THE MICROBIAL PROTEIN BIOREMEDIASE - A PROSPECTIVE AGENT IN CONSTRUCTION TECHNOLOGY

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Abstract

Now-a-days alternative approaches in construction technology are evolving rapidly in a bid to curtail the environmental hazards and low sustainability imparted by the prevalent technologies. Our study deals with the feasibility of application of a novel bacterial protein Bioremediase, on the most widely used cementitious material worldwide- Portland Pozzolana Cement, hitherto found to increase the compressive strength, flexural strength on Ordinary Portland cement based concrete and mortar samples. This silica leaching protein in its purified lyophilized form when impregnated into the Portland Pozzolana Cement based concrete and mortar samples, found to increase remarkably the compressive strength, flexural strength, resistance to commonplace environmental pollutants (water absorption and sulphate ion) as well as self-healing attributes of the test specimens. This Bioremediase protein exhibits sustainability across a wide time and temperature barrier. These findings along with the fact that this protein does not impart any negative repercussion on human health may render it as a prospective agent in construction technology.

Key words: Microbial protein, Concrete, Compressive strength, Sustainability, Portland Pozzolana Cement

1. Introduction

Biologically augmentation of strength of cementious materials has beckoned the researchers around the globe in recent times. Concrete, the commonplace construction material allover the world is ascribed with high compressive strength but modest tensile strength (Mehta, 1999). Its inadequacy of tensile strength paves way for counterbalancing via the use of reinforcements (e.g. steel rebar). However, even after reinforcement, cracks surface over concrete structure as fallout of applied structural loading, shrinkage and thermal deformations, most of them are, in true sense, inevitable and expected within the context of practicality (Jonkers, 2007). Commonly used reinforcement agents in construction materials corrode the structure within, thus bringing down the shelf life of the structures. Occurrence of cracks cut down the load capacity and stiffness of the concrete structure by yielding passage to ions-the chief culpable of concrete deterioration (Ramachandran, 2001). Chloride ions, oxygen and carbonating agents can pass through the cracks and end up in corroding reinforcing steel, which contributes to the extensive disintegration of concrete structure globally (Mehta, 1999). Hence occurrence of cracks is a prevalent form of damage in concrete structures. All these lead to appreciation of manufactural and maintenance cost of concrete based structure coupled with potential of environmental hazards. In this context, concept of biomineralization holds water.

Biomineralization is a metabolic process of formation of hard structures, surfaces or scales by combining minerals with organic compounds of some specific microorganisms (Skinner and Jahren 2003). According to Belkova (2005) metabolic activity of some specific microorganisms play a pivotal role in the transformation of many members of the periodic table. Specific microbial proteins influence the biomineralization process either through

guiding prevention or formation of mineral deposits (Boskey, 2003). This biominerology concept has been looked into very keenly for development of new bioconcrete material (Ramachandran et al., 2001; Ghosh et al., 2005), cleaning of concrete surface (DeGraef et al., 2005) and imparting microorganisms directly for inducing calcite precipitation in concrete crack (Bang et al., 2001; Rodriguez-Navarro et al., 2003).

In commercial purpose, the commonplace cement used is fly ash or slag based pozzolana cement, which is prepared by apposite mixing of ordinary portland cement and any pozzolana material such as fly ash, blast furnace slag, brick powder, rice husk ash etc. Portland pozzolana cement is generally slow setting and exhibits sulphate resistance attribute (Mehta, 1999). Owing to salvage of waste products such as fly ash and slag pozzolana cement fill the bill of eco-friendly construction ingredient. The present study furnishes a performance analysis of the bacterium BKH1 and its secretary bioremediase protein regarding compressive strength enhancement, tensile strength and self-healing attributes of portland pozzolana cement based specimens. Comprising with the observations of ordinary portland cement based specimens obtained earlier; we are trying to affirm the practical applicability of the bioremediase protein in fly ash/ slag based pozzolana cements as alternative approach to construction technology.

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 *Thermoanaerobactor fermicutes* (Biswas et al., 2010). In sealed glass pressure vials it can be cultured anaerobically (in presence of CO_2 atmosphere) (Chattopadhyay et al.,

1993) in a synthetic growth medium (containing $Fe(OH)_3$ – 0.1 M, Na_2HPO_4 – 0.6 g/L, 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 ^oC temperature (Ghosh et al., 2005). This bacterium has been found to survive up to pH 12.0 of the growth medium; however its growth rate is slowed down at this high pH level (Biswas et al., 2010). A few proteins are secreted by this anaerobic bacterium in the growth medium during its growth. One of the secretary proteins having molecular weight of 28 kDa has shown silica-leaching property (Biswas et al., 2010) similar to marine sponge (Cha et al., 1999). The protein is named as "Bioremediase", which is non-harmful, and eco-friendly processing additive.

