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Influence of bacteria megaterium on strength and durability properties of concrete partially substituted with metakaolin

Influencia de la bacteria megaterium en las propiedades de resistencia y durabilidad del hormigón parcialmente sustituido con metacaolín

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Abstract:
Concrete is one of the most used materials in the building sector. Inevitably, concrete gains strength and develops shrinkage cracks during the curing process. Shrinkage cracks provide an entry point for reactive fluids to get into the concrete core. Under favorable conditions, the fluids reduce the pH of the core and consequentially corrode the reinforcing bars. This research shows the use of Bacillus megaterium MTCC 3353, which produces calcium carbonate and closes the minute fissures. Part substitution of cement with metakaolin was also done in the study. It was seen that the bio-based concrete specimens enhanced the mechanical strength and durability parameters. The calcite precipitation was validated using a microstructure study. FESEM and XRD tests show the presence of calcite (calcium carbonate) inside the concrete structure. The bacteria were tested for their growth when superplasticizers were added to them. The bacteria showed normal growth compared to the conventional testing.
Keywords: *Mechanical properties; durability properties; microstructure; Bacillus Megaterium; Metakaolin.*

Resumen
El hormigón es uno de los materiales más utilizados en el sector de la edificación. Inevitablemente, el concreto gana fuerza y desarrolla grietas por contracción durante el proceso de curado. Las grietas por contracción proporcionan un punto de entrada para que los fluidos reactivos ingresen al núcleo de concreto. En condiciones favorables, los fluidos reducen el pH del núcleo y, en consecuencia, corroen las barras de refuerzo. Esta investigación muestra el uso de Bacillus Megaterium MTCC 3353, que produce carbonato de calcio y cierra las fisuras diminutas. En el estudio también se realizó la sustitución parcial de cemento por metacaolín. Se observó que las muestras de hormigón de base biológica mejoraron los parámetros de resistencia mecánica y durabilidad. La precipitación de calcita se validó mediante un estudio de microestructura. Los ensayos FESEM y XRD muestran la presencia de calcita (carbonato de calcio) en el interior de la estructura de hormigón. Se probó el crecimiento de las bacterias cuando se les añadieron superplastificantes. Las bacterias mostraron un crecimiento normal en comparación con las pruebas convencionales.
Palabras Claves: Propiedades mecánicas; propiedades de durabilidad; microestructura; Bacilo Megaterio; Metacaolín.

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1. Introduction

Concrete is very common in building various structures since it has adequate strength and durability. Concrete gets its strength from a series of hydration reactions between cement and water releasing large amounts of Green House Gases. In recent times, the construction industry requires high-performance concrete thereby increasing the amount of cement in the concrete design. Concrete would be vulnerable to shrinkage cracks if the amount of cement is increased. These cracks are not structural but would act as a pathway for the reactive fluids to get into the core. The fluids would reduce the pH of the core, thereby reducing the passivation layer in the reinforcement. The reinforcement would have lesser resistance and would be susceptible to various durability issues. Bio-concrete is a way to reduce the above complications and increase the durability of the structure. This is done by introducing bacteria into concrete and creating a thriving environment for the same. (Wiktor and Jonkers, 2011) experimented with a two-component healing agent inside clay pellets. The pellets were inserted with a source of calcium and dormant bacteria media. During favorable conditions, the bacteria would feed on the calcium source and precipitate calcite inside the concrete core. (Grabiec et al., 2012), (Shehab El-Din et al., 2017) and (Achal et al., 2011) examined the strength and durability properties of bacterial concrete with the Bacillus genus. The results showed that the biocrete specimens had better strength and resistance to water penetration than ordinary specimens. (Andalib et al., 2016) experimented with numerous bacterial cell concentrations and observed that the optimal concentration for enhancing the strength parameters was 105 cells/ml. Extensive research on the strength and durability of the same bacterial concentration was done by (Pappupreethi and Ammakunnoth, 2017) and (Seshagiri et al., 2012) The research showed the enhancement of the properties and also showed that the lifeless bacterial cells act as natural fibers inside the concrete. (Krishnapriya et al., 2015) explored the numerous bacteria genus and found that the effect of Bacillus megaterium was significantly better than the others. (Manikandan et al., 2015) explored the thermal properties of bacteria and found that it was stable till a temperature of 90oC. (Achal et al., 2011) introduced fly ash as an admixture inside biocrete. This improved the mechanical and durability properties due to the formation of secondary cementitious material. (Jiang et al., 2015) and (Kim et al., 2007) experimented with numerous concentrations of metakaolin and found that the pozzolanic material increased the strength by secondary reaction with calcium hydroxide. (Muduli and Mukharjee, 2020) varied the percentages of metakaolin from 5 – 20% along with replacement of coarse aggregates and observed improvements in the properties (mechanical and durability)

