Research Article

An Experimental Study on the Strength and Crack Healing Performance of E. coli Bacteria-Induced Microbial Concrete

Md. Mahfuzul Islam, Nusrat Hoque, Moinul Islam, and Imteaz Ibney Gias

Department of Civil Engineering, CUET, Chattogram 4349, Bangladesh

Correspondence should be addressed to Nusrat Hoque; nusrat_hoque@cuet.ac.bd

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The most commonly used building material in the construction industry is concrete. However, the weak features of concrete are its low ductility and limited tension capacity and hence crack development with the increase in load. These cracks get more worsened by the intrusion of water and salt present in the composition and hence causing deterioration and reducing the longevity of the material. This study focuses on an innovative approach to mitigate concrete’s fractures and flaws by utilizing microbiologically induced calcite (CaCO₃) precipitation (MICP) excited by Escherichia coli (E. coli) bacteria to improve the performance of cementitious building materials. The study investigated the development of microbial concrete in plain water using only one culture density (OD₆₀₀ 0.5 ± 0.1). In this study, two water-to-bacterial mix ratios (75:25 and 50:50) were used and compared to the conventional concrete (100:0). 100-mm cube-sized specimens cured for a period of 7, 28, 90, and 365 days were tested for compressive strength, water absorption capacity, ultrasonic pulse velocity (UPV), and SEM analysis, which were performed on the samples at regular intervals. According to the results of these experiments, microbial concrete with the 50:50 ratio exhibited the highest strength for all curing times. From the water absorption test of samples, it is found that the absorption of the materials got reduced due to the infusion of microorganisms in concrete. On the other hand, the UPV test showed high velocity than the control samples for specimens with an OD₆₀₀ 0.5 ± 0.1. Scanning electron microscope (SEM) analysis performed on distinct concrete groups at the age of 28 days showed fewer voids in the concrete lumps due to the increase in water substitution rate caused by microbial culture.

1. Introduction

Concrete is recyclable, and it is a widely accepted and universally used construction material. It is a durable, strong, locally available, versatile, and has superb resistance to compressive loads until a limit. However, the cracking load in concrete is lower than the failure load [1], and it is acceptable until a certain limit [2]. The reinforcement is used in concrete to transmit the strength, and if the crack is present, it causes corrosion [3,4]. In practice, cracks in concrete also reduce the durability, permeability, and strength of the concrete. In the extreme winter situation, the situation also gets deteriorated as water seeps through these cracks and freezes, and causes a widening of gaps [3]. It is always necessary to repair those cracks because tiny little cracks can lead to massive-sized shots and shorten the concrete’s serviceability limit. Fixing problems can be complicated if damage occurs in places, which is difficult to reach. For repairing cracks in concrete, several traditional repairing systems are introduced, but they are very costly and not naturally available.

Self-healing concrete is a new type of concrete that has the ability to repair its cracks automatically. It is like healing of body wounds by secretion of some sort of body fluid. Among various methods of self-healing, the most common methods are biological self-healing, natural self-healing, and chemical self-healing process. Biological self-healing can be achieved by adding bacteria to the concrete. In self-healing concrete, bacteria are used along with calcium nutrients known as calcium lactate. This product is then added while
preparing the concrete mix in wet condition. These induced bacteria can be in inactive stages for up to 200 years and become active as soon as it comes in contact with water seeping through the cracks in concrete. This initiates the germination of bacterial spores, which feeds on the calcium lactate consuming oxygen. This process transforms the soluble calcium lactate into insoluble limestone. When this limestone gets hardened, the crack is being filled up [4]. This method of adding bacteria to concrete is known as direct method and is the most common method of preparing self-healing concrete. This is the easiest and cheaper method compared to other methods, namely, encapsulation method, although the cost of self-healing concrete is usually high; however, concrete with self-healing mechanisms can minimize costing by eradicating the need for either costly repair of concrete or new concrete and increase the structure’s durability. Various physical and chemical treatments have been experimented so far to protect the concrete from damage, but very few of them proved to be fully compatible in terms of non-reversible action and sustainability. Therefore, the use of biological techniques could be focused on [5].