2.2. Purification of bacterial protein from growth medium

About 100 ml bacteria grown culture medium (6 – 8 days old) containing bacteria in the magnitude of 10^8 bacterial cells per ml was taken in a tube and centrifuged. The supernatant of the centrifuged culture medium was taken in a round bottom flask and lyophilized (Freeze dryer FD-1, Rikakikai, Toshiba) to dust powder (approximately 600 mg powder obtained from 100 ml bacterium grown cultured medium). The dust powder was then dissolved in 10 ml deionized distilled water and 20 ml of ice-cold acetone was added to it and kept at 4 ^oC for overnight. The crude proteins thus precipitated were separated by centrifugation and lyophilized to dust crude protein powder (about 200 mg obtained from 600 mg dust powder). The crude protein was then dissolved in 2 ml of deionized sterile water and loaded on Sephadex G-100 column (100 cm ×1 cm). Fractions (1 ml each) were collected through fraction collector (Eyela DC-1000). Measuring the optical densities of the fractions at 280 nm monitored the protein containing fractions. Biosilicifiation activity of each column-purified

protein containing fractions was performed using tetraethoxyorthosilicate (TEOS) as substrate. Those fractions showed biosilicification activity were pooled, concentrated by lyophilization and similarly eluted through the same Sephadex G-100 column. Protein containing eluted fractions were then pooled and dialyzed. The powder bioremediase protein was obtained after lyophilization (80 mg approximately) and stored in screw capped plastic container at room temperature for further work.

2.3. Preparations of mortar samples for compressive strength

Mortar samples were prepared by using commercially available fly ash pozzolana cement 43 grade (IS 8112*)*. Standard Ennor sand (IS 650) was used by mixing with cement (3:1 w/w) for mortar samples preparation. Cement to water ratio was kept fixed at 0.4 for all samples preparation. Standard mortar cubes of following dimension (70.6 mm x 70.6 mm x 70.6 mm) were cast as described by Ghosh et al. (2005) as follows:

Control mortar cubes – Cement and sand mixture only.

Bacterial cells incorporated mortar cubes – Cement + sand + bacterial cells (at three different concentrations as 10^4, 10^5, 10^6 cells /ml of water used).

Bioremediase protein incorporated mortar cubes – Cement + sand + bioremediase protein powder (1, 2, 3 and 4 µg/g cement used).

No additional nutritional material, only excluding those present in the diluted cultures, was supplemented in the mortar cubes during casting. All the samples were cured under water as well as in open air after 24 h of casting. The compressive strengths of the mortar cubes were measured after 3, 7, 14, 28, 60,120 days of curing.

2.4. Preparation of mortar samples for crack repairing test.

The samples preparation was similar as described earlier. Small bars of standard dimensions (68mm x 5mm x 15mm) were impregnated on the top surface to create artificial fissures in the mortar samples. After 24h of casting the small bars were taken out. The cracks formed in the mortar samples were cured in water for 7 days. After that, either normal cement-sand mixture filled up the artificial cracks or BKH1 cells (10^4–10^6 cells/ml water used) incorporated cement-sand mixtures or bioremediase protein (1- 4 µg/g cement) incorporated cement-sand mixtures. All the samples were cured under both water and air after 24 h of recasting. The compressive strengths of the mortar cubes were measured after 3, 7, 14, 28, 60,120 days of curing.

2.5. Preparation of mortar samples for sulphate resistance test.

Mortar samples for sulphate resistant test were similarly prepared by using normal cement-sand mixture as well as bacterial cells/ bioremediase protein incorporated with cement-sand mixture as stated earlier. After 1 day, the samples were removed from the cassettes and their masses were recorded. The samples were cured under sulphate solution (5% $MgSO_4$, pH 7.0) for 120 days are shown. After curing, the samples were removed from the tank, air-dried and their masses again determined. From the differences of final and initial mass, the percentage of mass increment was determined. This will determine the amount of sulphate solution entered within the samples.

2.6. Preparation of mortar samples for water absorption test.

Mortar samples by using normal cement-sand mixture as well as bacterial cells/ bioremediase protein incorporated cement-sand mixture were prepared as previously described. After 28 days of water curing, the samples were removed, dried and their masses were

recorded. Then the samples were immerged in a water tank for 30 minutes. After that, the samples were removed from the water and their masses were recorded immediately. The samples were again kept in water for 24 hours. Their masses were similarly recorded after 24 hours of water curing. From the difference in values of the masses the percentage of mass increment were determined, which would determine the amount of water entered within the samples.

2.7. Preparation of mortar specific beam for flexural strength test.

Specific beams (3 beams for each category) were prepared by using normal cement (PPC) - sand mixture for control specimens and purified bioremediase protein (3 µg/g cement used) incorporated cement-sand mixtures for experimental specimens. The dimension of the standard beam was 200 mm x 50 mm x 50 mm. The beams were cured for 28 days under water and their flexural strength were determined in 4-point condition.

