Microstructure Analysis of Microbial Concrete: A Comparative Study

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ABSTRACT

This paper represents a critical comparison between conventional concrete and concrete developed using "Bacillus cereus" and "Escherichia coli" (E. coli) bacterial culture to determine the effect of the genus Bacillus and non-Bacillus bacteria on concrete microstructure. The durability and uniformity performance of concrete specimens were evaluated through Ultrasonic Pulse Velocity (UPV) test and Water Absorption Capacity (WAC) test for different curing ages. Scanning Electron Microscopy (SEM) analysis was conducted to visualize the Microbial Calcite Precipitation of bacterial concrete. UPV and WAC tests showed that concrete specimens containing bacterial culture possess more density and uniformity than the conventional one. SEM analysis established the presence of bacterial impressions and calcite layer inside the concrete, which contributed to the improvement of impermeable characteristics of concrete. Concrete specimens containing "Bacillus cereus" showed better performance than "E. coli" in all aspects.

1. INTRODUCTION

Being one of the most used materials worldwide, the assessment in the context of the durability of the concrete is now a significant challenge for Civil Engineers. The techniques of having concrete of variable strengths are very available nowadays. But the methods of keeping them durable for year after year are not so many.

The role of the concrete is to bear the compressive or tensile loads and these generally lead to the crack development inside the concrete. The freeze-thaw actions, shrinkage may however, also be the reason behind crack development. Although this generally does not affect concrete significantly in terms of strength, the cracks created can be worked as the pathway to the waters, harmful foreign matters and chemical solutions (sulfates, acids, chlorides). This eventually spoils the homogeneity of the concrete matrix inside and degrades the quality of the rebar embedded, which leads to the corrosion later volume expansion and development of major crack inside the concrete. From the past decade, various eco-friendly approaches are taken to make the concrete more durable and uniform. In this regard, microstructure development and increment of impermeable capacity are taking a significant part.

The incorporation of bacteria inside the concrete as a self-healing agent as well as an agent to develop the mechanical and microstructure properties have been introduced by the researchers recently. The calcite-precipitating bacteria can be introduced to the concrete mix directly as a solution or also can be incorporated as a form of immobilized object [1-3]. The microbial precipitation greatly depends on the type of bacteria as well as the type of nutrients that has been used in the solution [4].

Therefore, the selection of the culture medium and bacteria is very important for this technique. Apart from this, the whole microbial process of self-healing by calcite precipitation inside the concrete varies in terms of some conditions like: pH, Calcium concentration, dissolved inorganic Carbon concentration, nucleation sites, etc. [5]. The technique provides long-term durability of concrete by introducing easy crack repairing way and reduces the cost of structural maintenance to a great extent. The use of bacterial culture to develop inner properties of concrete is also becoming more popular as it is totally eco-friendly and the result found is also very attractive. Incorporation of bacteria inside the concrete can decrease the water absorption capacity of concrete by about 60%-70% than the conventional concrete [6].

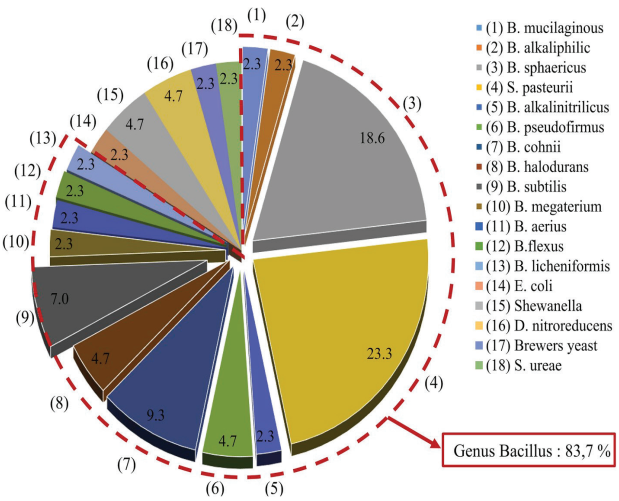

Fig. 1: Different types of bacterial usage in concrete [7]

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Several bacteria have been used by the researcher in this regard and most of which has given positive result in terms of durability assessment. But, in this specific context genus Bacillus has the highest rate of usage in this regard (about 84%) [Fig. 1]. Fig. 1 actually shows the application of different bacteria in cementitious material over the periods. For this experimental investigation, Bacillus cereus bacteria was chosen from genus Bacillus and Escherichia coli *(*E. coli*)* from non-Bacillus genus. The major target of this experiment is to observe the effect of bacterial culture in concrete microstructure properties through Ultrasonic Pulse Velocity (UPV), Water Absorption Capacity (WAC) and Scanning Electron Microscopy (SEM) analysis approach and to compare the effect of the genus Bacillus and non-Bacillus genus in concrete microstructure.

