Study on Development of Strength Properties of Bio-concrete

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Abstract The objective of this research is to identify a new calcite-precipitating bacteria and trying to improve the strength parameters of concrete. In concrete, cracks are the most vulnerable thing through which water, minerals, and other chemicals will ingress and contacts with reinforcement of concrete and corrosion of reinforcement and degradation of concrete will happen. Overall, the durability is greatly affected due to cracks and these cracks may be micro cracks which are impossible to identify and to assess the locations of these cracks in concrete. Inspection, maintenance, and repair of cracks will be difficult for large-scale infrastructure and most of the repair techniques are chemical based, expensive, and repair techniques can be used for large size cracks but not for micro cracks In order to increase the durability of concrete against these commonly observed cracks in concrete structures, autogenous pore refinement method can be adopted. By using the principle of biomineralization, bacteria forms the calcium precipitations which is usually called microbial-induced calcite precipitation (MIC). In the present work, a different bacterial colony is chosen to see that more improvement in terms of healing capacity of concrete can be achieved compared to Bacillus family, and the bacteria were identified such that which will grow in the high alkaline media, since concrete is highly alkaline material. Bacteria are cultured in the controlled medium to get the desired concentrations of cells, and it is observed that the compressive

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strength of concrete is improved more than 36% and there is an improvement in other strength parameters also. And also, it is noted that there will be an effect of cell concentration on the strength development. SEM and EDAX analysis reveals the deposition of calcium carbonate.

Keywords Concrete · Bio-concrete · Biomineralization · Strength

1 Introduction

Despite concrete's unmatched durability, it is susceptible to the damage associated with many actions. Some cracks are not a problem for concrete, but some can allow moisture into the concrete and damage the entire structure [1]. To overcome this problem, many chemical repair techniques are available but nowadays due to sustainable development, the research proceeded with less material consumption techniques, one such technique is microbial-produced $CaCO_3$ and these are all autogenous way of healing cracks. Among these, calcium carbonate is compatible with concrete mix and more environmental friendly In general, there are three ways getting the precipitation of calcium carbonate [2, 3]. One is dissimilation of sulfate reduction carried out by sulfate-reducing bacteria under axenic conditions. Second is the degradation of organic matter. Another way related to the degradation of urea by ureolytic bacteria. Among three pathways, degradation of urea by ureolytic bacteria is easier to operate and control [4, 5].

Urea is abundant in soil. Bacteria are isolated from soil is ureolytic, calcifying, and spore-forming bacteria and which has urease in its membrane [1]. The healing agents incorporated during concrete mix will flow into cracks and whenever concrete reaches adverse condition, bacteria forms spores and these spores will be in dormant state up to 200 years. This spore is highly resistant to temperature stress, salinity, extreme pH, and solvents [6].

1.1 About Calcifying Bacteria

Calcifying bacteria are obtained from the different sources like soil, water, ocean, caves, and concrete itself. Calcifying bacteria will produce an enzyme called urease which converts the urea into ammonia and carbon dioxide [7–9]. The following reactions obtained are:

$$CO(NH_2)_2 \rightarrow CO_2 + NH_4$$

The carbon dioxide obtained from urea will react with the calcium hydroxide of concrete as follows:

$$CO_2 + Ca(OH)_2 \rightarrow CaCO_3 \downarrow + H_2O \uparrow$$

Water evaporates in the chemical reaction and other compounds will settle down and which is insoluble in the water. The products obtained are calcium carbonate and water [10]. Calcium carbonate is insoluble in water and which fills in the cracks of concrete. Calcium carbonate is normally available in three forms such as calcite, aragonite, vaterite and among three, calcite is a more stable compound which has rhombohedra shape. It will form due to the presence of magnesium, manganese and orthophosphate ions. Calcium carbonate prevents the water uptake, permeability, carbonation, and chloride ion attack into the concrete and it enhances the durability and strength properties of concrete, mainly compressive, tensile, and split tensile strength [11].