2.8. Statistical analysis.

For each testing experiment, 6 samples are prepared for each category of testing. Every experiment was repeated twice and data was presented by averaging of 12 (n = 12) samples. Standard deviation for each data was determined and presented. The percentage of increment was calculated with respect to the control data.

3. Results and discussion

The purpose of this study was to observe the effect of the bacterial cells (BKH1) and its protein (bioremediase) on the mortar samples prepared by using fly ash based pozolanna cements. The positive effects of bacterium BKH1 and its bioremediase protein have already been studied on mortar/concrete samples by using ordinary Portland cements (OPC). But OPC

is not commercially available and in most cases, locally commercially available cements specially pozolanna cements are used for construction purposes.

Figure1 vividly describes the development of compressive strength of mortar cubes prepared by varying concentrations of bacterial cells using Portland Pozzolanic cement. The samples were cured for different days at room temperature in air and their compressive strengths were measured. It was noted that compressive strength of the mortar cubes augmented with addition of the bacterial cells at every stages of curing compared to the control specimens (devoid of the bacterial cells). The utmost 40.6% increment in regard to control after 28 days of curing and 41.8% increment after 120 days of curing were observed due to incorporation of bacterial cells directly to the cement-sand mixtures. The maximum increment in compressive strength was attained at the bacterial concentration of 10^5 cells per ml of water used in mortar preparation.

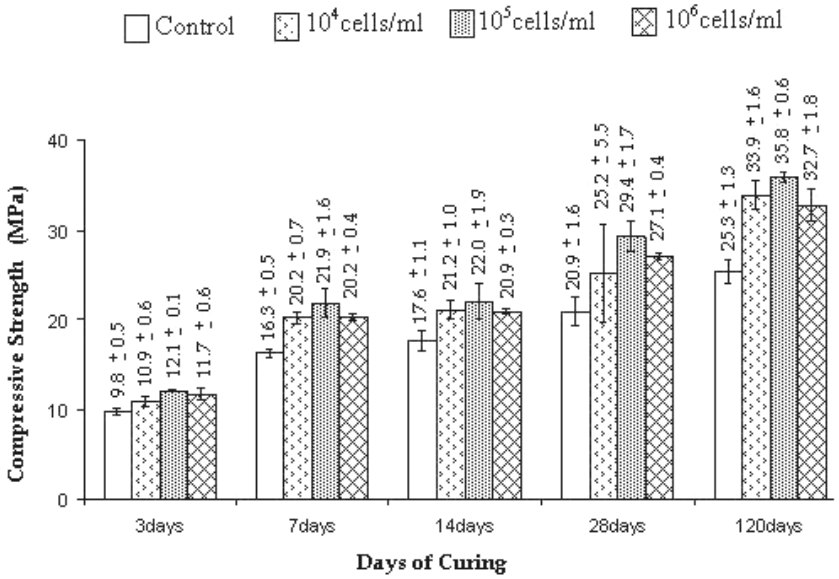

Figure 1. Mortar compressive strength with BKH1 cell under air curing

The observations of same stature were noted when the mortar samples were cured in water (Figure 2), where 39.4% increment in compressive strength was registered for 28-days cured samples and 42.4% increase in case of 120-days cured samples impregnated with bacterial cells at a concentration of 10^5 cells per ml of water used in mortar preparation. Concrete is one of the most heterogeneous materials. Mortar cubes were prepared manually. Manual compactness of the mortar samples sometimes vary and this may reflect in the strength measurement. But overall results showed consistency in the compressive strength increment.