Microbiologically induced calcite precipitation (MICP) is a method that could be adopted to solve the cracking problem, which can help to get long-lasting and eco-friendly concrete [6].

Figure 1 shows the actual imagery with a different interval of the crack healing process, which shows a gradual reduction of crack width with the time (0 day, 1 week, 2 weeks, etc.) reported by Wang et al. [7]. The crack had nearly healed fully by 3 weeks. Cracks up to 1 mm width can be independently screened, depending on the dose of bacteria and lactate-based nutrients. The autonomous waterproofing of 0.4-mm large cracks is sufficient for a dose of 15 kg/m³ of the auto-healing agent per m³ of the concrete mix (Figure 2).

Self-healing concrete can be illustrated as concrete, which has the capability of repairing itself back to the original state. It is a green technology that embeds self-activating bacteria into concrete and fixes its cracks. Since the material used for this technique can be grown in the laboratory, it does not pose a risk to natural resources. Hence, this method can be an effective technology for the improvement of the strength of concrete [9].

Bacterial impacts on the crack and self-healing treatment offer cleaner, more sustainable, longer-lasting material and reduce the cost of repairing the cracks in long term. By reducing absorption, permeability, and diffusion as the key mechanisms for carrying concrete, the durability of concrete can be increased [10]. Several studies have documented the effect of bio-based healing agents on the permeability and water absorption of concrete. Cracks in concrete structures can be reduced by the presence of bacteria as can be seen from previous literature as described later. Sarker et al. [11] used E. coli bacterial strain on concrete mix and suggested from the mortar test that it enhances concrete strength and the cement quantity can be reduced by using microbes without compromising the strength.

Safiuddin et al. [12] studied the effect of mixing Bacillus subtilis and Escherichia coli on the time required for crack the mitigation and mechanical properties of concrete by mixing them with a percentage by weight of cement. The result shows that 3% Bacillus subtilis is the optimum dosage for self-healing of concrete, whereas Escherichia coli mixed at the dose of 3% by weight of cement can increase the strength up to 60%.

The application of microbial concrete has been a research issue since long [13–15]. Xu et al. [16] used porous ceramsite particles as microbial carrier applying heat treatment and NaOH soaking and found that heat treatment can improve the loading content of ceramsite without reducing the concrete strength.

Zhang et al. [17] studied the concrete crack healing capacity using two microbial consortia under anaerobic (MC-Aa) and anoxic (MC-Ao) conditions and neurolytic pure-culture bacteria (Bacillus cohnii). Feng et al. [18] performed 3-point bending test by forming 0.3 mm width cracks on the beam bottom and found that the microcracks were healed by calcite precipitation due to the bacterial metabolic activity. Mondal and Ghosh [19] studied different levels of bacterial concentration of Bacillus subtilis and concluded that as the bacterial dose increases, crack healing also improved. Algiafi et al. [20] studied the factors influencing urea hydrolysis and bacterial growth so that the calcium carbonate precipitation inside concrete fissures can be modeled exactly. The authors also concluded that self-healing bacteria can be a future sustainable strategy to extend concrete life span. Vijay and Murmu [21] studied the effect of Bacillus subtilis strain bacterial concrete on the addition of calcium lactate. Nirala et al. [22] used Escherichia coli bacteria with 5%, 10%, and 15% by mass and observed a healing of fissures and improvement in compressive and tensile strength at the curing period of 7 days. Sumathi et al. [23] achieved a notable amount of healing in cracks at the age of 1 month using Bacillus subtilis bacteria. On the contrary, Balam et al. [24] achieved about 90 percent of surface healing at the age of 28 days using Bacillus cohnii culture by at the rate of 105 cells/mL. Vahabi et al. [25] used higher grade concrete with Bacillus subtilis bacteria with a concentration of 10 ml, 20 ml, and 30 ml and observed healing properties of concrete. Xu et al. [26] used Sporosarcina pasteurii at the concentration of 105 cells/mL and showed that the water absorption is reduced by four times when S. pasteurii is present. Bacterial calcite deposition resulted in a roughly eightfold reduction in chloride permeability, extending the life of concrete. Xu et al. [27] studied the effect of crack healing potential of reinforced concrete incorporated with ureolytic microbial self-healing agents immobilized in porous ceramsite particles and found that bacteria can heal up to 450 μm cracks within 120 days.