1.2 Types of Bacteria

1. *Bacillus sphericus*
2. UN-INDENTIFIED

Here, the first term is species name and the second term is genus name, and all three bacteria can precipitate the calcium carbonate by forming the spore and this spore is made of different compositions like water, cytoplasm, DNA, and calcium dipicolinate acid, and all these bacteria are best compatible with the concrete conditions and which are non-pathogenic in nature. *Bacillus sphaericus* is rod-shaped bacteria and it is an aerobic bacterium [12]. It is used as an insecticide for mosquitoes at the larva stage and recently, it is gaining popularity in civil engineering because of its calcifying property. These bacteria are isolated from soil and cultured in the laboratory at the minimum cost. The UN-INDENTIFIED bacteria is isolated from a source and cultured in the Biotechnology department laboratory, R.V. College of Engineering and this will survive in the pH of 13. After analyzing its function with the concrete, it is to be identified. Culturing techniques are done to enhance the growth and to access the particular bacteria while restricting the growth of other bacteria in the sample [13].

1.3 Objective of Project Work

The main objective is to study the strength enhancement of M25 grade of concrete and comparison of strength with the controlled specimen's strength for different concentration of cells of bacteria such as *Bacillus sphericus* and UN-INDENTIFIED bacteria.

2 Properties of Materials

2.1 Bacterial Strain and Nutrients

Bacillus subtilis and *Bacillus sphaericus* brought from Gene Bank, CSIR-Institute of Microbial Technology, Chandigarh and UN-INDENTIFIED bacteria was developed in the Biotechnology Department, R.V.C.E. Pure culturing of these bacteria were done in theBiotechnology Department.

3 Methodolgy

1. Culturing of bacteria
2. To study the strength behavior of concrete

4 Experimental Investigation

4.1 Culturing of Bacteria

Culturing of pure colony of bacteria was done in the laboratory while restricting the growth of other bacteria in the media and the media was prepared from different chemical compositions. For media to be in the solid form, agar-agar nutrient was used and to get the proper homogeneity in the mix, preferred media was kept in the incubator [14–16]. After the incubation, media was adjusted for required pH and kept for the autoclave along with the Petri plates and test tubes to kill the bacteria which were there in the water. Autoclaving was done for 45 min and during this time inside, the pressure will be developed and the temperature was about 118 °C. After 45 min, the pressure will get down within 15 min of switching off e tautoclave and specimens kept in the autoclave were taken out and allowed for cooling. Liquid media was transferred to plates and tubes in the laminar air flow [17, 19].

4.2 Inoculation of Bacteria

Once the media was transferred into Petri dish plates and tubes, the media become solid because of the agar content. Bacteria were inoculated with the help of inoculating needle usually called nichrome which is made of nickel and chromium. Initially, inoculation was done in the laminar airflow for only one or two Petri dish plates to check the growth of pure colony of bacteria and kept for incubation for 24 h at the room temperature. Once the pure colony of bacteria was obtained,

Fig. 1 Media of bacteria

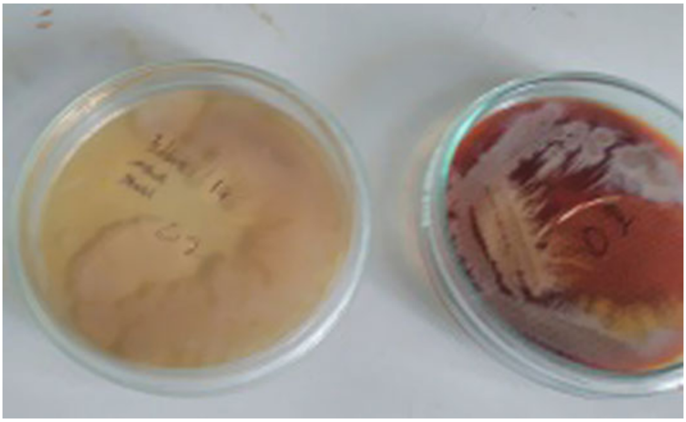


Fig. 2 Bacterial growth

subculturing was done with the inoculation of pure colony bacteria into other Petri dish plates [7, 8]. From these plates, inoculation was done for test tubes and kept for incubation (Figs. 1 and 2).