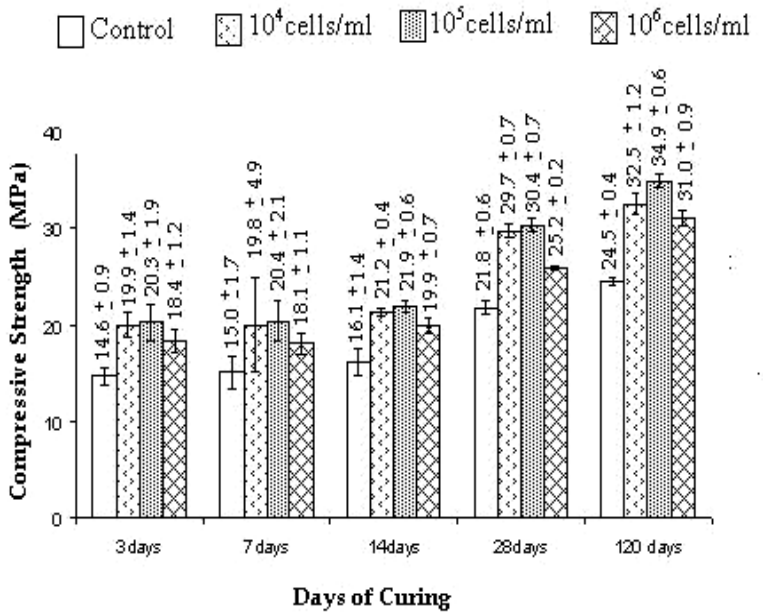

Figure 2. Mortar compressive strength with BKH1 cell under water curing

Sephadex G-100 column- purified pure bacterial bioremediase protein -admixed mortar samples when cured at room temperature in the air, displayed sharp increment of compressive strength at all ages (3, 7, 14, 28 and 120 days curing ages respectively) in match against normal control samples (Figure 3). Maximum compressive strength was attained at the purified protein

concentration of 3 µg per g of cement used (45% and 47.4% higher in magnitude compared to control in case of 28-days and 120-days of air curing respectively.

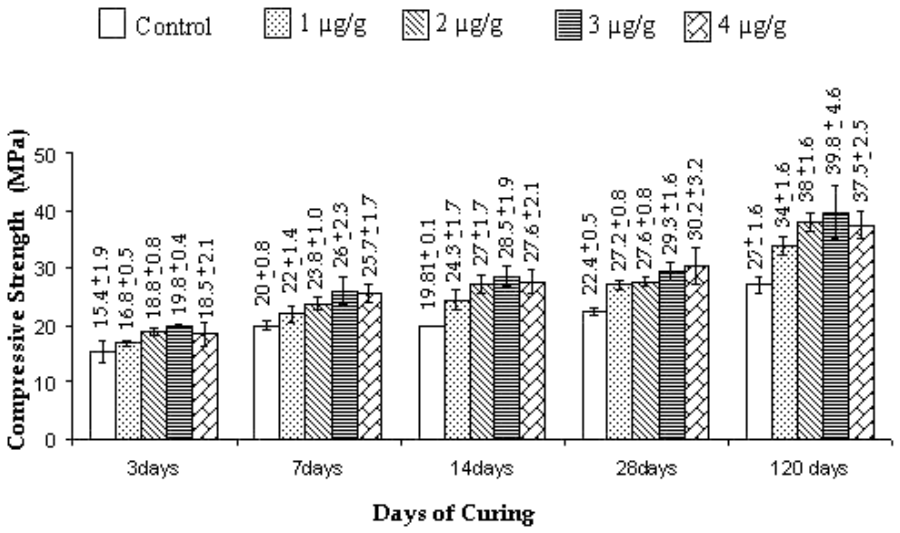

Figure 3. Mortar compressive strength with bioremediase protein under air curing

Similar trend of results were obtained when compressive strength of pure protein-admixed water cured mortar samples were noted (Figure 4).

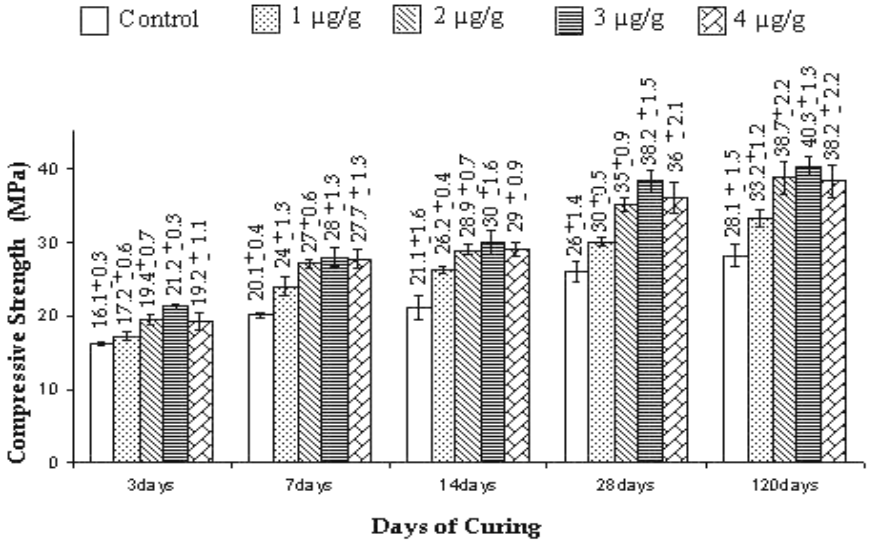

Figure 4. Mortar compressive strength with bioremediase protein under water curing

To figure out the feasibility of the bacterial protein in practical repairing circumstances, the artificially crack healing study by this novel biomaterial requires special attention. In Figures 5 and 6, compressive strength of mortar cubes where artificially generated cracks were repaired by normal cement-sand paste and of those cubes where the repairing material was bacterial cells (of different cell concentrations) admixed cement-sand paste respectively were furnished. It clearly displayed bacterial cell of 10^5 cells per ml concentration having an extra edge in terms of crack repairing efficacy (40.6% strength increment in 28-days air-cured samples and 38.9% strength increment for 28 days water-cured samples). This crack-repairing efficacy was further increased with increasing days of curing (45.5% for air curing and 45.3% for water curing respectively when incorporated with bacterial cells at 10^5 cells/ml).

