the concrete.[7,8,12] The biosilicification activity is prolonged inside the concrete through the gene transformation into the spore forming *B. subtilis* stain for realization of sustainability and true self-healing phenomenon (Fig. 1 & Table 1).

This study demonstrates that the transformed *Bacillus subtilis* bacterial strain possesses better efficacy for the strength and durability of the incorporated mortar specimens (Figs. 2A & 2B) due to the formation of Gehlenite along with calcite precipitation inside the mortar matrices. The increased compactness of the mortar sample is reflected by the results of ultrasonic-pulse velocity tests of the samples (Fig. 2C). The compressive strength, split tensile strength and flexural strength are the important factors of concrete which support the stability and longevity of the concrete based structures. Our experimental results confirm that TBC possess the property for overall increasing mechanical strength (Fig. 3). It is already reported that the bacteria *Bacillus sp.* is able to deposit calcite phase inside the concrete matrices when incorporated to the mortar samples.[2, 6, 10, 24-26] The biochemical activity of the bioremediase-like protein has been found to synthesize Gehlenite phase inside the concrete/mortar matrices.[7] These two phases are

synergistically developed inside the TBC matrices and exhibited better mechanical strength by filling the micro pores inside the samples (Fig. 3A & 3B). Bond strength of the TBC shows better performance than others, as greater split tensile strength of TBC (Fig 3C & 3D). The presence of adequate amount of Gehlenite beside calcite in the TBC matrices makes the concrete denser and produce stronger interfacial transition zone (ITZ) between aggregates and the matrix, which increases the bond strength between the reinforcement bar and surrounding concrete. The RCPT result suggests that small amounts of free chloride ions are permitted through the less porous TBC matrices implying better durability of the samples (Fig 3E).

The self-healing study of cementitious material incorporated with the bacterial cells of BKH2, T-JMJ107 and *B. subtilis,* it is clear that these bacterial strains are able to fulfil the self-healing behaviour to some extent. It is observed that both the bacterial strains BKH1 and BKH2 can survive inside the concrete matrices only for 7 – 8 days.[7, 12] The genetically modified *Bacillus sp*. has been investigated for green and sustainable (self-healing) concrete as it can survive inside the concrete in dormant phase over an extensive period which is not possible for other bacterial strains like *E. coli* bacterium (T-JMJ107).

The T-*B. subtilis* bacterial cells are found to act as a proper self-healing agent to catalyse the process of autonomous repair of micro and macro cracks inside the concrete and thereby increasing the strength and durability of the structures. Endospores remain in dormant phase as they are capable of surviving without nutrients and are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants.[28] In presence of the favourable environment, the endospore becomes active i.e., it transforms from the dormant phase to the vegetative phase. In this experiment, it is observed that the genetically enriched *B. subtilis* (T-*B. subtilis*) bacterial spore became active after a prolonged time of dormancy under favourable environment (introduction of LB in air cured cracked mortar sample), initiating the biosilicification and bio-mineralization processes (Fig. 6). This subsequently leads to the self -healing of the mortar as the cracks are filled with the newly formed needle-shaped nano-calcium aluminium silicate (Gehlenite) phase beside calcite inside the cementitious matrices (Fig. 7).

The non-transformed *Bacillus subtilis* bacterial cells (host cells) produce a protein which can synthesis calcite phase only inside the mortar samples. No such self-healing phenomenon was observed in the other bacterial cells like (wild type and transformed *E. coli*) incorporated mortar samples as they do not have the spore forming ability and perish.

Microstructures analysis of the self-healing material obtained from *B. subtilis* and T-*B. subtilis* bacteria treated mortar samples show contrasting textures of their matrices (Fig. 7). The matrix of the *B. subtilis* amended mortar samples is appeared to be amorphous, showing no signature of conspicuous crystal growth. On the other hand, the TBM samples show crystalline matrix where the individual crystals can be recognized. The formation of crystalline calcium carbonate phase inside the matrices of *Bacillus sp.* bacteria incorporated mortars is elaborately described in several studies earlier.[5, 22-27]The development of calcite and Gehlenite phases within the TBM matrices are shown in the Figure 7 which clearly demonstrates the true self-healing behaviour of the T-*B. subtilis* bacterial strain in cementitious composite.