4.3 Preparation of Broth

Preparation of broth involved the same nutrients except agar and broth will be in the liquid form because of not adding the agar. After adding all nutrients except agar in required water, the solution was kept in the shaker for proper mixing of nutrients and adjusted to required pH. After pH setting, the broth was sealed with a cotton plug which was kept for the autoclave as explained in the preparation of media. Inoculation of bacteria was done in the laminar airflow and kept in the shaker for 24 h or more [20]. After, one or two days, Bacterial growth was observed with more turbidity and growth of bacterial cells was checked in the microscope (Figs. 3 and 4).

Fig. 3 Broth before inoculation of bacteria

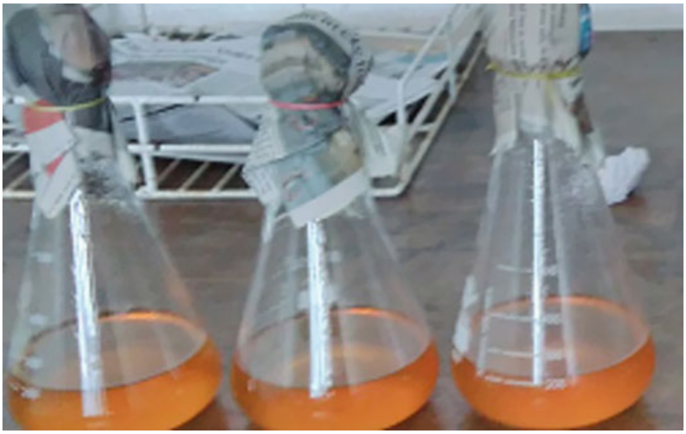


Fig. 4 Growth of bacteria after inoculation of bacteria

Table 1 Details of mix design

S. No.	Grade of concrete	Water (kg)	Cement (kg)	Fine aggregates (kg)	Coarse aggregate (kg)
01	M25	197	438	730	1033

4.4 Mix Design

Mix design uses codes like IS 456-2000 and IS 10262-2009 and with using these codes, mix design of M25 was done to find the proportion of coarse aggregate, fine aggregate, and cement [21–23]. Obtained proportions are shown in Table 1.

5 Results and Discussions

5.1 Slump Test

Slump test was conducted on the fresh concrete for M25 grade concrete values obtained from the test are listed in Table 2.

For RCC work, IS 456-2000 has specified the slump value of 90–100 mm and values got from the tests are within the limit and broth added while mixing was not affected the workability of mixes.

5.2 Compressive Strength Test

The compressive strength of different grades with different concentrations of bacteria as well as controlled specimens for 28 days and 56 days has been shown in Figs. 5 and 6.

Figure 5 shows the strength of M25 grade of concrete mix at different days for different bacteria and strength of UN-INDENTIFIED bacteria, and *B. sphaericus* is increased by 13.9 and 7.9% at 28 days and similarly, strength increment of 20.5 and 12.64% for 56 days occurred compared to normal concrete mix.

From Fig. 6, it is seen that strength of B-S and U-N is slightly increased at 28 days but there is an increase in strength at 56 days by 22.5 and 16.87% for UN-INDENTIFIED and *Bacillus sphericus*.