In microbially induced calcium carbonate precipitation (MCIP) or microbiologically induced calcite precipitation (MICP) via urea hydrolysis, substantial amounts of carbonates are produced rapidly through ureolytic microbes. Urea hydrolysis via the enzyme urease inside a calcium-rich atmosphere is investigated commonly because of its simplicity.

The decomposition of urea into carbonate and ammonium is amplified by the microbial enzyme going through
the chemical process as in equations (1) and (2). It is obvious from the chemical reaction that one mole of urea is hydrolyzed intracellularly to produce one mole of ammonia and one mole of carbamate, which then hydrolyzes naturally to generate one mole of ammonia and carbonic acid.

\[
\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \xrightarrow{\text{BACTERIA}} \text{NH}_2\text{COOH} + \text{NH}_3 \tag{1}
\]

\[
\text{NH}_2\text{COOH} + \text{H}_2\text{O} \rightarrow \text{NH}_3 + \text{H}_2\text{CO}_3 \tag{2}
\]

When it comes in contact with water, the pH levels increase as these molecules then split into bicarbonate and hydroxide ions:

\[
\text{H}_2\text{CO}_3 \rightarrow 2\text{H}^+ + 2\text{CO}_3^{2-} \tag{3}
\]

The series of events occurring during this ureolytic calcification was observed by Hammes and Verstraete [28] and emphasized more on the role of pH and calcium metabolism. Various physiological processes [28] create an alkaline atmosphere by the stimulation of the bacteria. Figure 3 depicts the series of events occurring throughout microbially induced carbonate precipitation (MICP). Positively charged cations (e.g., Ca\(^{2+}\) and Mg\(^{2+}\)) get adsorbed upon the cell surface due to the nucleating area created by the heterogeneous electronegativity loaded bacterial cell membrane. Neutral pH environments facilitate the presence of various anionic (negative charge) groups, and these anionic charges get dominant over the bacterial cell surface resulting in the secretion of divalent cations (positively charged) on interaction. As shown by Eqs 4-5, the bacterial cell membrane plays a vital role in the CaCO\(_3\) precipitation like a nucleation site.

\[
\text{Ca}^{2+} + \text{Cell} \rightarrow \text{Cell} – \text{Ca}^{2+} \tag{4}
\]

\[
\text{Cell} – \text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{Cell} – \text{CaCO}_3 \tag{5}
\]

The microbes serve as a nucleation site, assisting in the formation of calcite that can gradually seal cracks and pores throughout concrete, improving its durability. This micro-biologically induced calcite precipitation (MICP) is the product of a complicated sequence of biological processes. This process leads to the formation of CaCO\(_3\) crystal form, which expands and develops as the bacteria produce calcium lactate nutrition. The crystal formation grows until the entire void is filled. Hence, this natural and biochemical method increases the sustainability of concrete.

However, the subject of bacterial precipitation is still dubious. Several researchers argue that precipitation is indeed the by-product of metabolism that happened accidently. Wherever others consider, this is a distinct environmental phenomenon that can be beneficial for humans [29, 30].