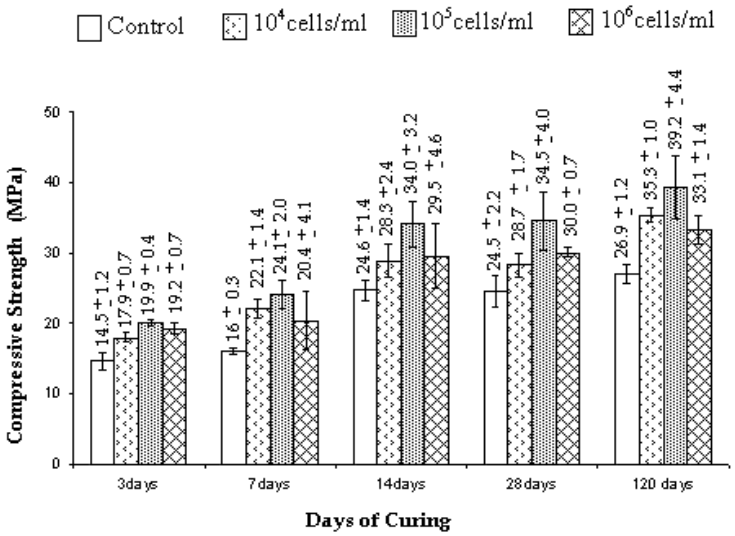

Figure 5. Compressive strength of the mortar whose crack was repaired by BKH1 cell incorporated cement-sand mixture (air curing)

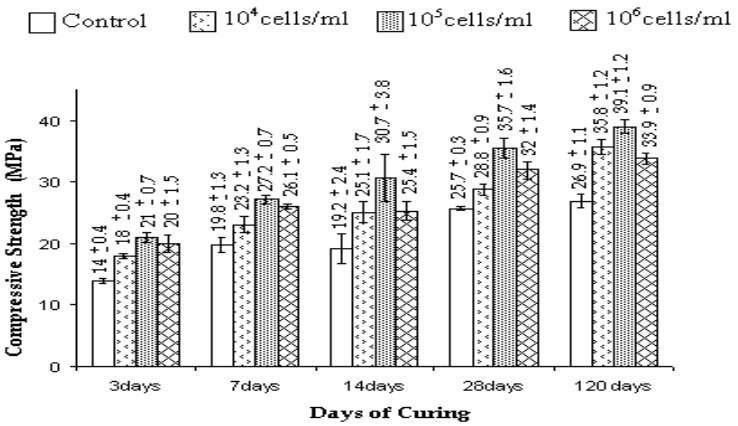

Figure 6. Compressive strength of the mortar whose crack was repaired by BKH1 cell incorporated cement-sand mixture (water curing)

Bioremediase protein amended cement-sand paste was found to be similarly competent compared to normal cement-sand paste in context of crack-repairing ability. It was observed that the purified protein in concentration of 3 µg/g of cement used displayed highest potency (compressive strength augmentation 43.8% for air curing and 46.8% respectively for 28-days air-cured and water-cured samples) (Figures 7 and 8).

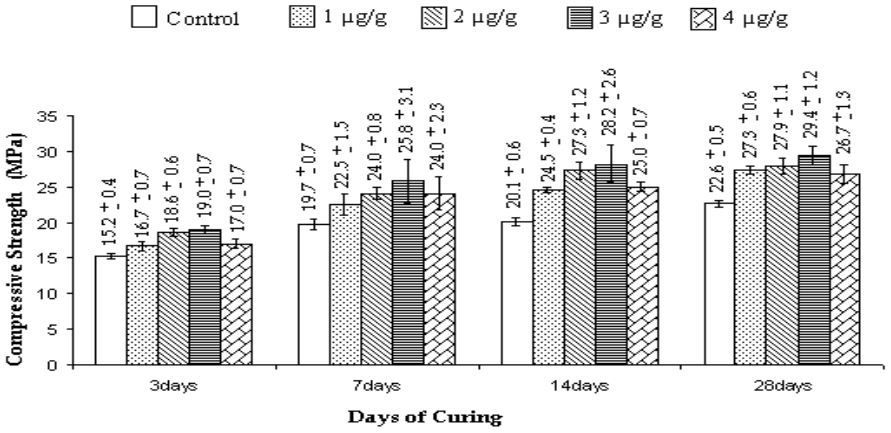

Figure 7. Compressive strength of the mortar whose crack was repaired by bioremediase protein incorporated cement-sand mixture (air curing)