In this durability assessment investigation after the successful incorporation of Bacillus cereus and E. coli bacterial culture inside the concrete several curing period (days) was completed. Ultrasonic Pulse Velocity test, Scanning Electron Microscopy analysis and Water Absorption Capacity test was conducted to analyze and compare the relative resistivity and durability of different samples having different contents of bacteria.

The ultrasonic pulse velocity (UPV) test is the widely used non-destructive test used to determine the quality and structural integrity of concrete after construction. An ultrasonic pulse is allowed to pass through the cast sample and the velocity of the pulse is determined. The denser and higher the integrity of the inner matrix of the concrete, the more durable will be the concrete. The cylindrical specimens having diameter of 100 mm and height of 200 mm was used in this test. The UPV test generally provides improved and more accurate result with the aging of concrete. Rather than testing at 28 days curing period, if prolonged cured samples than this can be tested the more reliable result can be found [8]. So, UPV test was conducted on the specimens for 60 days and 200 days of curing age. The Water Absorption Capacity (WAC) test result resembles the development of resistivity to water penetration and also gives a good indication about the inner microstructure of the concrete specimen. To analyze the reduction of the porosity, Water Absorption Capacity test was conducted at 200 days curing age. Scanning Electron Microscopy (SEM) analysis was also conducted on the prepared samples in order to see the actual image of the concrete sample composed of the desired ingredients on micro-scale and from this analysis, the void presents and the dispersion of various constituents inside the sample can easily be determined. From this respected analysis, the microscopic images of the microstructure of concrete specimens were observed and the effect of bacterial culture and their relative comparison were made.

This environment-friendly and bio-chemical concrete is the most efficient one among all for making the structure durable. This technique can reduce the maintenance cost of construction by lessening the frequent expensive repairing works.

2. EXPERIMENTAL PROGRAM

2.1 Preparation of Bacterial Culture

Bacillus cereus and E. coli, both facultative anaerobic, spore-forming, rod-shaped bacterium which is easily accessible from the environment, were selected as the bacterial agent for this experimental investigation. Both of the bacteria were selected due to their carbonate precipitation capacity. "Bacillus cereus," a representative from genus Bacillus, was selected for the comparison with "E. coli," a representative of genus Escherichia. Both of the bacteria are easily accessible from nature. So the cost-effective approach was also one of the vital points for selecting those respective bacteria. Rather than depending on cell count technique, optical density was chosen to control the cell concentration of the bacteria in the culture.

An optical density of 0.5±0.1 was chosen in this form of study depending on the previous result found in the optimum optical density investigation. LB media mechanism was chosen for the growth and maintenance of both bacterial strains. Autoclaving and seeding process was maintained according to the prescribed rules. Flow diagram of detailed experimental program is represented by Fig. 2. The prepared bacterial cultures used in concrete mixing are shown in Fig. 3 and Fig. 4.