Table 2 Slump values for both types of bacteria on different concrete mixes

S. No.	Concrete grade	Value of slump (mm)
1	M25	90
2	M25, 10^6 cells	92
3	M25, 10^7 cells	90

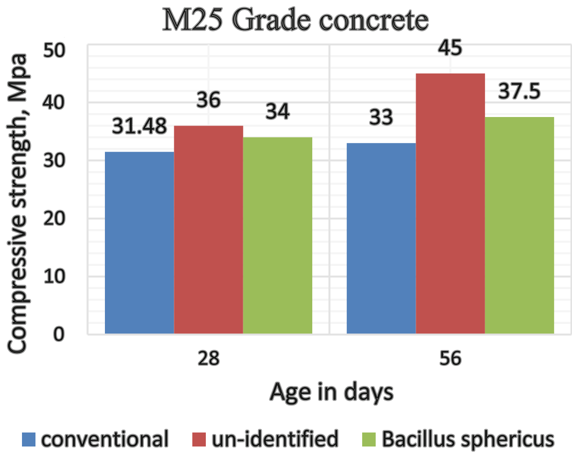


Fig. 5 Compressive strength of M25 at 10^6 cells

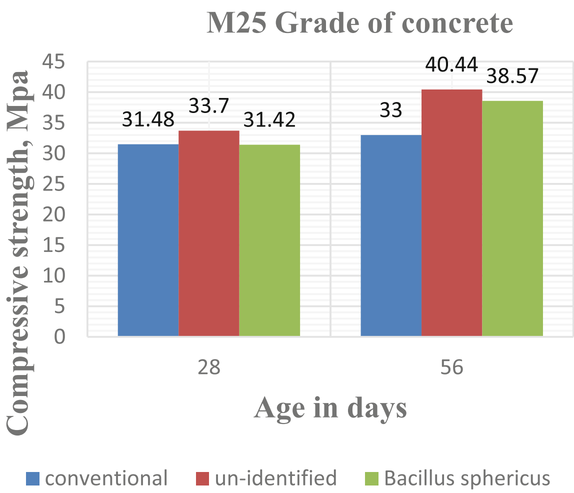


Fig. 6 Compressive strength of M25 at 10^7 cells

5.3 *Split Tensile Strength*

Test results got from different types of bacteria and bacterial concentration when added to the concrete mix is listed in Table 5.4.

Figure 7 reveals the split tensile strength of conventional and bacterial added concrete mix. From Fig. 7, it is found that increment of strength occurred in all the cases but at the concentration 10^6, there is increase of strength by 6.12 and 3.26% for BS and UN, and increase of strength occurred for *B. sphaericus* and UN-INDENTIFIED bacteria by 2% (10^7 concentrations).

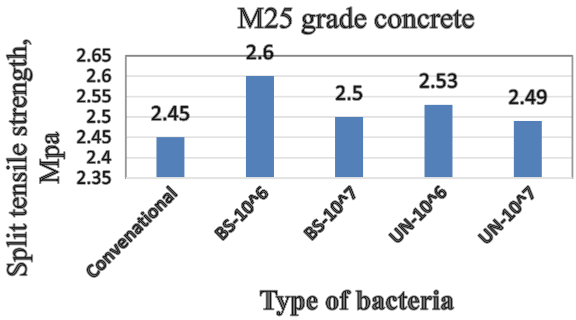


Fig. 7 Split tensile strength of 25 grades of concrete mixes

5.4 *Modulus of Elasticity*

Figure 8 shows the modulus of elasticity of M25 grade of concrete with and without bacteria. It is noticed that elasticity of concrete at 10^6 concentrations is increased by 3.6% for UN-INDENTIFIED bacteria and there is a decrease of modulus of elasticity by 1.48% for Bacillus *sphericus*. For 10^7 concentrations, it is observed that decrease of elasticity for BS and increase of elasticity by 1.5% for UN-INDENTIFIED bacteria.

Figure 9 reveals the flexural strength of concrete mix with and without bacteria. Flexural strength of *B. sphaericus* has been increased for both the concentrations by 9.13 and 7.2% but there is no increment of strength occurred at the concentrations like 10^6 and 10^7 for UN-INDENTIFIED bacteria.

