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Bacterial based admixed or spray treatment to improve properties of concrete

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Abstract. The capability of calcite precipitation by microbes is a well-known natural phenomenon and is now successfully employed to improve properties of concrete. The calcite precipitation by microbes will be affected by the process in which bacteria is introduced into concrete. The present study highlights different ways of microbial incorporation into concrete and its effect on the properties of resultant concrete. The bacteria were introduced into concrete either during casting or curing of concrete. The efficiency of the proposed microbial treatment was monitored in terms of strength development, water impermeability, pH of the pore solution, microstructure and level of $CaCO_3$ precipitated. It was observed that both admixed treatment or spray treatment during curing were effective in calcite precipitation. The procedure involving spraying of bacterial culture onto concrete surface was efficient as well as economical.

Keywords. Biomimicry; concrete; microbes; curing; calcium carbonate precipitation; strength.

1. Introduction

Man has always taken inspiration from the nature in solving some of the routine problems. Biomimicry or 'biomimetic' is the term coined for such inspirations that are applied in the practical world. It is defined as 'learning from and then emulating natural form, processes, and ecosystems to create more sustainable designs and commercial end products [1]. The field of biomimetic is highly interdisciplinary, and the vast field of application of bio-inspired designs attracts engineers, computer scientists, material scientists, physicists, chemists, etc. Infact, biomimicry can be applied to any field in order to obtain desired results. One such latest application of biomimicry involves the use of biology into concrete. Bioinspired engineering solution for civil infrastructure has drawn lot of interest from the scientific community during the last decade. It is inspired by the natural phenomenon which demonstrates the precipitation capability of bacteria from natural habitats to precipitate calcium carbonate [2]. Microbial induced calcium carbonate precipitation (MICP) is one of the bio-cementation technology that has been successfully employed to improve both strength and durability characteristics of concrete [3–5]. MICP via urea hydrolysis is an easily controlled mechanism in which high amounts of carbonates are produced by the ureolytic bacteria in short time period. MICP comprises of series of complex biochemical reactions. As part of metabolism, some bacterial species produce urease, which catalyzes urea to carbamate and ammonium, as shown in the following equations:

$$\underset{\text{Urea}}{CO(NH_2)_2} + \underset{\text{Water}}{H_2O} \rightarrow \underset{\text{Carbamate}}{NH_2COOH} + \underset{\text{Ammonia}}{NH_3} \quad (1)$$

$$\underset{\text{Carbamate}}{NH_2COOH} + \underset{\text{Water}}{H_2O} \rightarrow \underset{\text{Ammonia}}{NH_3} + \underset{\text{Carbonic Acid}}{H_2CO_3} \quad (2)$$

These products formed in Eqs. (1) and (2) further equilibrate in water to form bicarbonate and 2 moles of ammonium and hydroxide ions, resulting in increase of the pH as shown in the following equations:

$$\underset{\substack{\text{Ammonia}\\\text{Ions}}}{2NH_3} + \underset{\substack{\text{Water}\\\text{Ions}}}{2H_2O} \leftrightarrow \underset{\text{Ammonium}}{2NH_4^+} + \underset{\text{Hydroxide}}{2OH^-} \quad (3)$$

$$\underset{\substack{\text{Hydroxide}\\\text{Ions}}}{2OH^-} + \underset{\substack{\text{Carbonic}\\\text{Acid}}}{H_2CO_3} \leftrightarrow \underset{\substack{\text{Bicarbonate}\\\text{Ions}}}{CO_3^{2-}} + \underset{\text{Water}}{2H_2O} \quad (4)$$