Bacterial specimens were cast and immersed in an exclusive media for gaining strength. The media consists of 50g of $CaCl_2$ and 20g of CH_4N_2O mixed in a liter of water. Initially, urea decomposes to carbamic acid and NH_3. Subsequent disintegration forms H_2CO_3 and carbonates as the by-products. The pH of the core would increase due to the above reactions creating CO_3- ions. Lastly, the Ca^{2+} ions from the calcium source combine with CO_3^- ions forming calcium carbonate.

This study deals with the usage of Bacillus megaterium in concrete to enhance the properties. Cement was partially substituted with metakaolin in three percentages (10%, 12.5%, and 15%). The following were the objectives of the study.

- *To understand the growth pattern of bacteria with superplasticizers.*
- *To prepare the control and bacterial specimens and test their mechanical strength*
- *To check the durability properties of ordinary and bacterial concrete*
- *To validate the calcite precipitation of bacterial specimens using microstructure study.*

2. Materials

2.1 Concrete

53 grade OPC cement was used as the binder for casting the concrete. The properties of cement and metakaolin are tabulated in (Table 1). Fine aggregates in compliance to (Buraeu of Indian Standard, 1987) *were used along with coarse aggregates passing through IS 12.5mm and retained in IS 20mm sieves. Metakaolin conforming to (Buraeu of Indian Standard – 1967) was substituted as a part substitute for cement in the three percentages.*

Table 1. *Properties of cement and metakaolin*

Physical	**Cement**	**Metakaolin**
Specific gravity	3.11	2.6
Mean size	17 µm	32 µm
Color	Grey	White
Chemical	**Cement**	**Metakaolin**
Silicon dioxide	23.8	51.24
Aluminum Oxide	5.8	46
Ferric Oxide	3.4	0.66
Titanium dioxide	-	0.67
Calcium oxide	62.6	0.09
Magnesium oxide	1.36	0.03
Sodium oxide	1.13	0.09
Potassium oxide	1.17	0.03

2.2 Bacillus Megaterium

Bacteria were brought from Microbial Type Culture Collection, Chandigarh in a dormant state (Figure 1). The bacteria required a particular broth for their growth specified in (Table 2). The broth was decontaminated using a cooker, after which the bacteria is introduced into the broth and allowed to incubate for 1 day. This creates a yellowish-white solution having a random bacterial concentration. Serial dilution of the mother culture was done to bring the bacterial concentration to the required level (105 cells/ml).

Figure 1. *Mother culture before serial dilution*

Table 2. *Properties of bacteria*

Genus Name	Bacillus
Species Name	Megaterium
Type	B
MTCC no.	3353
Derived	Kerala, India
Temperature	35ºC
Nutrient Broth	Glucose (5.0g), Peptone (5.0g), Extract (3.0g), NaCl (5.0g) mixed distilled water
Incubation Period	24 hours
Sub-culturing Period	30 days

Figure 2. *Serial dilution process and subsequent concentration check using a bio spectrometry*

The concentration of bacteria was evaluated using bio spectrometry (Figure 2). The bacterial solution was kept in the equipment and visible light was passed through it. The equipment will measure the optical density value and this can be substituted in (Equation 1) derived by (Manikandan et al., 2015) to get the bacterial concentration.

$$Y = 8.59 * 10^7 * X^{1.3627} \tag{1}$$

Where X represents the optical density value.