6. Conclusion

Genetically improved endospore forming *B. subtilis* strain enhances the autonomous healing property of cementitious composites. The formation of Gehlenite phase by the enriched microbes with high longevity inside the mortar/concrete is the main reason behind the increment of its strength and durability. Development of a newly transformed strain and exploration of its overall property for sustainable green concrete would be a promising area for future construction technology. This study will provide an eco-friendly, pollution free, non-hazardous biotechnologically improved way for fulfilling the mostly desired "green" self-healing concrete for modern.

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Conflict of interest: None

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Figure legends:

Figure 1: (A) host cell *B. subtilis* (B) T- *B. subtilis* in agar Plate containing ampicillin; (C) SDS-PAGE images of protein profiles.

Figure 2: (A) Compressive strengths of T-*B. subtilis* amended mortars; (B) Comparison of compressive strengths for different bio-mortars; (C) Ultrasonic Pulse Velocity for different bio-mortars.

Figure 3: (A) Compressive strengths; (B) Tensile strengths and Flexural strengths; (C) Bond strength of different bio-concretes (Plane bar); (D) Bond strength of different bio-concretes (Deformed bar); (E) Rapid Chloride Permeability Test; (F) Slump Test of Concretes.

Figure 4: Self-healing study of different bio-mortars by (A) compressive strengths (B) Ultrasonic Pulse Velocity measurement.

Figure 5: Images of the cracked and healed surfaces of mortars.

Figure 6: FESEM images of the progress of Gehlenite formation inside the mortar in presence of T-*B. subtilis* in different days.

Figure 7: FESEM images of self-healing materials obtained from (A) *B. subtilis* (B) T-*B. subtilis* incorporated cementitious matrices (C) TEM images of T-*B. subtilis* incorporated cementitious matrices (D) XRD spectra of self-healing materials obtained from (i) *B. subtilis* and (ii) T-*B. subtilis* incorporated cementitious matrices.

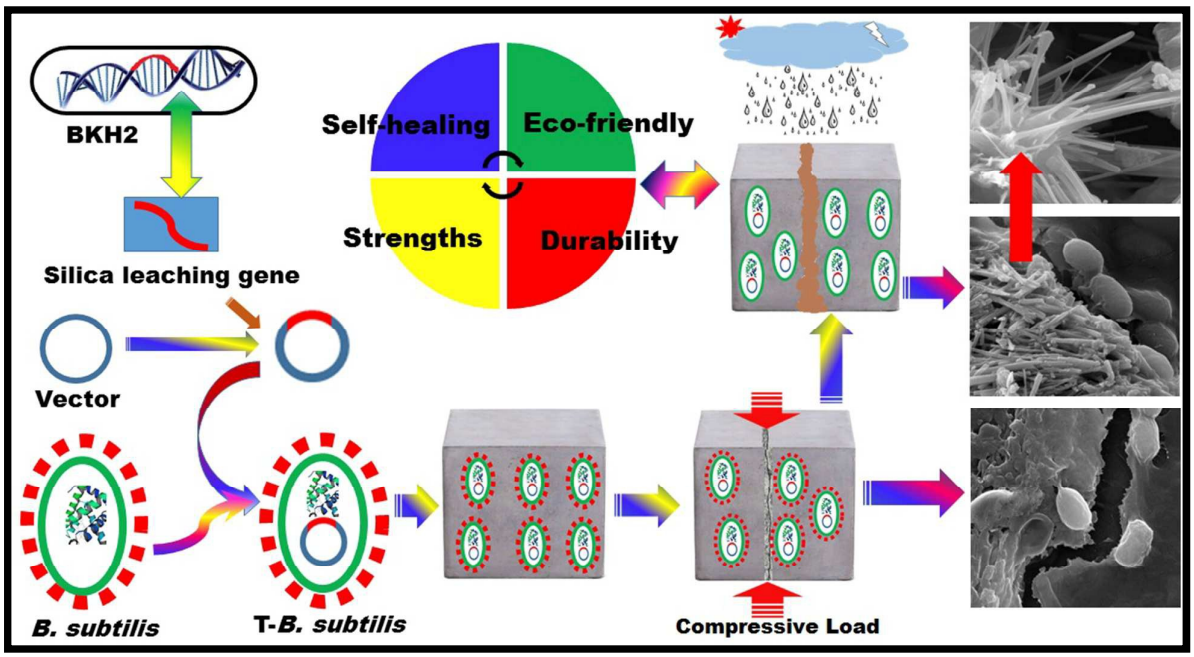
Table 1: Biosilicification activity of protein(s)