Since the cell wall of the bacteria is negatively charged, the bacteria draw cations from the environment, including Ca^{2+}, to deposit on their cell surface. The Ca^{2+} ions subsequently react with the $(CO3)^{2-}$ ions, leading to the precipitation of $CaCO_3$ at the cell surface that serves as a nucleation site (Eqs. (5) and (6)):

$$\underset{\text{Calcium Ions}}{Ca^{2+}} + \text{Cell} \rightarrow \text{Cell-}Ca^{2+} \quad (5)$$

$$\text{Cell-}Ca^{2+} + \underset{\text{Bicarbonate Ions}}{CO_3^{2-}} \rightarrow \underset{\text{Calcium Carbonate}}{\text{Cell-}CaCO_3} \quad (6)$$

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This technology of precipitation of carbonate crystals is called mineral precipitation technology and has been successfully demonstrated in the repair of limestone monuments [6–8], consolidation of sand-columns [9, 10], crack remediation in concrete [4, 11], improvement in properties of building materials [12] and enhancement of strength and durability of RC structures [3, 13–17].

It has been well reported that MICP leads to improvement in the properties of resultant concrete. However, the precipitation of calcium carbonate by bacteria is dependent on various factors like concentration of dissolved inorganic carbon and of Ca^{2+} ions, the pH of the pore solution, the presence of nucleation sites for crystal development, temperature and salinity of the suspension, etc. [13]. Some of these factors will be affected by the process in which the bacteria is introduced in concrete, viz. during casting of concrete or at later stage during the service life of concrete. There are different ways by which bacteria can be introduced into concrete. It can either be admixed into concrete during casting, or the concrete can be cured with the bacterial culture. Also, a spray treatment can be provided to concrete, in which the surface of concrete can be sprayed with the bacterial culture. The effect of different modes of application of microbial treatment has not been investigated so far. The present study is aimed at understanding the effect of different ways of incorporation of bacteria into concrete on the properties of resultant concrete.

The bacteria isolated from cement samples (*Bacillus* sp.) are used in the investigation and is introduced into concrete either during casting or curing or is sprayed on the concrete surface during 28 days of curing. To gain better insight into the efficiency of the bacterial treatment, some parameters of the study are carried out separately on surface concrete and on the core of bulk concrete. The results are compared with the non- bacterial treated specimens.

2. Materials

The common constituents of concrete are cement, fine aggregates and coarse aggregates. Ordinary Portland Cement (OPC) conforming to IS 12269-1987 [18] were used for preparing concrete specimens. Well-graded natural river sand conforming to IS 383-1970 [19] with fineness modulus of 2.89 was used as fine aggregate. Crushed stone with a maximum size of 20 mm was used as coarse aggregates.

Another vital constituent of microbial concrete is microorganisms. The bacteria used in the present study were isolated from commercially available cement, since the isolated bacteria were to be used in concrete that has a highly alkaline environment. The isolation of bacteria was carried out by using enrichment culture technique [20]. The most efficient non-pathogenic strain, *Bacillus* sp. CT-5 was used in the study.

Using *Bacillus* sp. CT-5 strain, bacterial culture was prepared, which was used in place of water to prepare bacterial concrete specimens. To prepare bacterial culture, 0.65 g of Nutrient broth (commercially available standard nutrient broth (NB) consisted of (g/l) casein hydrosylates 15.0, peptone 5.0 and NaCl 5.0 g/l (HiMedia, Mumbai, India)) was added in 80 ml of water and kept for autoclaving for 20 min at 120°C. The autoclaved NB medium was inoculated with 1% of overnight grown bacterial strain. The solution was kept on shaker at 120 rpm and its optical density (OD_{600}) at 600 nm wavelength was monitored with the help of UV-Vis spectrophotometer. The shaking was done till the OD_{600} of bacterial culture reaches 0.5. Bacterial culture grown in nutrient broth was supplemented with filter sterilized 2% urea (w/v) and 25 mM calcium acetate solution (w/v) to obtain final solution. This bacterial culture was used instead of water to prepare concrete specimens.