High-performance concrete is used in numerous applications and requires superplasticizers (SPs) to enhance some of its properties. Bacteria was checked for its compatibility and growth when SPs were added to it. Three generations of SPs namely Lignosulfonate (LS), Sulponated Naphthalene Formaldehyde (SNF), and Polycarboxylate (PC) were used for the study. (Figure 3)

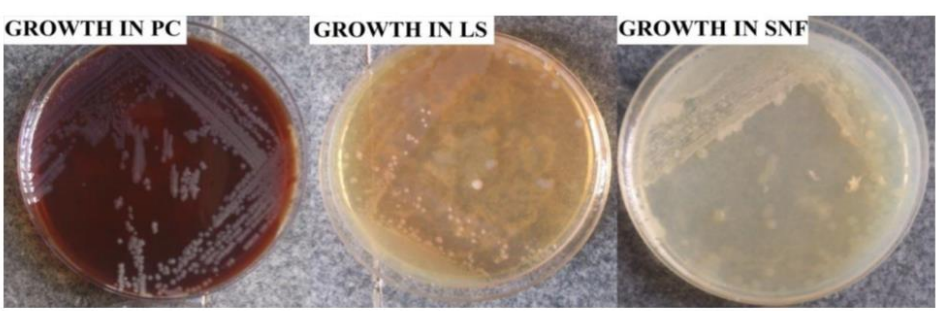

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Figure 3. *Serial dilution process and subsequent concentration check using a bio spectrometry*

Bacteria were grown inside the petri dish after adding each SP and it was observed that the growth was normal and was not affected by the admixture.

3. Methodology

3.1. Compression Strength test

The specimens (Ordinary and bacterial concrete) were proportioned using (Buraeu of Indian Standard, 2000) and (Buraeu of Indian Standard, 1982) – 2009. The specimens were cast inside a prefabricated mould of side 100mm and allowed to gain strength for 24 hours. Ordinary concrete was cured in normal water for the required number of days (7, 14, and 28) in a controlled environment. Bacterial specimens were prepared by adding 5 ml of the serially diluted water during the mixing process. The bacterial specimens were cured using a broth (50g of $CaCl_2$ and 20g of CH_4N_2O) for the required number of days. Cement was substituted using metakaolin in the percentages of 10, 12.5, and 15 for both ordinary and bacterial specimens. The specimens were cured in their respective media and tested on the 7th, 14th, and 28th day for their compressive strength to conform to (Buraeu of Indian Standard, 1959).

3.2. Flexure Strength test

The specimens were prepared and cured similar to the compression strength test. The specimens were cast inside the mould of size 500 mm x 100 mm x 100 mm and cured in a separate medium for the required number of days. The specimens were taken out and tested using a flexure testing machine on the respective days (7th, 14th, and 28th day) according to (Buraeu of Indian Standard, 1959).

3.3. Water Absorption test

Specimens of cube side 100 mm were cast in a prefabricated mould and cured for 28 days in the respective media. The specimens were kept inside the oven for a day and the dry weight was taken as W1. The oven-dried specimens will be immersed for 2 days inside water and the corresponding weight was taken as W2. The water absorption was calculated according to (ASTM, 2008) ASTM C 642-06 standard (Equation 2)

$$Water\ absorption\ (\%) = \frac{W2 - W1}{W1} * 100\% \qquad (2)$$

3.4. Acid attack test

This test shows the interdependency of durability with the strength parameter and measures the percentage of mechanical strength decrement due to the infiltration of chloride ions. The test requires 100mm cubes cast and cured in the same manner as the above tests. Specimens (Figure 4) were immersed inside a solution of diluted acid (5% sulphuric acid), taken out on the 7th, 14th, and 28th days and tested for compression strength (Equation 3)

$$Decrease\ in\ compression\ strength = \frac{Strength\ before\ immersion - Strength\ after\ immersion}{Strength\ before\ immersion} * 100 \qquad (3)$$