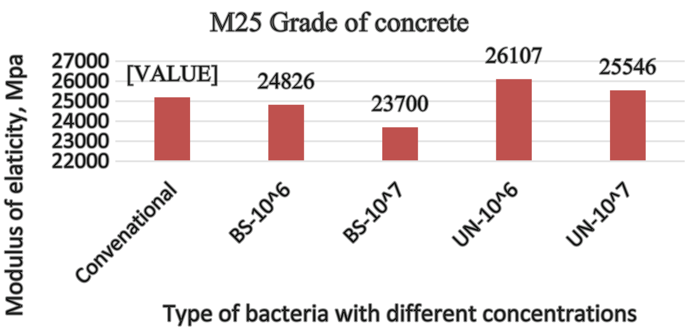


Fig. 8 Modulus of elasticity of M25 grade of concrete at different concentrations

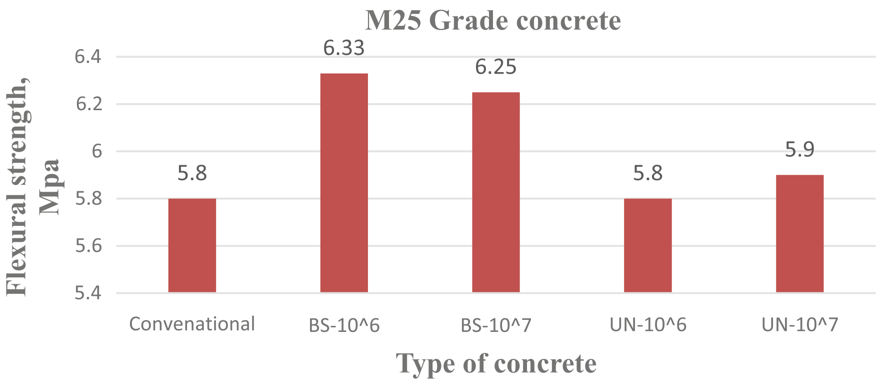


Fig. 9 Flexural strength for different concrete mixes

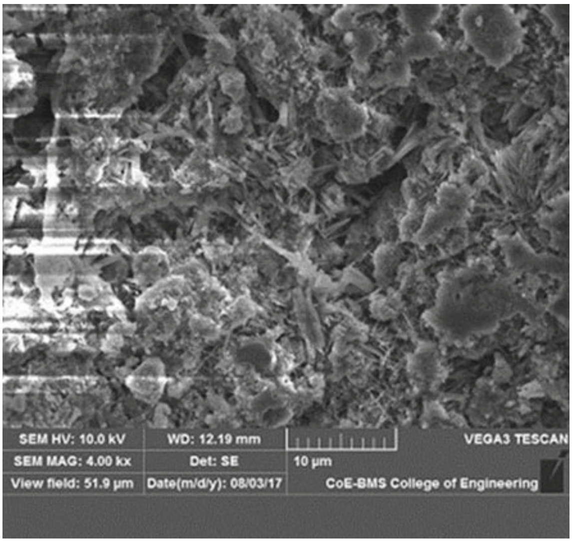
5.5 Analysis of Concrete Microstructure by SEM and EDAX

In order to know the microstructure and elements of concrete mix, SEM and EDAX tests were conducted on all the samples of specimens and results of these are discussed below.

5.5.1 Microstructure of Controlled Mix

Figures 10 and 11 reveals the presence of large amount of C-S-H gel and ettringite in the normal mix.