2.1 *Mix proportions and specimen preparation*

The reference concrete mix was designed as per IS10262:2009 [21] to obtain a 28-days compressive strength of 35 MPa. The final mix proportions of the reference mix were 1:1.54:2.86 of cement, sand and coarse aggregate, with the water-cement ratio (*w/c*) of 0.47. For the mixes made by using bacteria during casting, the bacteria culture was used instead of water, by keeping the bacterial culture - cement ratio as 0.47.

Cube specimens of 150 mm size were prepared for all mixes.

Since the major objective of the study is to investigate the effect of microbial treatment provided to concrete, the treatments were divided into four major categories:

(1) Microbial treatment provided during the casting stage; in which the specimens were cast with the bacterial culture.
(2) Microbial treatment provided during the curing stage; in which the normally cast specimens were dip in the bacterial culture for curing.
(3) Microbial treatment provided both during the casting and curing stage; in which the specimens were cast and cured in the bacterial culture. In this case, the casting of specimens was done by using the bacterial culture, instead of water.
(4) Microbial treatment provided by spraying at the surface of the specimens only; in which the specimens were cast with water. Thereafter, bacterial culture was introduced by spraying of bacterial culture on the concrete surface at the regular intervals of time. The spraying was done at the regular interval of 2 days and the culture was sprayed on all the faces of cube.

For achieving various treatment processes, the specimens were cast either with water or the equivalent amount of bacterial culture, and then were cured either in water or the culture as the case may be. The nomenclature of the

Table 1. Details of microbial treatment provided to concrete specimens.

Sl. no.	Mix designation	Mode of bacterial introduction
1.	MC0R0	Control specimens with no bacteria
2.	MC1R0	Bacterial culture used for casting + cured in water
3.	MC0R1	Water is used for casting + cured in bacterial culture
4.	MC1R1	Bacterial culture used for casting + cured in bacterial culture
5.	MC1R2	Bacterial culture used for casting + sprayed with bacterial culture after 24 h of casting

specimens according to the treatment involved is enlisted in table 1. In the nomenclature 'M' stands for mix, followed by the method of casting (C0 indicates that casting is done with water, while C1 indicates casting of concrete specimens by bacterial culture). It is further followed by the type of curing the specimens were subjected to: R0 indicates conventional method of water curing; R1 indicates curing of specimens by dipping them in bacterial culture for required number of days; while R2 indicates curing by spraying of bacterial culture on the surface of concrete specimens.

The following tests were performed to check the efficiency of the proposed treatment:

2.2 *Compressive strength test*

To study the compressive strength of concrete, 150 mm cube specimens were casted and compacted using a vibration machine. After 24 h of casting, the cubes were demoulded and then cured either in water or in the bacterial culture at room temperature until compression testing at the age of 3, 7, and 28 days as per IS 516:1959 [22]. Before testing, the samples were wiped with a paper towel to remove excess liquid from the surface. The tests were performed in triplicate and average value was taken as representative compressive strength.

2.3 *Water permeability test*

Impermeability of concrete was measured in terms of resistance to water penetration. Water permeability test was conducted for all mixes at the end of 28 days of curing. For the test, 150 mm concrete cubes were prepared for each mix and then were cured in the corresponding media as mentioned above. After 28 days of curing, all the specimens were air-dried for 12 h and were analyzed as per DIN 1048 [23]. The test was continued for 72 h, in which water was allowed to penetrate into the specimen at the atmospheric pressures of 1, 3, and 7 bars sequentially for 24 h each. After 72 h of water penetration, each specimen was split into two halves for determination of water penetration depth. The average value obtained from three specimens was taken as the representative water permeability.

2.4 *$CaCO_3$ precipitation and pH measurements*

Both these measurements were carried out on the powdered samples taken at two levels from the solid concrete specimens. One set of samples were taken from the top 1–10 mm of the concrete cube to represent the surface layer of concrete and the other sample was taken from the core of concrete, i.e., at 70–80 mm from the surface. The sample collected was powdered and was used to measure the amount of calcium carbonate present and the corresponding pH at that level.