Figure 4. *Specimens before and after acid attack test*

3.5. Field Emission Scanning Electron Microscope (FESEM) test

The microstructure of concrete was studied to show the precipitation of bacteria, which helps in increasing the strength and durability. This test was done by using a tiny piece (1 m^3) of the specimen after the strength testing (Figure 5). The specimens were cleaned, oven-dried and sputter-coated using Q150T Turbo-Pumped Sputter/Carbon Coater. This was done for ordinary concrete and optimal bacterial proportion (BC10 for M30 and BC12.5 for M40).

Figure 5. *FESEM specimen after the sputter coating process*

3.6. X-Ray Diffraction test

X-ray diffraction provides the chemical composition of the specimen. The specimen taken for this test was taken after the strength testing and was ground down to the required powder form. The specimen was kept inside the apparatus, which started to scan the specimen at different angles starting from 10o to 90o. The testing was done for both ordinary concrete and optimal bacterial proportion (Figure 6).

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Figure 6. *XRD sample hammered down to the required size*

4. Results and Discussions

4.1. Compression Strength Test

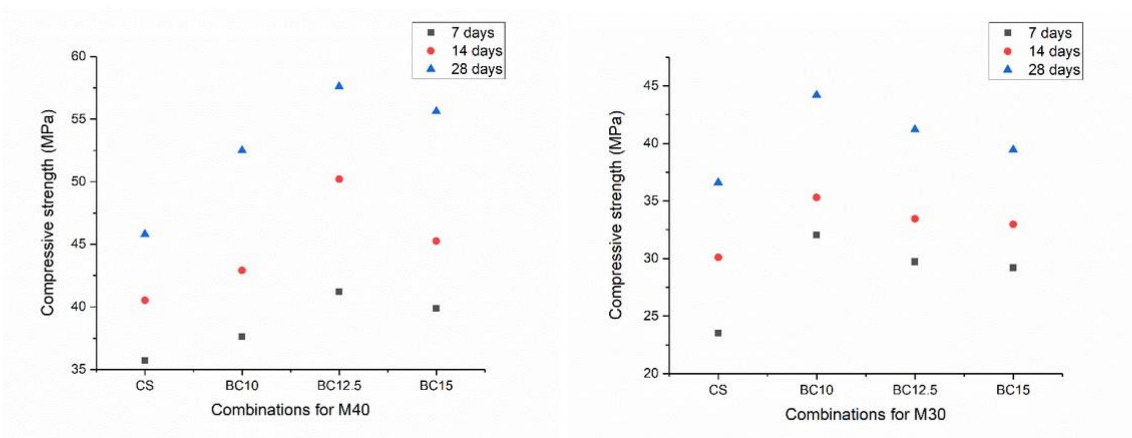

Figure 7. *Compression strength results for mixes M30 and M40*

The compression test showed that all the bacterial specimens performed better than the conventional concrete. The combination BC10 showed the highest 28-day compression strength (44.89 MPa) than BC12.5 (41.26 MPa) and BC15 (39.18 MPa) and CS (36.78 MPa) for M30 as shown in (Figure 7a). The combination BC12.5 showed the highest 28-day compression strength (57.24 MPa) than BC10 (52.64 MPa) and BC15 (54.63 MPa) and CS (46.87 MPa) for M40 as shown in (Figure 7b).