Fig. 10 SEM analysis of normal concrete mix



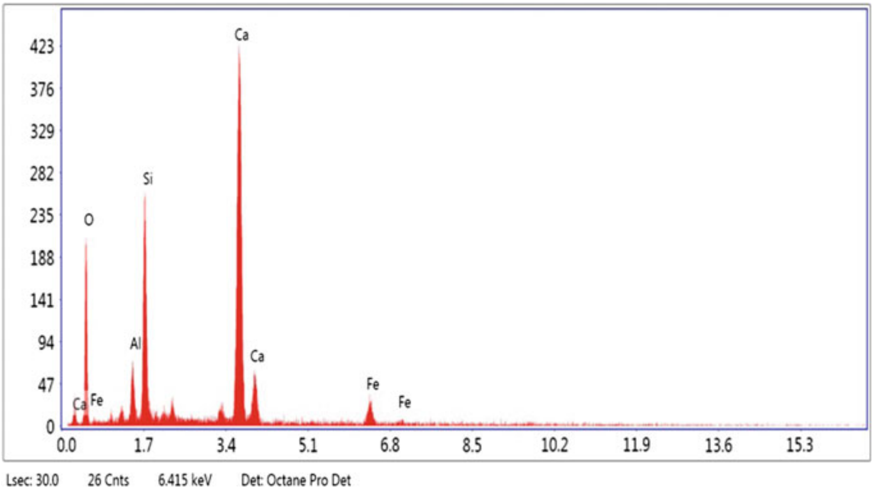


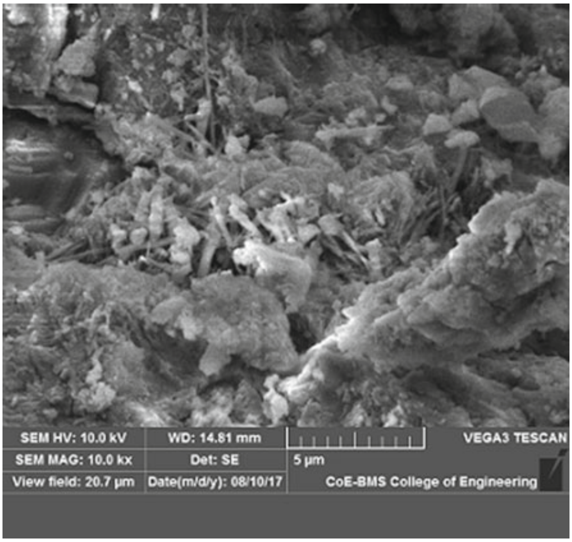
Fig. 11 EDAX analysis of normal concrete mix

5.5.2 Results of Unidentified Bacteria

Figure 12 exhibits the presence of ettringite, C-S-H gel, and white precipitation which indicates the calcium deposition. Figure 13 shows energy dispersive X-ray analysis of M25-10^6. Calcium-to-silicate ratio is 3.17 and this indicates the higher strength compared to controlled specimens.

Figure 14 shows the presence of large amount of C-S-H gel. Figure 15 describes the different compositions of M25-10^7 and Calcium-to-silicate ratio obtained is 5.5 which indicates higher strength of M25-10^7.