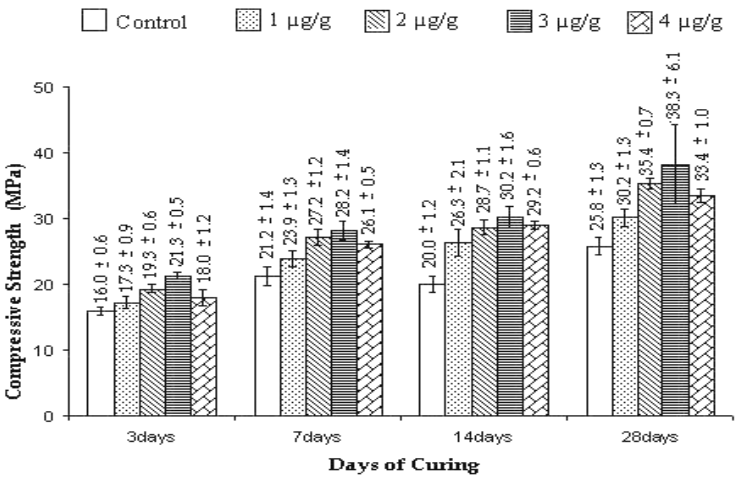

Figure 8. Compressive strength of the mortar whose crack was repaired by bioremediase protein incorporated cement-sand mixture (water curing)

Flexural strength of specific mortar beam has been analyzed by using bioremediase protein incorporated cement-sand mixture to the beams. Different concentration of protein was used in preparation of different beams. The flexural strength of bioremediase protein incorporated mortar beams was found higher compared to control beam. The maximum flexural strength increment was 33% with 3µg/g bioremediase protein incorporated samples (Figure 9).

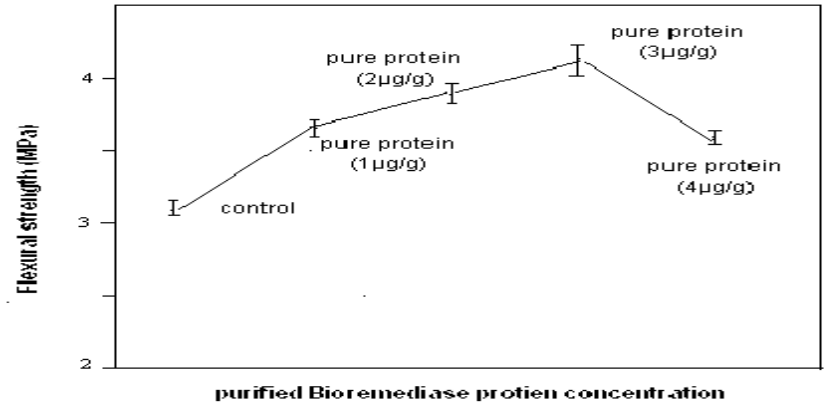

Figure 9. Flexural strength of mortar bar

Durability analysis of BKH1 cells or its bioremediase protein on cement mortar specimens is very crucial for sustainable construction purposes. It was noted that mass of mortar samples impregnated with bacteria and also with the bioremediase protein were less altered as compared to the control samples. An average increase of only 2.2% and 4.7% in mass were noted for mortar samples impregnated with bacterial cells concentration of 10^5 cells per ml after 30 min and 72 h respectively (Table 1).

Table 1. Water absorption (28days) using BKH1 cells

Mass / Mortar samples	Initial Mass (g)	Weight after 30 min (g)	% Increment in mass	Wt. after 72 h (g)	% Increment in mass
Control	735.5 ± 0.4	756.8 ± 0.4	2.9	790.5 ± 0.6	7.5
10^4 cells/ml water	730.5 ± 0.3	747.0 ± 0.2	2.3	772.0 ± 0.4	5.7
10^5 cells/ml water	733.2 ± 0.5	749.2 ± 0.5	2.2	767.6 ± 0.4	4.7
10^6 cells/ml water	720.3 ± 0.4	736.9 ± 0.3	2.3	765.2 ± 1.0	6.24

Data are presented mean ± SD; N = 12

Whereas the bioremediase protein incorporated mortar samples showed less increase in mass (only 1.7% and 3.7% after 30 min and 72 h respectively when protein was used at 3µg/g cement) due to water absorption (Table 2).