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4.2. Flexure Strength Test

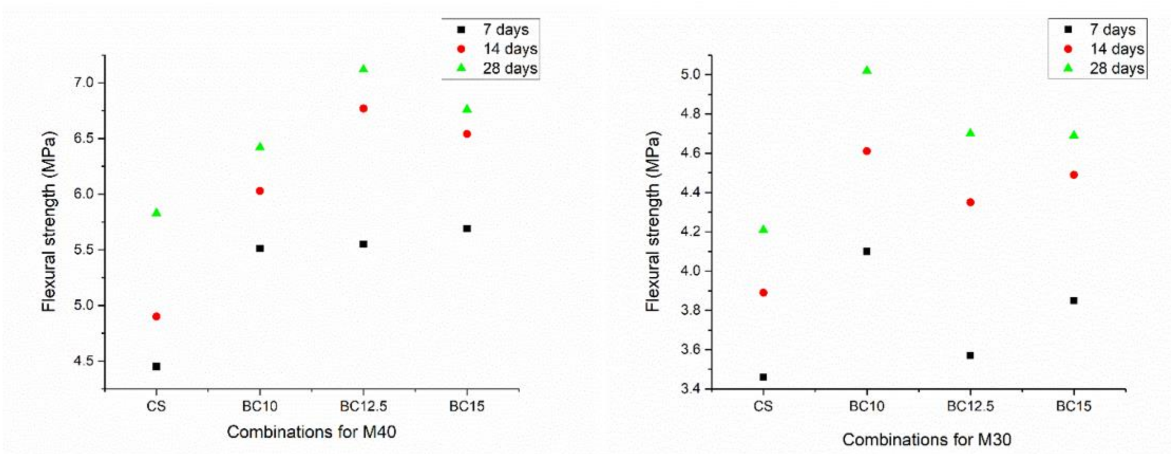

Figure 8. *Flexure strength results for mixes M30 and M40*

Similar results were seen for the flexure testing in which all the bacterial specimens achieved better results compared to ordinary concrete. BC10 showed the highest 28-day flexure strength of 5.03 MPa compared to the other combinations BC12.5 (4.72 MPa) and BC15 (4.67 MPa) for the M30 mix. BC12.5 showed the highest 28-day flexure strength of 7.09 MPa compared to the other combinations BC10 (6.42 MPa) and BC15 (6.7 MPa) for the M40 mix (Figure 8).

The strength testing showed that combinations of BC10 and BC12.5 performed better than their respective counterparts in M30 and M40. A similar trend was seen for the 7th and 14th-day strength values for both the mixes.

4.3. Water Absorption Test

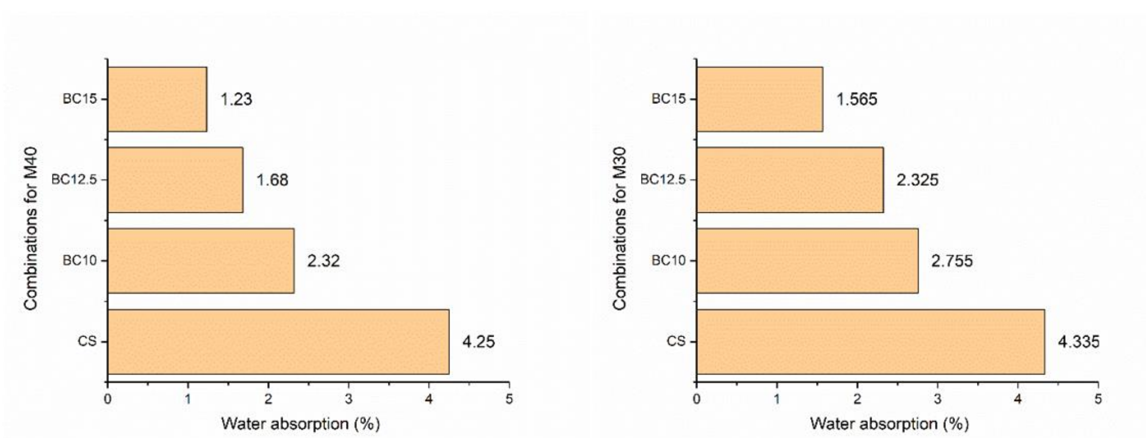

Figure 9. *Water absorption (%) results for mixes M30 and M40*

The (Figure 9) shows that the percentage of water absorption was directly proportional to the amount of metakaolin added in place of cement for both mixes. This can be accounted for by the reaction of metakaolin *with calcium hydroxide producing secondary insoluble cementitious material, thereby reducing the penetration of water. BC15 absorbed 1.565% of water, followed by BC12.5 and BC10, with 2.325% and 2.755% of water absorption respectively for the M30 mix. BC15 absorbed 1.23% of water, followed by BC12.5 and BC10, with 1.68% and 2.32% of water absorption respectively for the M40 mix. Ordinary concrete ended up with more water absorption values of 4.335% and 4.25% for M30 and M40 respectively.*