The amount of calcium carbonate synthesized by the microbes was calculated by EDTA titration measurements [24, 25]. For this test, 0.5 g of the powdered sample was mixed with 3 ml HCl, 4 ml NaOH and 43 ml water. A drop of an indicator, hydroxylnaphtha blue was added to the obtained solution which gives light pink colour to solution. The pink coloured solution so obtained was further titrated against EDTA. During titration, the pink colour solution changes its colour from pink to violet at a certain point. The change of colour confirms the presence of calcium carbonate in the concrete sample. The amount of EDTA added for per gram of sample can be correlated with the amount of $CaCO_3$ formed. The test was performed at the age of 28 days and was done in triplicate for each case.

pH of the powdered specimens was monitored so as to check the integrity of the passive layer formed around the steel rebar, which remains stable only at high pH levels. The pH of the sample was tested by Suspension method, which was adopted because of its feasibility and simple procedure. This method has been used to measure pH of concrete by various researchers (Rasanen *et al* [26], Behnood *et al* [27], Sharma and Goyal [28]). For measuring pH of concrete mix, 3 g of powdered concrete sample was taken and was mixed with water in ratio of 1:9 in test tube and left undisturbed overnight. After 24 h, this sample was tested by pH meter to get pH of the mix. The pH measurements were conducted at 3, 7 and 28 days of curing.

2.5 *SEM and XRD*

The broken specimens from compressive strength studies were further analyzed with SEM and XRD. The samples were collected from the top surface of concrete in order to discover evidence of bacterially precipitated crystals. Both

the tests were performed at 28 days of curing of concrete specimens. For SEM, the samples were gold coated with a sputter coating Emitech K575 and then examined at accelerating voltages from 30 to 35 kV. XRD spectra were obtained using an X'Pert PRO diffractometer and performing investigations at diffraction angle (2θ) ranging from 10° to 80°.

3. Results and discussions

The following section discusses the effect of microbes on the compressive strength of concrete, water permeability, $CaCO_3$ precipitation, pH and the microstructure analysis of various mixes prepared using microbes.

3.1 *Compressive strength development*

Figure 1 summarizes the 3, 7, and 28 days compressive strength obtained by applying different bacterial treatments to concrete and is compared with the corresponding control specimens. From figure 1 it is clear that the compressive strength of microbial treated specimens is significantly higher than control specimens, irrespective of the bacterial treatment provided to the concrete. The improvement in compressive strength caused by microbial treatment can be attributed to the deposition of $CaCO_3$ within the pores of cement-sand matrix. These depositions help in plugging the pores present in concrete [9, 15–17].

For all the bacterial treatments, maximum increase in strength occurred at 3 days of curing as compared to 7 and 28 days. The percentage increase in compressive strength for MC1R0, MC0R1, MC1R1 and MC1R2 specimens as compared to MC0R0 were found to be 60%, 67.44%, 68.60% and 65.69%, respectively, at 3 days of curing. However, the relative increase in compressive strength at 7 days of curing in MC1R0, MC0R1, MC1R1 and MC1R2 as compared to MC0R0 was observed to be 22.4%, 25.0%, 18.67% and 21.51%, respectively. Similarly, the percentage increase in strength at 28 days of curing was 21.11%, 18.33%, 13.88% and 23.88% for MC1R0, MC0R1, MC1R1 and MC1R2 as compared to MC0R0, respectively.

This was due to fact that till 3 days, bacteria grows properly and completely adopts the environment inside concrete. The nutrients were available in abundance during this period and microbes were able to grow fast and precipitate calcite. The calcite formation and further hydration of concrete subsequently filled the pores present in concrete, leading to reduced porosity. The reduction in porosity could have prevented further ingress of nutrients, leading to the arrival of anaerobic condition for bacteria present in concrete. Due to this, the activity of bacteria slowed down, with bacteria converting themselves into spores.