Nevertheless, based on the past studies as summarized earlier, the current study presents the crushing of concrete with the results obtained from UPV and water absorption test including SEM analysis for *E. coli* microbial culture ratio mixed with water in comparison with normal water. In direct application method of bacteria, bacterial spores and calcium lactate are added into concrete directly when mixing of cement is completed. The utilization of these microorganisms and calcium lactate does not change the typical properties of cement. At the point when water interacts with these microscopic organisms, they develop and feed on calcium lactate and deliver limestone. Consequently, the cracks are fixed. But in the case of an encapsulation method, bacterial spores are added with encapsulated nutrients in a concrete matrix. Hence, direct application method is chosen for this study for its easiness in use.

On the other hand, *Escherichia coli*, otherwise called *E. coli*, is a micrometer Gram-negative, non-spore-forming bacteria that are ordinarily found in the lower digestive tract of warm-blooded life forms. The primary benefit of inserting *E. coli* bacteria in concrete is that it continually hastens CaCO\(_3\). Positive increments in compressive strength test results due to the addition of *E. coli* in concrete were also
reported by past researchers including Vijay et al. [3]. Hence, *E. coli* bacteria are used in this study and two arbitrary ratios (75:25 and 50:50) of plain water to microbial culture were chosen to find out the effectiveness of *E. coli* bacteria in concrete. The study aims to see the effect of bacterial injection in concrete to improve cracks and to maintain good compressive strength.

2. Materials

Ordinary Portland cement (OPC) ASTM type 1 complying with ASTM C-150 has been chosen as the cementitious material in the experimental work. Locally available natural sand passing through 4.75-mm sieve and retaining on 0.075-mm sieve has been used as a fine aggregate, and crushed stone with a nominal size of 12.5 mm has been chosen as a coarse aggregate in this experiment.

This study uses *Escherichia coli* (*E. coli*) bacteria that feed on carbon dioxide instead of traditional feed like sugar and other organic matter. *E. coli* is a well-known bacterium which has many other uses in the real world like synthesizing useful chemicals such as insulin, creating synthetic forms of human growth hormone. This bacterium can intrude into concrete cracks and can remain dormant for many years even at high temperatures. It is a nontoxic bacterium that reproduces quickly by splitting method, according to research. Therefore, one of the advantages of this bacterium is its easy culture within a short time. Plain water with a pH value of 7 and zero turbidity was used for the study. Sylhet sand with an absorption capacity of 2.78% and specific gravity of 2.51 is used as fine aggregates, whereas locally available stones with a specific gravity of 2.74 and absorption capacity of 2.33% are used as coarse aggregates.

3. Experimental Procedure

3.1. Preparation of Bacterial Culture. *Escherichia coli* bacteria had been used in this experiment, which was collected from the Microbiology Department of another local university. The media used was nutrient broth, which was made from peptone, beef extract, and a slight amount of NaCl. All composition materials were taken in a 300 ml conical flask in proper quantity and then mixed with water and stirred slowly for the preparation of nutrient broth. To make the media germ free, the media was sterilized for exactly two hours in sterilization autoclaves. *Escherichia coli* spores were then injected into the prepared media by using a needle. The spore-injected media was then settled in the refrigerator by maintaining an ambient temperature. This process allows the bacteria to germinate in a binary fission manner. The growth period of bacteria and germination time helps to determine the bacterial concentration.

The next stage was to investigate different bacterial groups. However, before those steps, the properties of these prepared samples had been determined. Optical density (OD) measurement of bacterial culture having an optical density of OD600 0.5 ± 0.1 yields better strength [31]. Based on those studies, this research work used an OD value of 600 0.5 ± 0.1.

Generally, in spectrophotometer, the wavelength can be set from 420 to 660 nm. In this study, a wavelength of 600 nm has been set to track the growth of *E. coli*. It is crucial that the cells are in an excellently physiological process of growth. The estimated relationship between absorbance and colony-forming units (CFU) (the number of viable bacteria within a sample) may differ as the cell size differs with growth phase (lag, log, and stationary). The concentration of cells differs from the optical density and was therefore estimated using the following relation:

\[ Y = 8.59E10^7 X^{1.362}, \]

where X is reading at optical density 600 nm, and Y is cell concentration per ml.

Figure 4 represents the whole procedure related to preparation of the bacterial culture.