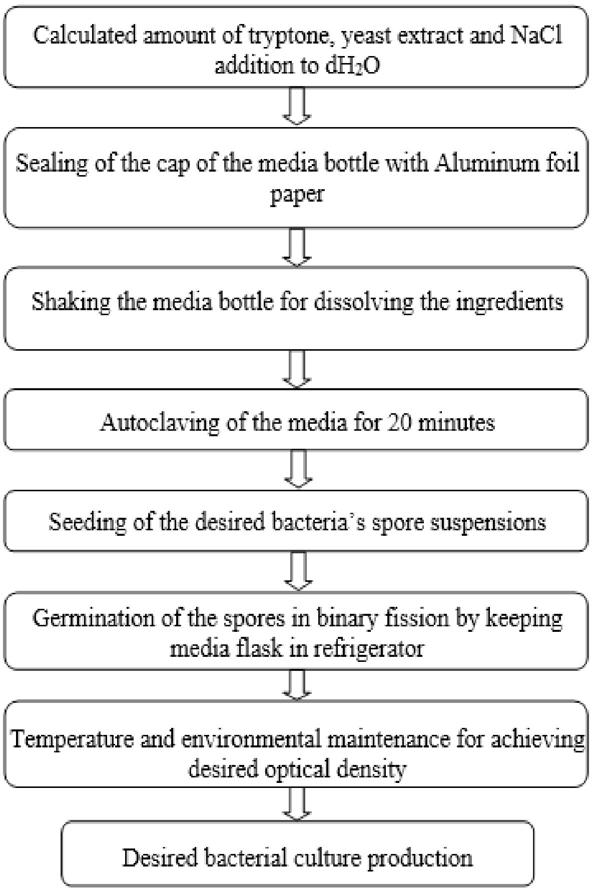


Fig. 2: Process followed for bacterial culture preparation

Fig. 3: Bacillus cereus bacterial culture

Fig. 4: E. coli bacterial culture

2.2 Mix Proportions, Specimen Details and Material Properties

Concrete specimens were prepared according to ACI standard procedure. Three different mixtures with Portland Composite Cement (PCC) (CEM II) were done with design strength of 20 MPa. Mix ratio was derived as 1.0:2.57:2.72. Cylindrical concrete specimens of 100 mm diameter and 200 mm high were cast for all respective tests.

Locally available sand was used as fine aggregate. The coarse aggregate was 12.5 mm nominal size crushed stone. Table 1 and Table 2 report the properties of aggregates used in this experimental investigation.

Table 1: Properties of Fine Aggregate

Fineness Modulus	2.53
Specific Gravity	2.62
Absorption Capacity	2.76%
Water Content	6.81%

Table 2: Properties of Coarse Aggregate

Unit Weight	1581.4 kg/m^3
Specific Gravity	2.76
Absorption Capacity	2.31%
Water Content	5.96%

Bacillus cereus and E. coli enriched bacterial cultures were prepared having optical density (OD_{600}) 0.5±0.1. The bacterial cultures were added directly to the concrete mix after achieving desired growth stage and optical density.

Bacterial culture was added as a full replacement of water required for mix design. Concrete specimens with and without bacterial culture were cured in tap water following ASTM C1602 guidelines.

For UPV test all the specimens were cured for 60 and 200 days in order to observe the long-term effect of bacteria inside the concrete. For SEM analysis longer curing specimens were taken. Intermediate curing and testing weren't done in order to check the behavior of bacteria induced concrete for 60 and 200 days curing effect. In this experimental investigation, the major target was to see the impact of bacterial culture in concrete properties in the case of long-term. Being cured for 60 and 200 days, the specimens were kept in open air under laboratory conditions. The temperature was maintained in between 25 – 27 ºC. Table 3 represents the specifications of the specimens.

Table 3: Specimen specifications

Specimens	Bacterial concentration (%)	Bacterial agent
M-1	0	None
M-2	100	Bacillus cereus
M-3	100	E. coli

3. TESTING LAYOUT

This study investigated the effects of bacteria in concrete due to long term curing. Three different types of concrete specimens were prepared with and without microbial water.

For each test, three specimens of every group were tested and the average of them was taken as the result.

3.1 Ultrasonic Pulse Velocity (UPV) Test

UPV test can evaluate the inner quality of concrete specimens. It can assess whether the inner core of the concrete specimen is dense and uniform or if there is void. UPV test was done on cylindrical specimens by placing a pulse transmitter on one face of the cylinder and a receiver on the opposite side. A timing machine was used to find out the results. Table 4 shows the chart to categorized specimens depending on UPV results. Depending on this chart, the tested specimens were categorized according to their results.

Table 4: Quality grading of concrete

Pulse Velocity (km/sec)	Concrete Grading
Above 4.5	Excellent
3.5-4.5	Good
3.0-3.5	Medium
Below 3.0	Poor/Doubtful

3.2 Scanning Electron Microscopy (SEM) Analysis

SEM analysis was conducted for following two major targets.