Samples	Total protein (mg)	Activity (Units)	Sp. Activity (Units/mg)
Crude bioremediase like protein (BKH2)	5	144	28.8
Purified bioremediase like protein (BKH2)	2	410	205
Crude whole cell protein (*B. subtilis*)	5	---	---
Crude whole cell protein (Transformed *B. subtilis*)	5	186	37.2
Purified bioremediase like protein (Transformed *B. subtilis*)	2	570	285

One unit activity of bioremediase protein is expressed as µg of silica released/mg of protein

Graphical Abstract:

TEXT: Genetically modified spore forming *B. subtilis* bacterial cells for eco-friendly sustainable self-healing bio-concrete.



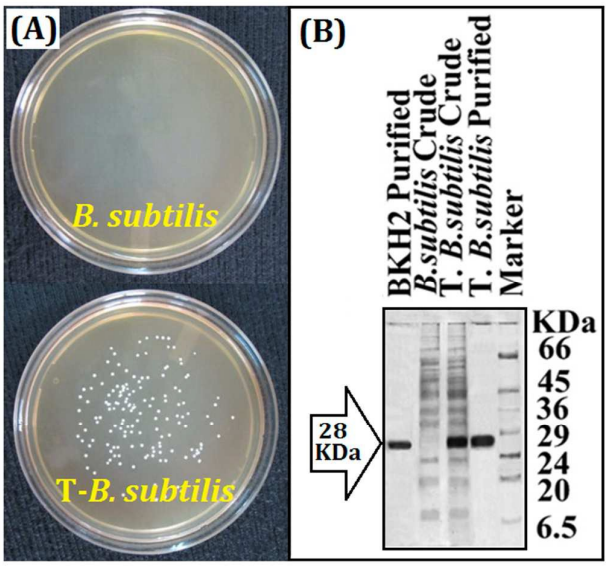


Figure: 1