On comparing the effect of bacterial treatment on the properties of concrete, it is observed that the strength development of concrete is affected by the bacterial treatment. The mixes in which bacteria were introduced during either casting or curing stage had the percentage increase of 60 to 68.6% at 3 days. The mix in which bacteria was introduced at casting stage, followed by curing with bacterial culture (MC1R1 mix) had highest increase of 68.60% in 3 days compressive strength. It is because when bacterial culture is mixed with the ingredients of concrete, it is uniformly distributed inside the concrete and leads to uniform precipitation of calcium carbonate, and hence densification of concrete matrix. It indicates that the best results of bacterial treatment can be obtained only when bacterial culture is used both at casting and curing stage.

Also, two types of bacterial curing procedures were adopted in this test programme. In one curing procedure (adopted in MC1R1) the specimens were submerged in bacterial culture till the end of curing, while in the second curing procedure (adopted in MC1R2), the culture was sprinkled periodically onto the surface of specimens. The

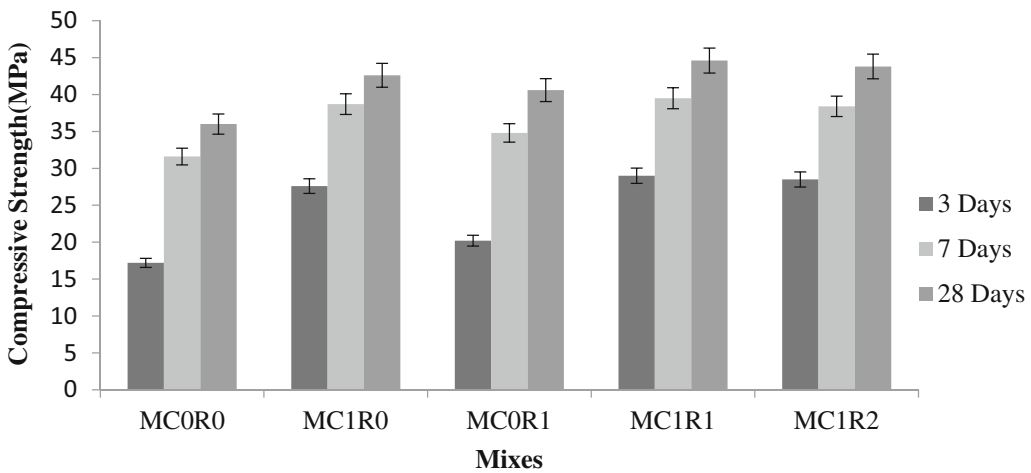

Figure 1. Compressive strength development for various mixes at different ages.

Table 2. Penetration depth and calcite precipitation of samples treated with microbes.

Sample	Depth of penetration (mm)	EDTA consumed in titration (ml)	$CaCO_3$ precipitation (mg/g of concrete)
MC0R0	33.24±3.62	1	5.01
MC1R0	8.87±2.11	5.2	26.03
MC0R1	9.94±1.86	4.9	24.52
MC1R1	6.15±1.58	7.2	36.04
MC1R2	6.64±2.32	6.5	32.54

later procedure required lesser amount of bacterial culture, hence reducing the cost of treatment. As can be observed from figure 1, the compressive strength development of mixes in both curing procedures is almost same. It indicates that curing procedure involving periodic sprinkling of bacteria culture can be easily adopted without any loss in terms of compressive strength development.

3.2 *Water permeability test*

The water permeability test results for all mixes are presented in table 2. These results indicate that the permeability of bacterial concrete is significantly lower than the control concrete. The water penetration depth reduced dramatically by bacterial intervention, invariably in all the treatments adopted in the study. The lower permeability of bacteria treated specimens can be attributed to the denser interfacial matrix formed due to calcite precipitation between the pore structure of concrete matrix. Similar observations regarding improvement in porosity of concrete by using bacteria was reported by Achal *et al* [29]. Van Tittelboom *et al* [30] also reported that use of *bacillus sphaericus* in concrete resulted in a decrease in water permeability.