- To compare the micro-structural difference between conventional concrete and bacterial concrete; and
- To observe and compare micro-structural differences between Bacillus cereus and E. coli induced concrete

For SEM analysis, powdered samples were collected from the core of each specimen. SEM analysis was conducted according to the standard procedure. In SEM analysis, Interfacial Transition Zone (ITZ) was focused specially to visualize the bacterial mineral deposition, which produces a filler effect in the cement-sand matrix. Fig. 5 represents the SEM analysis machine that was used to conduct the respective analysis.

Fig. 5: SEM analysis machine used for this study (Model: JSM 7600F, JEOL-JAPAN)

3.3 Water Absorption Capacity (WAC) Test

Water absorption capacities of cylindrical specimens were evaluated for 60 and 200 days of curing periods. It was done to observe the effect of bacteria on concrete permeability during long run. The test was conducted according to ASTM C642 standard. To perform the test, the specimens were initially taken out from the curing environment and they were weighed during their SSD condition (W_0). Then the specimens were placed in to an oven at 100°C for 24 hours and the mass of the dry condition (W_1) was taken. Following equation was used to find out the result.

$$\text{Water absorption } (\%) = [(W_0 - W_1) / W_1] \times 100 \quad (1)$$

4. TEST RESULTS AND DISCUSSION

4.1 Ultrasonic Pulse Velocity (UPV)

UPV test was conducted on concrete specimens having 0% bacterial culture, 100% Bacillus cereus enriched microbial culture and 100% E. coli enriched microbial culture with curing ages of 60 and 200 days. In the case of the UPV result, higher pulse velocity represents higher uniformity and density of the specimens. UPV test was conducted with a large gap between two curing ages in order to observe the effect of bacteria at long run. It was done to ensure whether any negative changes occur during long curing period. Table 5 and 6 represent the results of the UPV test and the categorization of concrete specimens depending on it.

Table 5: UPV results and categorization of specimens (60 days curing age)

Group	Pulse Velocity (km/s)	Concrete Grading
M-1	3.50	Good
M-2	3.63	Good
M-3	3.58	Good

Table 6: UPV results and categorization of specimens (200 days curing age)

Group	Pulse Velocity (km/s)	Concrete Grading
M-1	3.67	Good
M-2	3.89	Good
M-3	3.78	Good

For M-2 and M-3 mixes, the UPV results were much higher than the conventional one for both curing ages. For M-2 mix, the result was the highest one. It may be due to the usage of bacterial culture of "Bacillus cereus" which has the ability of more $CaCO_3$ precipitation inside concrete. The pores between cement-sand matrices were filled up by the addition of bacterial culture. Apart from M-2, a better result was also found in M-3 mix. M-3 mix possesses E. coli bacterial culture.

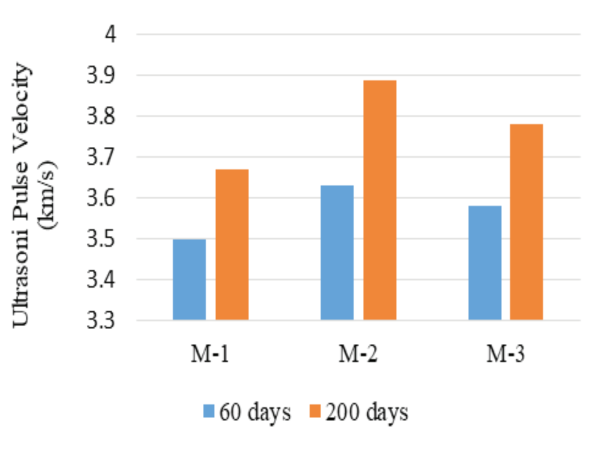
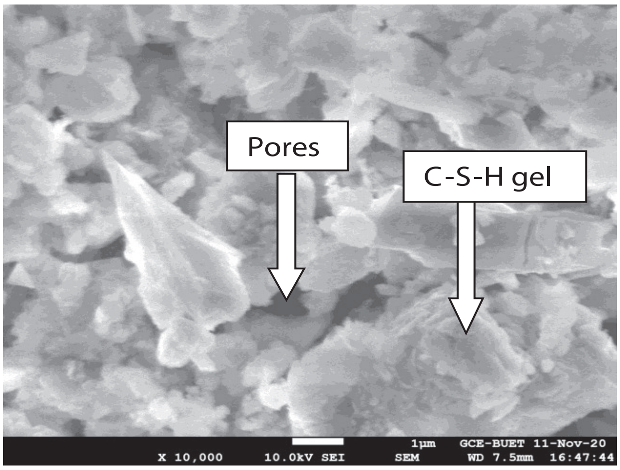
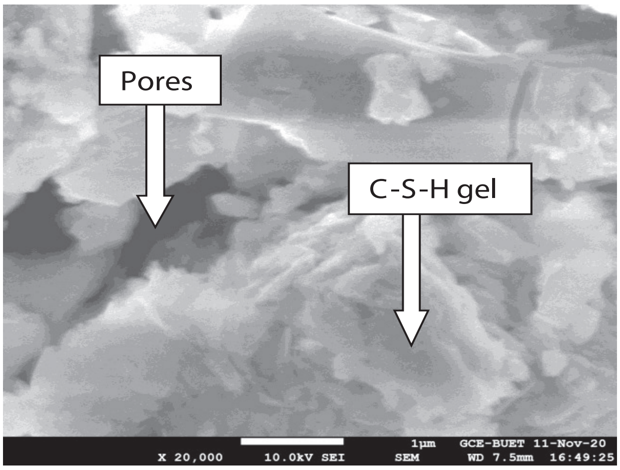