Table 2. Water absorption (28days) using bioremediase protein

Mass / Mortar Sample	Initial Wt. (g)	Weight after 30 min (g)	% Increment in mass	Wt. after 72 h (g)	% Increment in mass
Control	735.7 ± 0.5	754.8 ± 0.4	2.6	789.7 ± 0.4	7.3
1µg/g	733.9 ± 0.5	750.2 ± 0.5	2.2	781.3 ± 0.7	6.5
2µg/g	730.7 ± 0.2	747.4 ± 0.2	2.3	769.4 ± 0.1	5.3
3µg/g	725.5 ± 0.3	737.7 ± 0.2	1.7	752.6 ± 0.2	3.7
4µg/g	728.5 ± 0.3	744.5 ± 0.4	2.2	765.6 ± 0.2	5.1

Data are presented mean ± SD; N = 12

The results of sulphate resistance tests distinctly asserted the positive influence of these biomaterials on cement mortar specimens. It was noted that mass of mortar samples impregnated with bacteria and also with the bioremediase protein were less affected as compared to the control samples. An increase of only 4.5% in mass was noted for samples impregnated with bacterial concentration of 10^5 cells per ml (Table 3).

Table 3. Sulphate resistant test with BKH1 cells incorporated mortar samples

Mass / Mortar samples	Initial Mass (g)	Final Mass (g)	Average % of Increment
Control	735.0 ± 0.5	796.4 ± 0.6	8.5
10^4 cells/ml water	731.6 ± 0.4	783.4 ± 0.5	7.1
10^5 cells/ml water	737.5 ± 0.4	770.5 ± 0.5	4.5
10^6 cells/ml water	734.2 ± 0.5	789.2 ± 0.5	7.5

Data are presented mean ± SD; N = 12

Similarly 4.6% of mass increment was seen in bioremediase protein admixed mortar samples with protein concentration of 3 µg/g of cement used (Table 4). It is thus evident from these data that bacterium/bioremediase protein incorporated biomaterials are less prone to sulphate attack compared to normal cement-sand mortar.

Table 4. Sulphate resistant test with bioremediase protein incorporated mortar samples

Mass / Mortar sample	Initial Mass (g)	Final Mass (g)	Average % of Increment
Control	738.2 ± 0.3	799.5 ± 0.4	8.15
1µg/g	735.2 ± 0.5	785.0 ± 0.5	6.5
2 µg/g	736.1 ± 0.2	790.1 ± 0.3	6.95
3 µg/g	737.8 ± 0.4	771.2 ± 0.4	4.6
4 µg/g	730.6 ± 0.2	771.1 ± 0.3	5.5

Data are presented mean ± SD; N = 12

Water absorption test and sulphate resistant test both thus confirm that bacterium BKH1 or its bioremediase protein amended biomaterials are more durable compared to normal mortar.

Preserving them at two extreme conditions the activity of the protein remained almost unaffected. In performing these experiments, bioremediase protein powder was stored at 65 ^{o}C and -20 ^{o}C temperature respectively for 6 months. The protein was then used in preparation of mortar sample as stated earlier. The compressive strength of the stored bioremediase protein

impregnated mortar samples was found to increase in similar fashion as observed in fresh protein samples earlier (Table 5). More than 40% strength improvement was noticed against the stored protein impregnated mortar samples. This result suggests that the bioremediase protein can be stored and used for practical construction purposes without having any sophisticated storage facility.

Table 5. Effect on compressive strength by the protein stored at two extreme conditions

Sample specification	**Compressive Strength (MPa)**	
	Protein stored at 65 ^{o}C	Protein stored at –20 ^{o}C
Control mortar	25.8 ± 1.13	25.2 ± 1.17
Mortar with protein (1 µg/g)	30.7 ± 1.36 (18.6% ↑)*	30.2 ± 1.79 (19.7% ↑)*
Mortar with protein (2 µg/g)	37.1 ± 1.27 (43.8% ↑)*	36.6 ± 1.50 (41.2% ↑)*
Mortar with protein (3 µg/g)	37.7 ± 1.29 (46.1% ↑)*	36.19 ± 1.39 (43.6% ↑)*
Mortar with protein (4 µg/g)	36.2 ± 2.09 (40.3% ↑)*	34.31 ± 1.80 (36.2% ↑)*

- ↑ indicates increment, Data are presented mean ± SD; N = 12. The value within parenthesis indicates the % increment with respect to control.

Biochemical assay for bioremediase protein using ordinary Portland cement and pozolanna Portland cement respectively as substrate clearly indicated the activity of the protein was more in PPC than OPC in all-experimental conditions (Figures 10 & 11).