4.4. Acid attack test

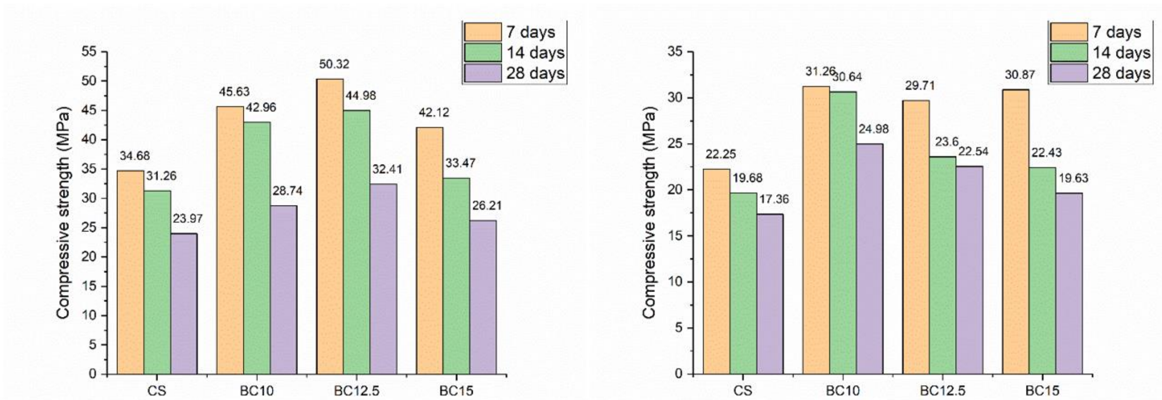

Figure 10. *Compression strength values after 7,14 and 28 days of immersion in an acidic environment for mixes M30 and M40*

After immersing in a very severe exposure condition, the specimens were tested for their compressive strength. The strength of all the specimens was indirectly proportional to the time of exposure in the acidic environment. In the M30 mix, it was seen that combination BC10 exhibited the least reduction of compression strength with a value of 39% compared to BC12.5 (44.3%) and BC15 (42%) respectively. In the M40 mix, it was seen that combination BC12.5 exhibited the least reduction of compression strength with a value of 43% compared to BC12.5 (45%) and BC15 (48%) respectively. Concrete showed a reduction in strength of 52% and 53% for both mixes (Figure 10).