Fig. 6: Comparison between UPV results through bar chart



(a)



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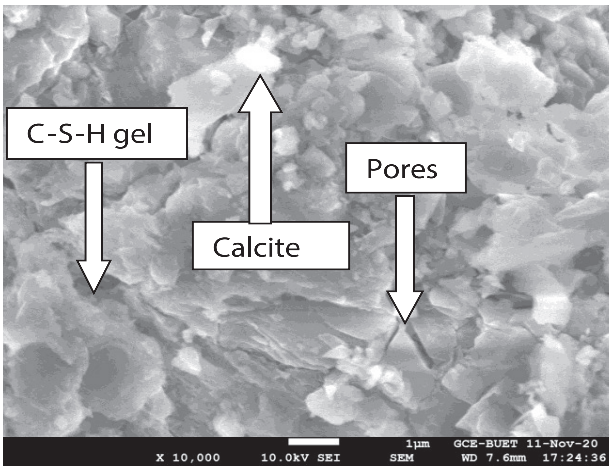

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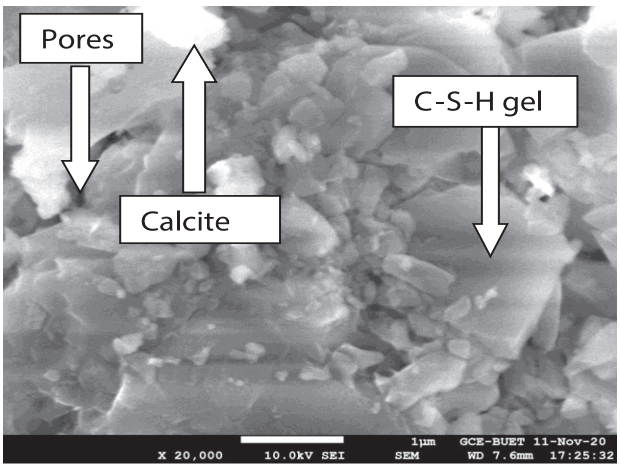

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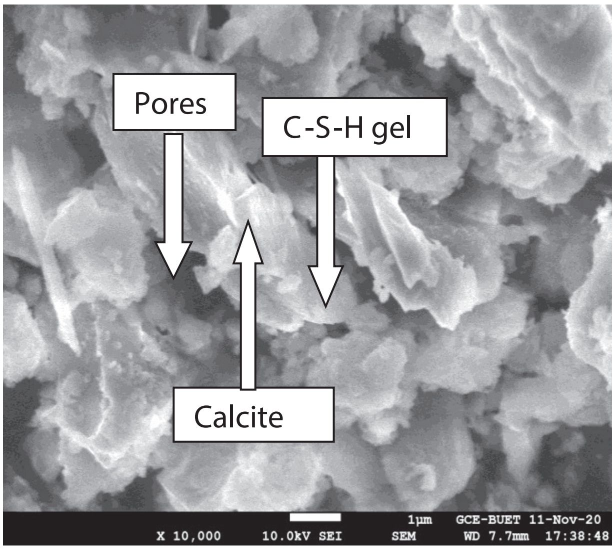