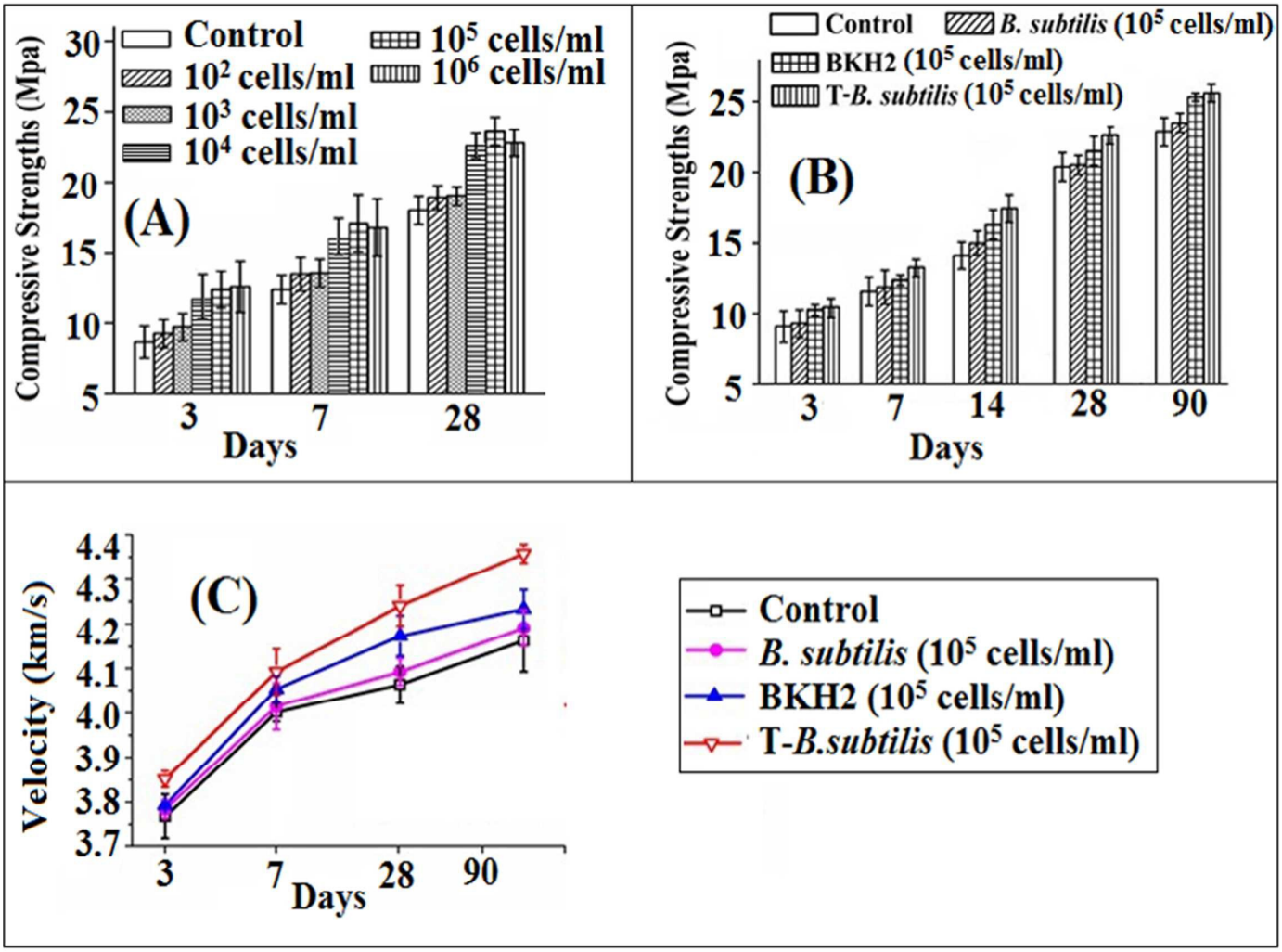


Figure: 2

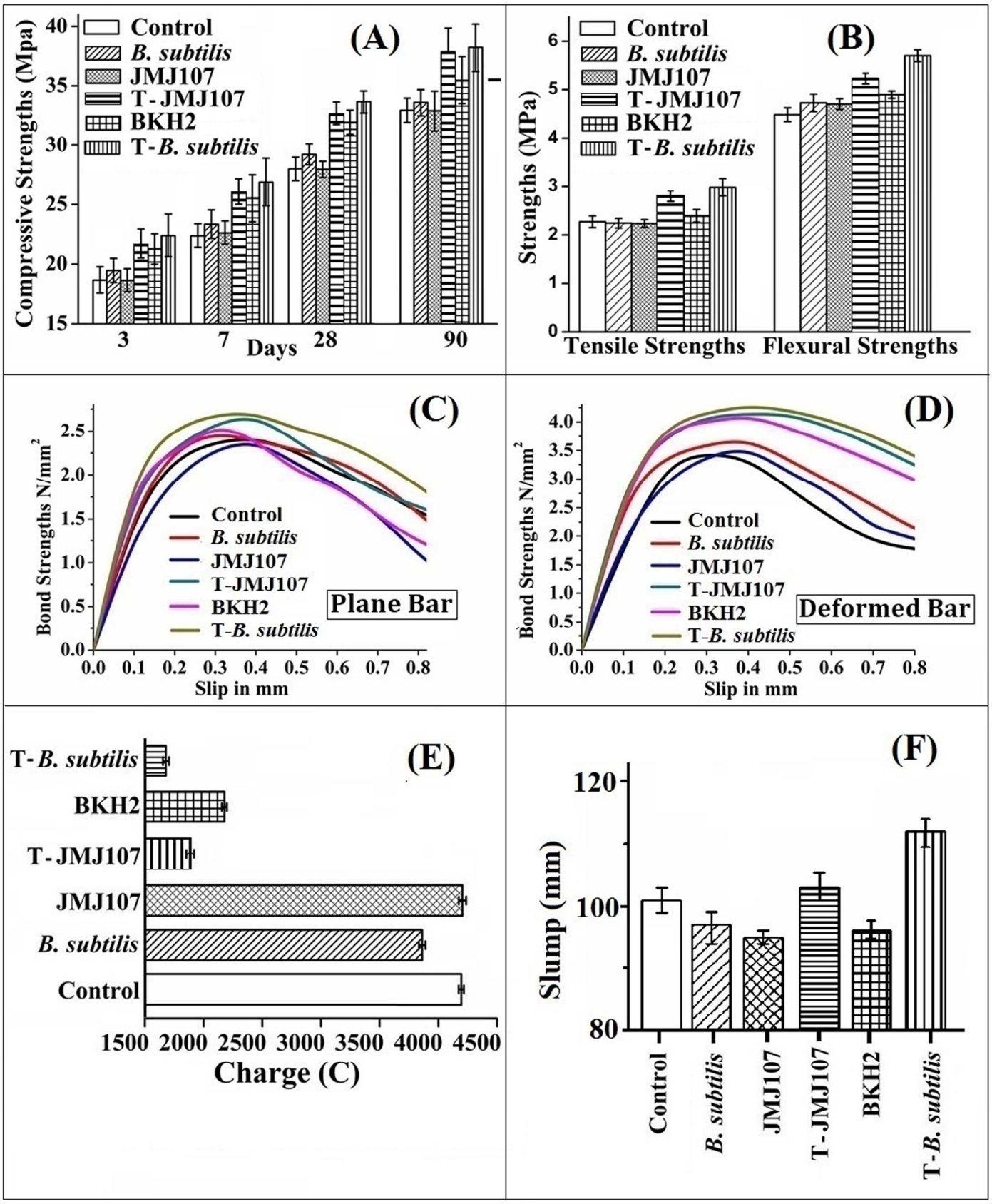


Figure: 3

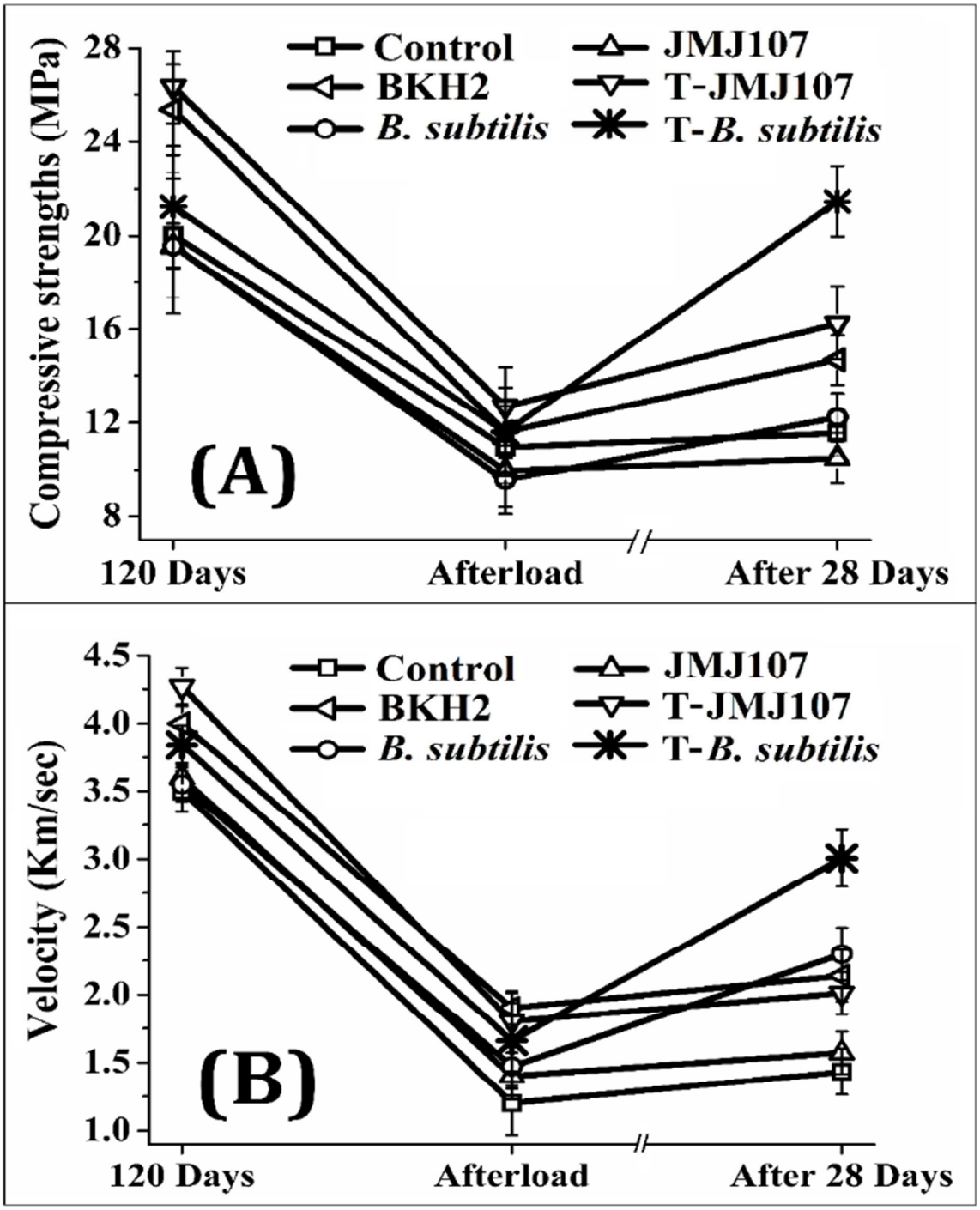


Figure: 4