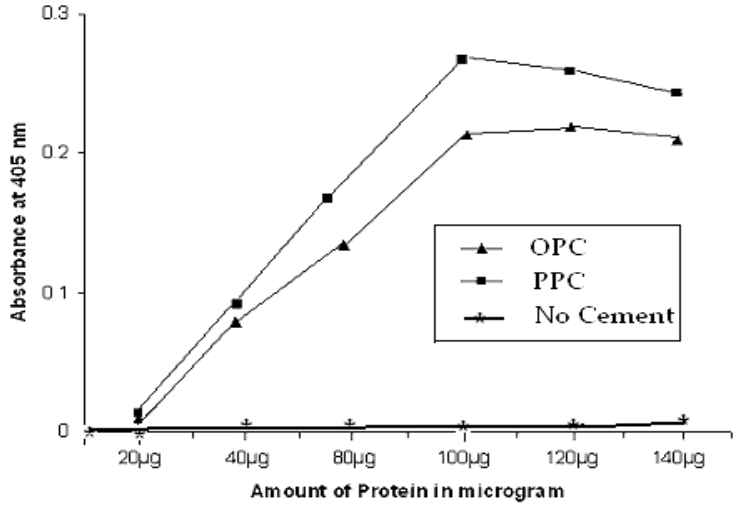

Figure 10. Bio-silicificaton assay of Bioremediase protein using cement (50 mg) as substrate

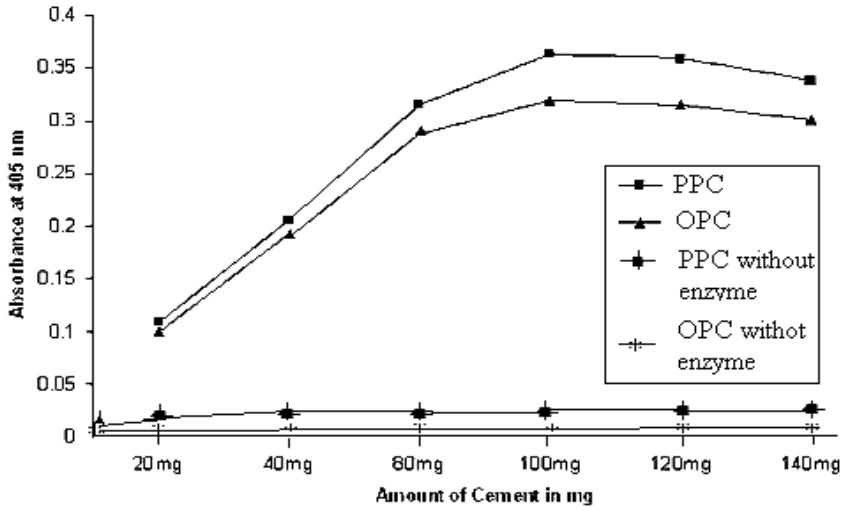

Figure 11. Bio-silicificaton assay of Bioremediase protein (100 µg) using different concentrations of cement as substrate

This is in agreement with the results obtained in PPC based mortar samples. The chemical composition of pozzolanas varies considerably. Of the active oxides, silica is normally considered to be the most important in the form of silicate and should not normally fall below 40% of the total; indeed some of the best pozzolanas have silica contents above 90% [1]. On the other hands, in ordinary Portland cements, silica contents vary from 19-23% only. Bioremediase protein can leach silica from silicate compounds and help to form calcium-aluminium-silicate by using the available silica within the concrete/mortar matrices [12].

Previously it was observed that only 25 to 30% strength increment was achieved by using ordinary Portland cement with BKH1 cells or its specific bioremediase protein incorporated mortar samples [9, 16]. This study showed 40-45% strength increment of PPC with the same bacterium or its bioremediase protein. The higher content of silicate in PPC helps to increase the activity of bioremediase enzyme. This clearly explained the higher strength improvement in pozzolanas cements when BKH1 cells or its specific protein (bioremediase) was used (Table 6). The increment of crack repairing capacity of bioremediase protein was also more significant in PPC than that in OPC (Table 6). The ultrasonic pulse velocity was also more increased that revealed more compactness of protein amended PPC mortar samples than OPC mortar samples. Sulphate resistance and water absorption tests confirmed that those two properties were more or less similarly increased.

Table 6. Comparative performance analysis using BKH1 Cell on two different Cement Systems at 28 days curing

Parameter / Cement Type	% Increment of compressive strength (MPa)	% Increment of compressive strength (MPa) After crack healing	% Increment of Ultrasonic pulse velocity	% Increment in mass by water absorption		% Increment in mass by sulphate absorption
				30 min	72 h	
OPC	24.74	14.15	4.00	1.67	6.39	4.83
PPC	45.00	50.75	13.97	2.20	4.70	4.50

4. Conclusion

Bioremediase protein secreted by the bacterium BKH1 is a potential additive agent for different types of cements. The increment of strength and other essential features of mortar/concrete materials are substantially higher for pozzolana cement based mortar/concrete materials than ordinary portland cement based specimens when admixed to bioremediase protein. Opulence of silica in fly ash pozzolana cements is behind the enhanced activity of the bioremediase protein that ushers a new hope in future construction technology.

Acknowledgement

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