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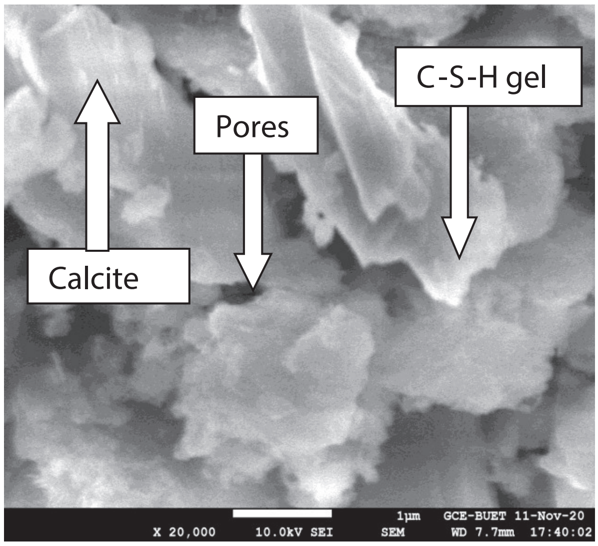

(f)

Fig. 7: SEM images of (a) M-1 (X 10000); (b) M-1 (X 20000); (c) M-2 (X 10000); (d) M-2 (X 20000); (e) M-3 (X 10000); (f) M-3 (X 20000)

Figure 6 graphically represents all the results obtained from the UPV test. As compared to the conventional one, both bacteria-induced concretes have given better results. The microbial carbonate precipitation in the concrete matrix is the main reason behind it. This kind of precipitation technique acts as a filler element that dense the microstructure of concrete. UPV test results strongly indicate this phenomenon. From SEM analysis, this phenomenon can be ensured. It can be seen that for both curing ages, M-2 concrete has given better results than M-3.

4.2 SEM Analysis

Figure 7 shows the scanning electron microscopic images of the samples, which comprises the microscopic images at a preferable magnification range and from these images, the relative comparison among these three types of concrete mixes (M-1, M-2 and M-3) can be carried out. Each of the samples was tested in powdered form.

Figure 7(a) and 7(b) show the formation of calcium silicate hydrate or C-S-H gel. For 0% bacterial culture, the CSH gel is relatively dispersed and less compacted. The formation of portlandite $Ca(OH)_2$ and calcite can also be observed in the samples. From these images, pores in microstructure can also be observed.

Sample incorporating Bacillus cereus has shown the best result foremost. Figure 7(c) and 7(d) show that the structural compactness and densification which are foremost maximum in this investigation among the other comparative elements. The C-S-H gel is well dispersed and the precipitation of the calcite crystal causes effective strength gain. The pores are at a minimum level, which can be counted as negligible.

M-3 mix in Figure 7(e) and 7(f) exhibit a better result than that of the control one. Here the structure shows relatively better compactness and denser formation. It shows better effective strength than M-1 mix due to this structural formation. This increased strength is because of the calcite production by the bacteria E. coli.

Among all concrete mixes, M-2 and M-3 possess contrasting textures. Being the conventional one, M-1 shows minimum crystal growth whereas the crystalline matrix can be seen in M-2 and M-3 mixes. So as compared to the conventional one, the samples having bacterial culture showed more developed microstructure.

Now, in between M-2 and M-3 mixes, SEM images of M-2 mix are looking denser, and the pores are lesser than M-3 mix. Concentrations of relatively large crystals can be seen in M-2 mix. The textural setting of M-2 mix resembles that coherence between particles of sand and the microstructural matrix is probably enhanced due to crystallization at the concrete matrix. Though the microstructure of M-3 mix is also promising but micro-structural analysis of M-2 mix shows denser and durable characteristics.

The analysis shows that concrete having bacterial constituent demonstrates better result in term of strength and compactness of the structure, which leads to the durability and sustainability of the structure in harsh conditions. Moreover, the comparison between the bacterial concretes exhibits that the concrete having Bacillus cereus has the better comparative preference to concrete having E. coli.