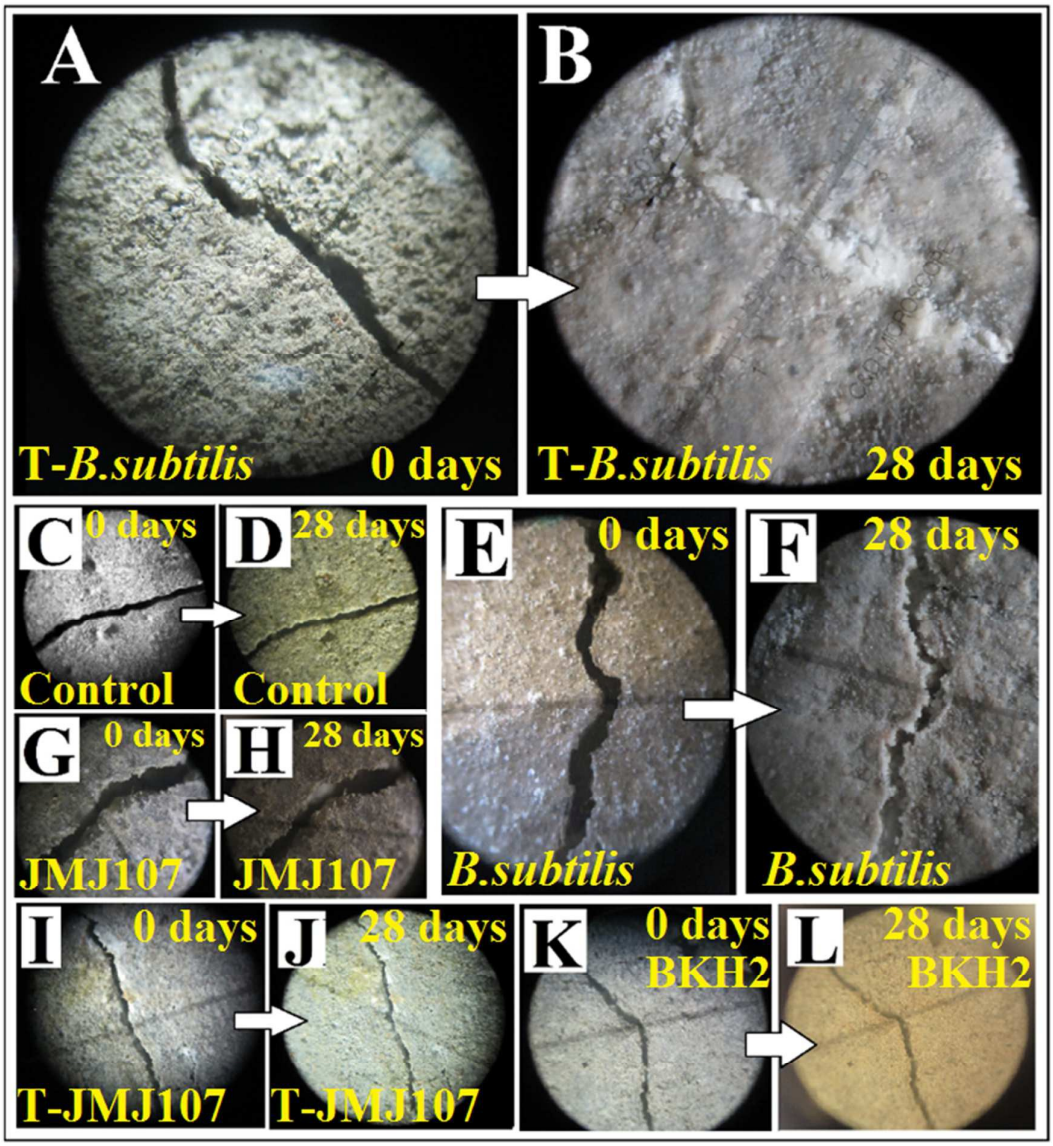


Figure: 5

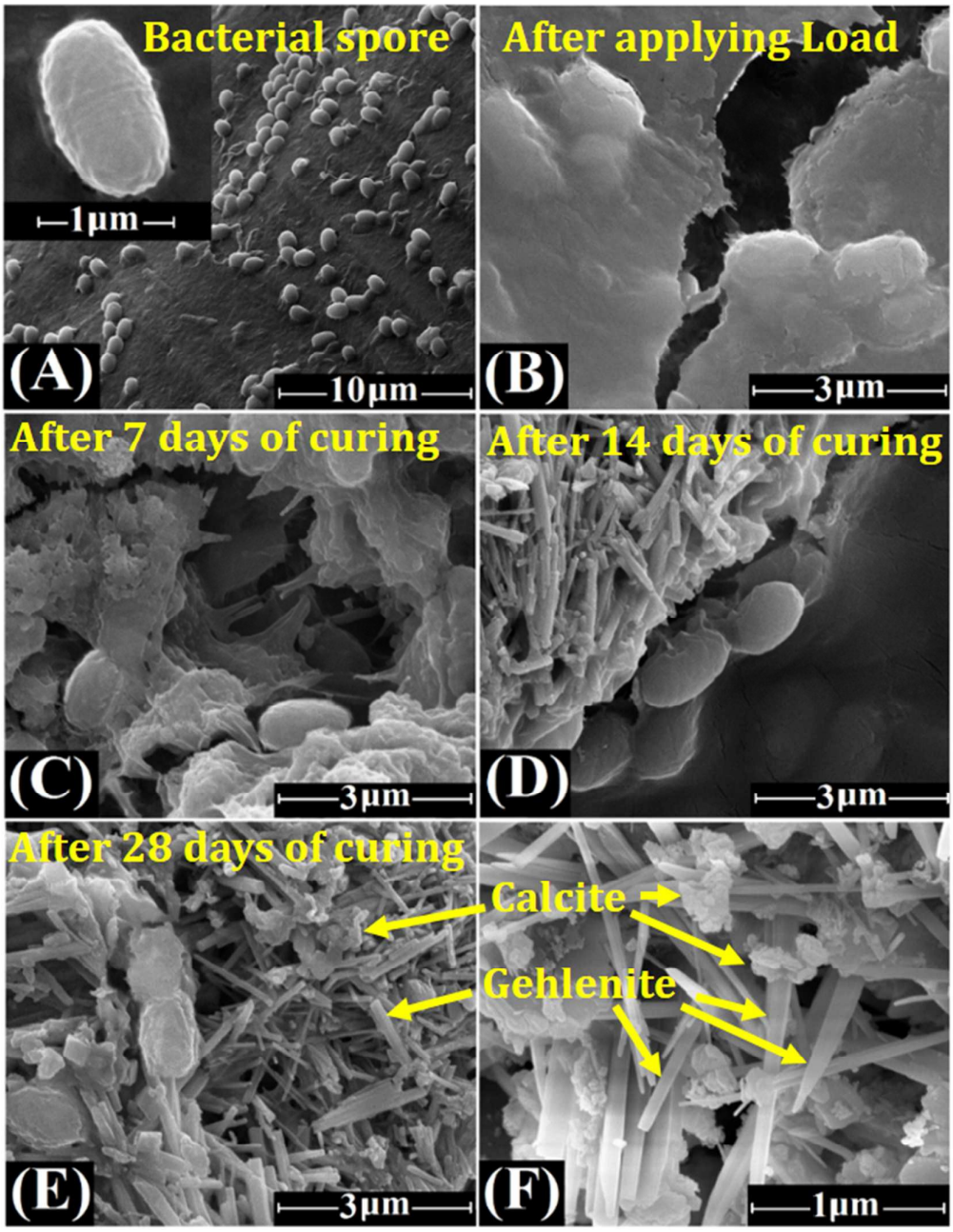


Figure: 6

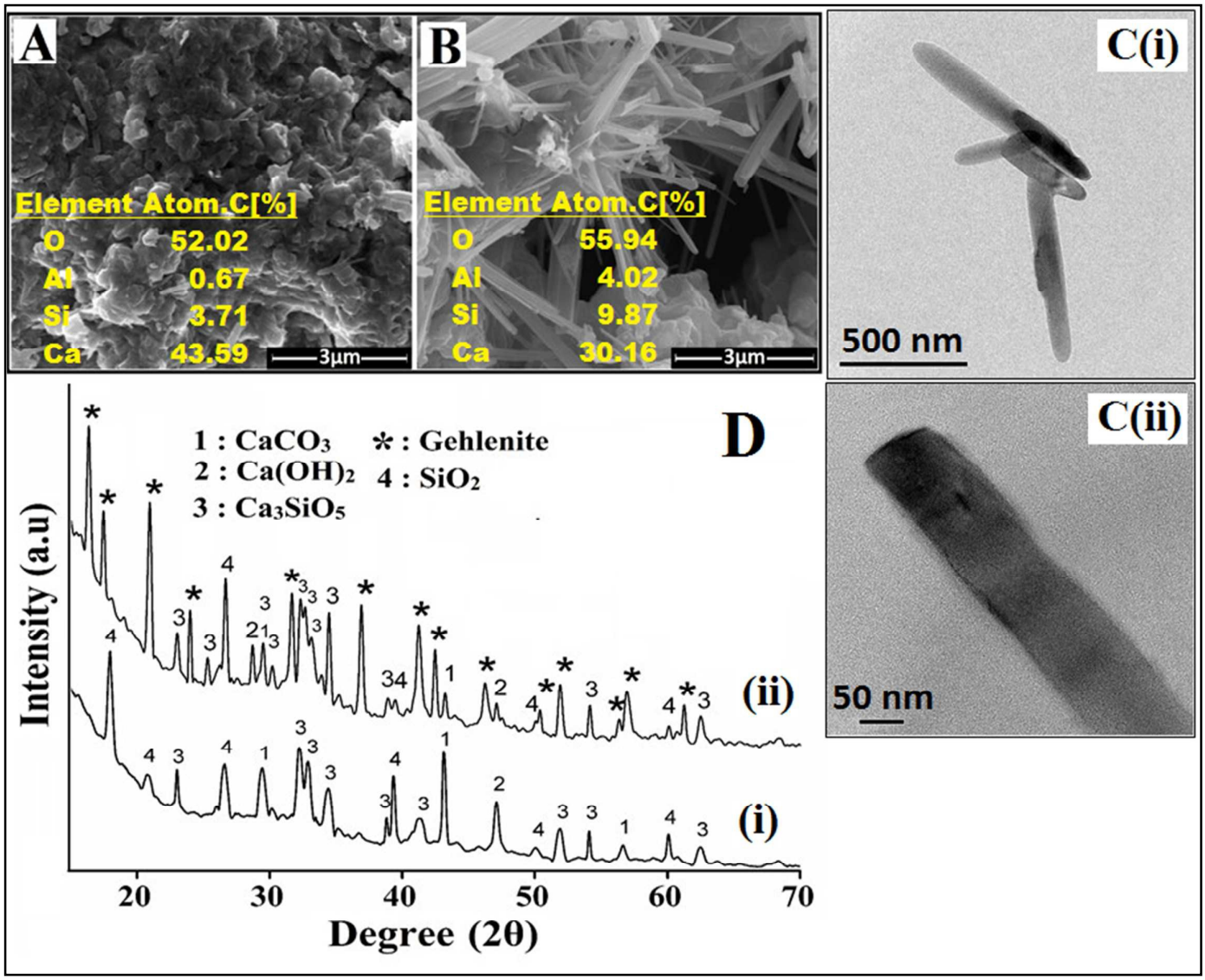


Figure: 7