3.2. Concrete Specimen Preparation. Cube samples of size 100 mm × 100 mm × 100 mm were made following ASTM standard procedure using the mix proportions as obtained from the proper mix design. Mix design was performed for two strength requirements: 25 MPa and 35 MPa. Cement: fine aggregate: coarse aggregate ratios for the two-strength category are 1:2.091:2.276 and 1:1.43:1.8 with water cement ratios of 0.5 and 0.395, respectively. Bacterial culture media containing the required number of microorganisms
mixed with water were used as the liquid substance. Two water: microbial culture ratios were 75:25 (75% of water and 25% of microbial culture) and 50:50 (50% of water and 50% of microbial culture). Prepared concrete is poured into mold for casting and removed from the mold after 24 hours. After preparing the samples, they were cured in plain water for various time spans.

3.3. Concrete Strength Using Ultrasonic Pulse Velocity (UPV) Measurement. The interior quality of concrete samples can be assessed using the UPV test. High velocity indicates strong concrete consistency, which can be attributed to density, uniformity, homogeneity, and other factors. On cube specimens, the UPV test was performed by putting a pulse transmitter on one side of the cube and a receiver on the other. Conventional relation between speed, distance, and time as shown below is used to compute the ultrasonic pulse velocity

$$\text{UPV} = \frac{L}{T},$$  \hspace{1cm} (7)

where $L$ is distance between transducers, and $T$ is transit time.

3.4. Absorption of Water by Immersion Method. Water absorption tests of concrete samples were measured using ASTM C1585 and ASTM C642 method. Water absorption in percentage

$$\text{water absorption in percentage} = \left( \frac{W_1 - W_2}{W_2} \right) \times 100,$$ \hspace{1cm} (8)

where $W_1$ is SSD weight of the sample, and $W_2$ is oven-dry weight.

3.5. Analysis Using Scanning Electron Microscopy (SEM). SEM analyses were performed to observe and analyze the microstructural changes between conventional concrete and E. coli generated concrete. Powdered samples were taken from the core of each sample for SEM examination. The SEM analysis was carried out according to the guideline. The interfacial transition zone (ITZ) was utilized in SEM research to observe bacterial mineral formation, which creates a filler effect in the concrete mixture.

4. Results and Discussion

4.1. Compressive Strength Test Result. Compressive strength results of various concrete mixes at different curing ages are presented in Figure 5(a).

The compressive strength test results at various ages show the trend as expected where compressive strength increases with ages. This strength gain may be attributed to the formation of more hydration products as the time progresses, which improves the bonding between the particles. But the rate of strength gain is not same for all

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Preparation of the bacterial culture. (a) Materials. (b) Materials poured into flask for Luria–Bertani media. (c) Preparation of media for sterilization. (d) Media kept in refrigerator for germination. (e) Prepared bacterial solution.}
\end{figure}
specimens and variation is obvious since the design and material proportions are not same.

As obvious from the plot in Figure 5(b), the highest strength gain is observed in between 7 and 28 days. Subsequently, the strength increment becomes gradual. The graph also implies that the strength of 25 MPa concrete increases more than the 35 MPa concrete. However, when the concrete samples were cured for 90 or 365 days of curing, the strength of 35 MPa concrete increases more than the 25 MPa concrete. For 35 MPa grade, the compressive strength increases up to 21.5% for Escherichia coli (50% bacterial culture). On the other hand, the strength increase is higher for water: bacterial media ratio of 50:50 than water: bacterial media ratio of 75:25 concrete.

However, in all cases, the strength of bacterial concrete is higher than normal concrete, which most probably is the result of the filling of the pores inside the concrete matrix by the precipitation of calcite due to the bacterial injection.