4.3 Water Absorption Capacity (WAC)

Water absorption capacity of concrete resembles its porous and permeable characteristics. Higher value in this regard represents more permeable structure. Increase of permeability decreases the concrete's durability. Table 7 and 8 represent the water absorption capacity values of concrete specimens at 200 days curing age. Long curing day was taken to observe the microbial carbonate precipitation of bacterial concrete samples.

Table 7: WAC results of specimens (60 days curing age)

Group	Water Absorption Capacity (%)
M-1	5.23
M-2	2.87
M-3	3.12

Table 8: WAC results of specimens (200 days curing age)

Group	Water Absorption Capacity (%)
M-1	4.51
M-2	1.91
M-3	2.08

From Table 7 and 8, it can be seen that with respect to M-1 mix, the water absorption capacities of M-2 and M-3 mixes decrease a lot. It is due to the use of bacterial culture which has the ability to reduce the pores in concrete matrix.

In between two microbial groups, M-2 concrete's absorption capacity is about 8-10% less than M-3. This explains that Bacillus cereus bacterial culture has a greater ability to precipitate carbonate in concrete microstructure which can be the main reason behind it. So, between M-2 and M-3 group, M-2 group shows the most promising result in the case of absorption capacity.

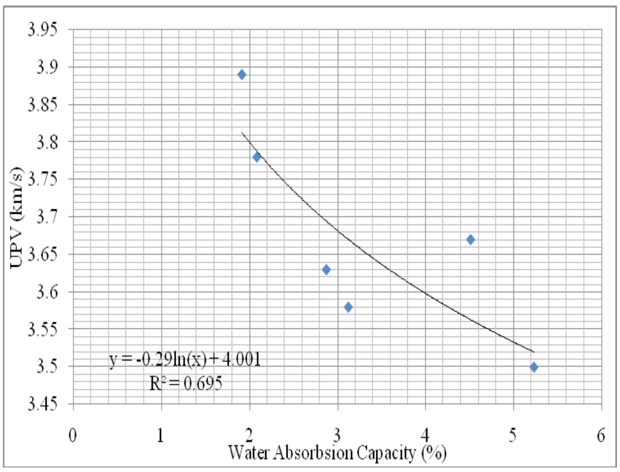

Fig. 8: Relationship between WAC (%) and UPV (km/s)

Later on, a relationship between water absorption capacity and UPV was developed. The graph in Fig. 8 resembles a parabolic shape from which it can be seen that for higher UPV value, the absorption capacity is lower. The correlation formula is found as,

$$Y = -0.29\ln(x) + 4.001$$

$$R^2 = 0.695$$

The regression value indicates an acceptable relationship between WAC and UPV data. It indicates that 69.5% data of the regression model fits actual observations.

5. CONCLUSION

This research was conducted to investigate the effect of bacterial culture on concrete microstructure through non-destructive tests and to compare the results. In this investigation, two different genus bacteria were taken as bio-agents in order to investigate their suitability and differences through the analysis of concrete microstructure. It was done in order to find out the most effective genus through a comparative study. A long gap between curing days was taken to observe the micro-structural changes more accurately. The obtained results from the investigation highlight the following conclusions:

1. Bacterial concrete incorporating E. coli and Bacillus cereus individually has higher pulse velocity than the conventional concrete, which provides comparatively better uniformity, densification and effective strength gain.
2. Concrete having Bacillus cereus demonstrates relatively higher pulse velocity than the one having E. coli, which proves the comparative betterment of the first one in terms of compactness and less porousness.
3. SEM image analysis shows comparatively densified and compacted inner structure of the bacterial concrete than the conventional concrete.
4. Having a relatively higher dispersion of CSH gel in microstructure, concrete incorporating Bacillus cereus may possess higher effective strength gain than the E coli incorporated one.
5. Concrete samples having bacterial culture possess lower permeability and water absorption capacity than conventional concrete.
6. Comparison between Bacillus cereus and E. coli showed that Bacillus cereus incorporated concrete absorbs less water and possess more compact microstructure.

7. A relationship between UPV and WAC has been developed which may be helpful in developing durable concrete.

8. Due to the high compactness and integrity of the inner structure of the specimens as found from test results, Microbial concrete is recommended to be a wise solution for making concrete durable, and the use of Bacillus cereus can be a better option in this case.

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