On comparing the relative performance of all bacteria treated specimens, it was observed that the procedure of bacterial application does not have significant effect on reduction in permeability. All mixes had extremely low depth of penetration. Even in the mix which was only cured with bacterial culture showed lower depths of penetration. This can be due to blocking of pores of the top surface due to calcite precipitation by microbes present in water used for curing. Since penetration is a surface phenomenon, even blocking of top surface pores are sufficient to reduce water permeability. Hence, it can be said that for durability of concrete against aggressive ions, any type of bacterial procedure will be efficient.

3.3 *$CaCO_3$ precipitation*

The amount of $CaCO_3$ formed is an indicator of bacterial activity in calcite precipitation and is correlated to the amount of EDTA added per gram of sample [31]. Generally, 1 ml of EDTA consumed is equivalent to 5.006 mg of $CaCO_3$ formed per gram of concrete sample. The values of $CaCO_3$ precipitated for various samples are shown in table 2. Data clearly indicates that larger amount of $CaCO_3$ is precipitated in bacterial mixes since EDTA consumed is more in these mixes. Among all bacterial treatments studied, maximum $CaCO_3$ is formed in treatment involving microbes at both casting and curing procedures (i.e., MC1R1 and MC1R2). During casting, the bacterial cells were added as an admixture to concrete and during curing, the specimens were cured either by spraying bacterial culture (MC1R2) or by dipping the specimens in bacterial culture (MC1R1). Since in both these treatments, bacteria was made available during both the casting and the curing process; therefore, calcium carbonate precipitation was achieved at both early stages of hydration (in the bulk concrete) and at the later stages of hydration (on the surface of concrete). The specimens which were sprayed with microbes also had sufficient amount of precipitates. It indicates that the same effect can be obtained by using lesser amount of microbes and nutrient culture. The cost of bio-deposition treatment depends on the price of the microorganisms and the nutrients used for its growth [8, 29]. Use of lesser amount of microbes and nutrients will economize the whole treatment with no compromise on the quality of concrete obtained.

3.4 *pH measurements*

One of the major durability concerns of reinforced concrete (RC) structures are its deterioration and premature failure due to corrosion of the steel reinforcement. The protection to rebar against corrosion is provided by the formation of a passive layer (thin protective oxide film) over the steel surface due to high alkalinity of concrete pore solution. This passive film is stable at the high pH of concrete pore solution. In order to check any adverse effect of the bacterial treatment on the corrosion process, pH of the concrete specimens was measured at two locations of concrete (at the surface and inside the concrete matrix). The values of pH were measured at 3, 7 and 28 days of casting for all specimens and are presented in figure 2. It can be observed that the pH of all the mixes at all ages remains well above 12.5. It indicates that the alkalinity of concrete is maintained uniformly that helps in the development of passive layer around steel rebar and hence prevent the rebar against corrosion due to aggressive environment. The bacterial treatment did not affect the pH of the pore solution