4.5. FESEM test

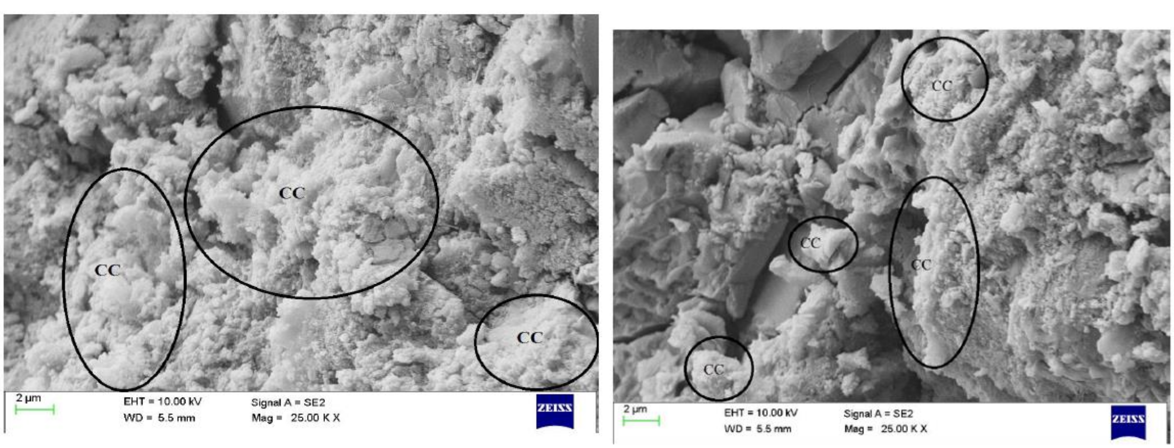

Figure 11. *FESEM images for the optimal combination for mixes M30 and M40*

FESEM images were taken at the size of 2µm with a 25000X zoom level for the combination BS10 and BS12.5 for the mix M30 and M40 respectively. The images showed the concrete core with evidence of calcium hydroxide and CSH gel. The images also showed a white insoluble material inside the core indicating the presence of calcium carbonate. These images are not concrete evidence of the presence of calcium. The presence of calcite can be reaffirmed using the subsequent XRD analysis (Figure 11).

4.6. XRD test

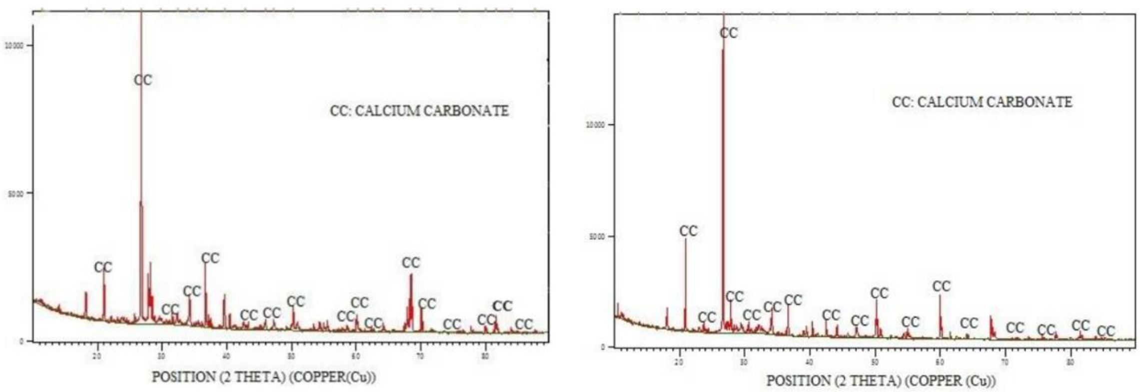

Figure 12. *XRD graphs for the optimal combination for mixes M30 and M40*

XRD graphs showed the chemical constituents of concrete. The graphs have peaks that indicate the presence of certain materials. The graphs showed the presence of calcite (calcium carbonate) at certain angles along with the usual constituents of the concrete core, validating the information of FESEM images. Similar images were taken for concrete which showed a very mild presence of calcium carbonate, which might have formed during the curing process (Figure 12).

5. Conclusions

This study dealt with the use of bacillus megaterium in concrete with partial substitution of metakaolin for cement. Two concrete grades, M30 and M40 were used for the study of mechanical and durability parameters. The following were the conclusions of the study.

• The mechanical strength was assessed in terms of compression and flexure. Bacterial specimens exhibited better strength than their concrete counterparts. BC10 and BC12.5 were the optimal mixes for the grades M30 and M40 respectively.

• The durability of concrete was evaluated for water absorption and resistance to acid attack. The water absorption reduced as the percentage of metakaolin increased. Resistance to acid was measured in terms of compression strength and all bacterial specimens performed better than ordinary concrete. BC10 and BC12.5 were the optimal mixes for the grades M30 and M40 respectively.

•FESEM images show the presence of calcite compound inside the bacterial concrete core (white-colored substance). The XRD results also showed the presence of calcite in the peaks at different angles showing the chemical composition.

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