Fig. 12 SEM analysis of M25-10^6 cells



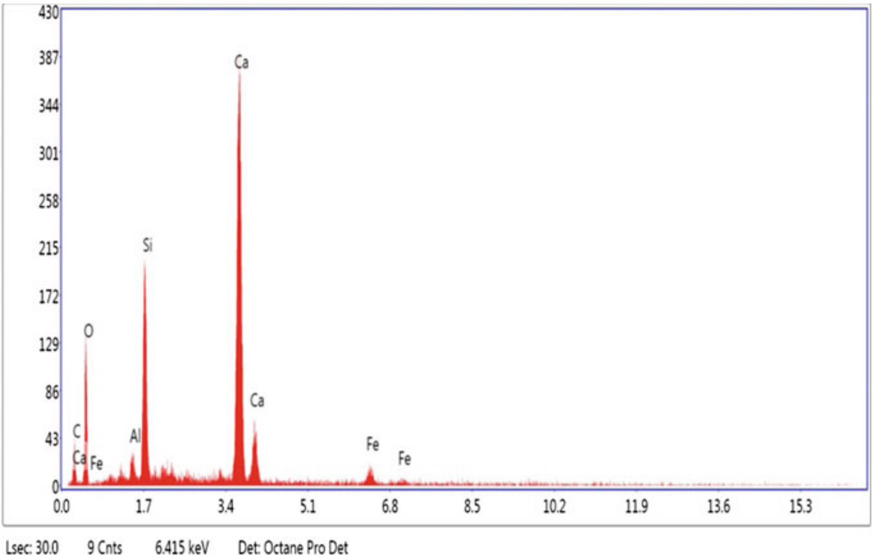


Fig. 13 EDAX analysis of M25-UN-10^6 cells

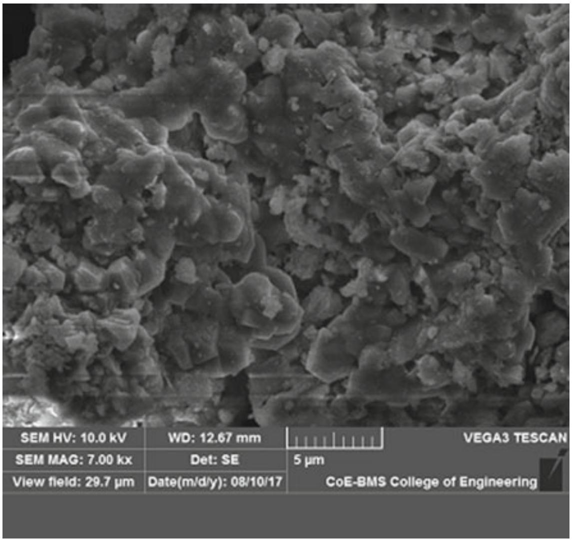


Fig. 14 SEM analysis of M25-UN-10^7 cells

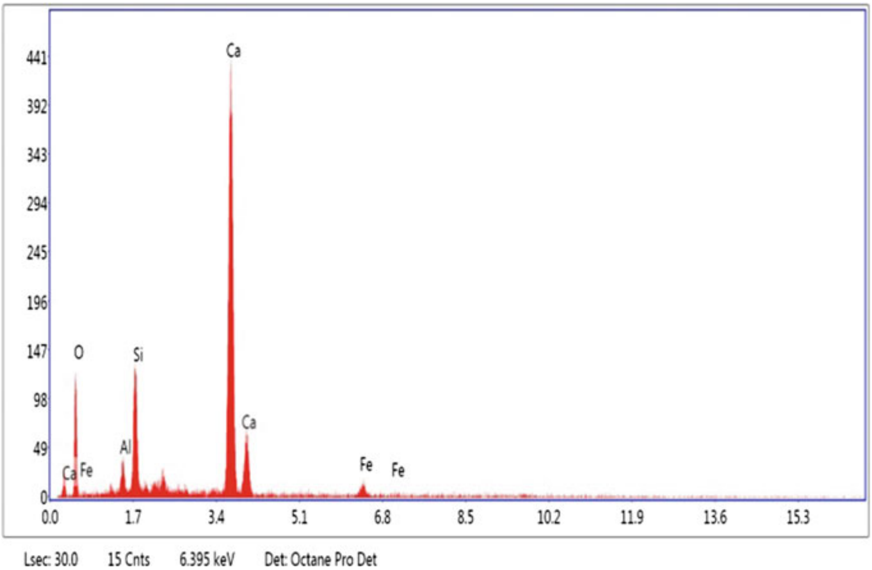


Fig. 15 EDAX analysis of M25-UN-10^7 cells

6 Conclusions

Based on the experimental investigations done on the controlled specimens, UN-INDENTIFIED bacteria and *B. sphaericus* concrete mix, the following conclusions drawn are listed below.

UN-INDENTIFIED Bacteria

- The percentage increase in compressive strength for 10^6 concentrations of cells is 14.35, 36.36% for M25 grade of concrete at 28 and 56 days.
- The percentage increase of modulus of elasticity for the concentration of 10^6 is 23.78%.
- There is no appreciable increase in the flexural strength but achieved the target strength for both concentrations.
- SEM and EDAX analysis are the basis for the increment of strength for different concentrations.

Bacillus *sphericus* bacteria

- The percentage improvement in the split tensile strength for 10^6 and 10^7 cells is 6.12, 2% for M25 grade of concrete at 28 days.
- The improvement of modulus of elasticity is occurred at 10^6 concentrations of cells by 31%.
- The percentage increase of flexural strength for 10^6 and 10^7 concentrations of cells is 9.13, 7.75% for M25 grade of concrete at 28 days.

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