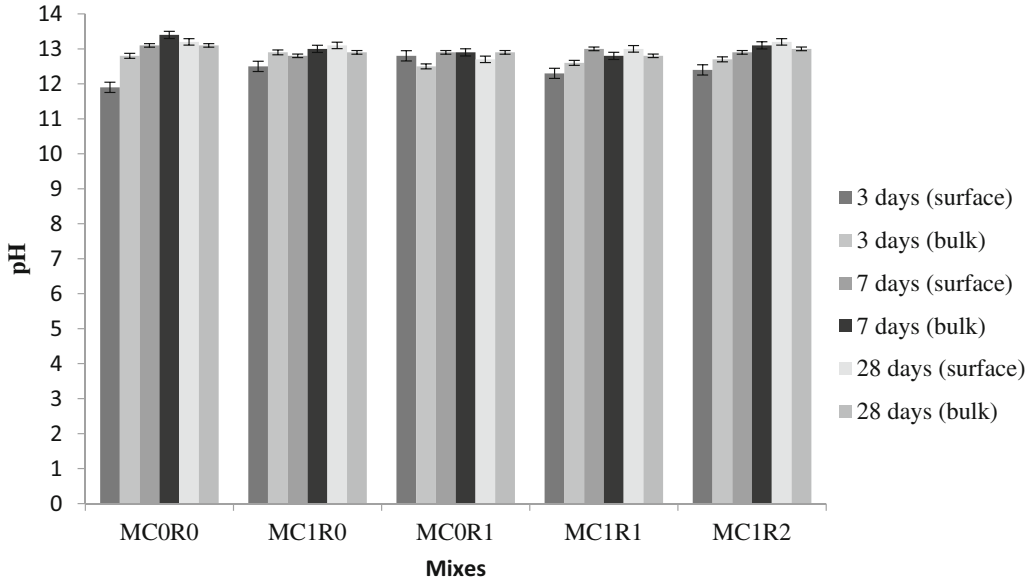

Figure 2. pH values of top surface and bulk concrete for all mixes at various ages.

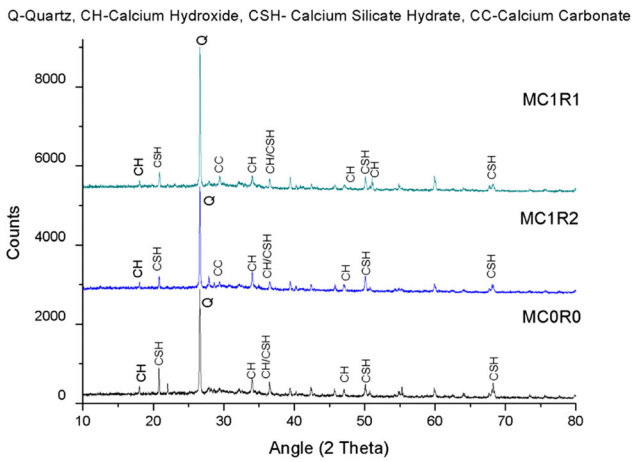

Figure 3. XRD patterns of MC0R0, MC1R2 and MC1R1.

adversely, and hence will not be detrimental to rebar corrosion.

3.5 *Microstructure analysis*

The top surface of all the specimens was characterized by SEM and XRD analysis. XRD analyses of the concrete sample from the top surface at 28 days revealed the presence of peaks of calcite crystals in microbial treated specimens. Such peaks are present along with other general peaks of control specimens as shown in figure 3. SEM analysis shows the presence of $CaCO_3$ crystals in association with bacterial cells which gives an evidence of the enhanced mechanical properties at different ages. As shown in figures 4 and 5, microbial treated specimens show the presence of rod shaped bacterial cells (BC) in close proximity with calcium carbonate crystals (CC). All the bacterial treated specimens show the presence of lamellar rhombohedral crystals of calcite and needle-shaped aragonite crystals of $CaCO_3$. The EDX analysis also confirmed the elemental composition of crystals with peaks showing high amount of calcium and carbon. The blurred SEM images around the calcium carbonate crystals of microbial treated specimens can be attributed to the presence of exopolymeric substances (EPS) around the conjunction of calcium carbonate crystals and the associated bacterial cells (figure 5). Exopolymeric substances (EPS) are organic polymers of high molecular weight of microbial origin which is secreted by microorganisms into their environment and are found to be responsible for binding of cells to the substratum [32]. Role of EPS in cell adhesion and calcium carbonate precipitation in concrete was also reported by Joshi *et al* [33].

4. Conclusions

The present study investigated the effect of various types of bacterial treatments on the properties of concrete. It was observed that all types of bacterial treatments enhanced compressive strength and reduced permeability of the resultant concrete. The treatment in which bacteria was introduced during casting results in uniform distribution and better strength and durability properties. The remarkable improvement in permeability of concrete by the surface treatment by bacteria indicates that it can act as surface sealant to enhance durability of concrete in aggressive

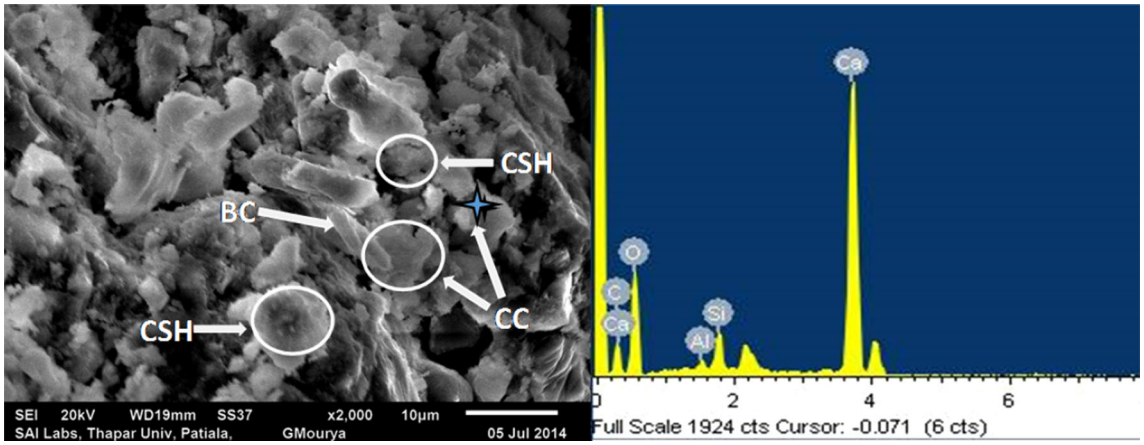

Figure 4. Presence of calcium carbonate crystals associated with bacterial cells in microbially treated specimens. (CC: Calcium carbonate, BC: Bacterial cell, CSH: Calcium silicate hydrate). ✦shows the spot of EDX analysis.

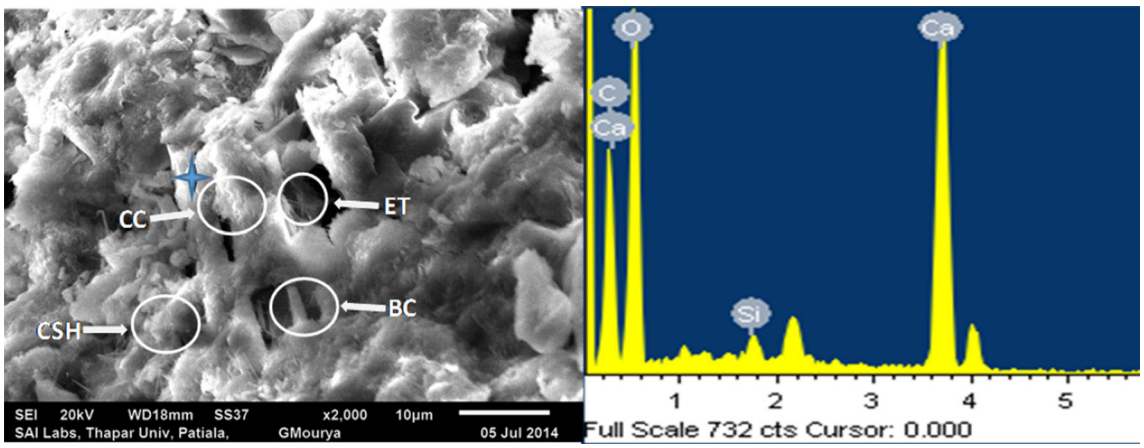

Figure 5. Rod-shaped bacterial cells present in concrete. (CC: Calcium carbonate, BC: Bacterial cell, CSH: Calcium silicate hydrate, ET: Ettringite). ✦shows the spot of EDX analysis.

environment. The procedure can be economized by spraying of bacterial culture onto concrete surface at regular intervals with no compromise on the resultant properties of concrete.

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