4.2. Ultrasonic Pulse Velocity Test. Figure 6(a) shows UPV results on different curing ages for designed concrete strength of 25 MPa and 35 MPa. Similar to compressive strength, UPV values of all specimens rise with increase in age irrespective of the bacterial doses. As such, the average gain in UPV values at 365 days curing corresponding to its value at 28 days varies from 6.15 to 16.21%
**Figure 6:** Variation of UPV test results and curing periods for 25 MPa concrete and 35 MPa concrete.
for control specimen. In bacterial concrete, the long-term UPV improvement varies from 4.49 to 16.49% for C25 concrete, whereas it is 3.81–9.39% for C35 concrete at 365 days compared with 28-day UPV values. For samples with longer curing period, the concrete mix is filled with more hydration products, C-S-H, and this leads to more solid and compact internal structures.

This in turns increases the velocity since the time required to travel the pulse is decreased.

According to the guideline of concrete quality, UPV above 4.5 km/sec implies excellent quality concrete, whereas UPV ranging 3.5–4 km/sec specifies good quality; UPV ranging 3–3.5 km/sec specifies medium quality; and below that value concrete is of doubtful quality.

From Figure 6(b), it is obvious that concrete samples are of medium quality for normal as well as microbial concrete for curing ages of 7 days and 28 days. On the other hand as the curing age increased (90 days and 365 days), the concrete quality raises from medium to good quality concrete for 25 MPa concrete group. However, for 35 MPa concrete, it remains still in the medium-quality range except for BC 50: 50–35 MPa at the age of 365 days. Another trend that is observed is that the UPV value of microbial concrete is higher than normal concrete, which implies the dense nature of microbial concrete than normal one.

4.3. Water Absorption Capacity Test by Immersion Method. Test results of water absorption are shown in Figure 7.

From Figure 7(a), it can be said that the use of microorganisms in concrete reduces the absorption of the material. When the water absorption rate is observed as a function of curing days as in Figure 7(b), it can be said that the water absorption decreases as the time increases. The lowest water absorption is observed for BC 50 : 50 35 MPa concrete, which suggested that as the bacterial concentration increases, there is a reduction in water absorption. That means microorganisms help concrete make more durable. The maximum reduction in water absorption is found to be 17.60% for Escherichia coli (50% bacterial culture).

4.4. Analysis of Scanning Electron Microscope (SEM). Changes in the microstructure of concrete due to the addition of E. coli bacteria can be studied by SEM analysis caused. Concrete specimens with a standard curing period of 28 days were taken from all types of samples (with and without culture for 25 MPa and 35 MPa mix) and studied at various magnifications. Figures 8 and 9 show the SEM morphology of the three concrete groups at 28 days. It shows that the bacterial inclusion has a significant impact on the microstructure of concrete. Conventional concrete (Figure 8) shows significant amounts of voids among the concrete samples. Voids decreased as the rate of water substitution by microbial culture rises.

The intrusion nature of bacterial concrete might be attributed to the compact microstructure. And it is obvious that the microstructure of concrete had a considerable impact on the hardness and durability properties of the material. Reduction in voids in microbial strain-infused concrete mixtures was indeed the primary cause for its better strength and durability properties when compared to the plain concrete group.

The cause for the high compactness of concrete containing microbial strains was also validated by SEM analysis. It exhibited the presence of calcite precipitation in bacterial concrete, which implies the presence of fewer cavities and more compact concrete. Calcite precipitation was detected as the white patches of areas in these images. The density of white patches seen in these photographs increased as the concentrations of microbes increased. As a result, it can be stated that the denser microstructure of concrete mixture is primarily responsible for the increase in strength and durability of concrete with the inclusion of microbial strain.

4.5. Comparison between Destructive and Nondestructive Testing Results. The common destructive test to determine the strength of concrete is the crushing of sample. On the other hand, UPV is the easiest nondestructive test to determine the compressive strength. The comparison in strength obtained from these two methods is presented in this section.

The test results show that the UPV and compressive strength of concrete mix are significantly affected by bacterial doses and curing age. The experimental investigation was carried out at curing ages of 7, 28, 90, and 365 days. To establish the relationship between UPV and compressive strength, all the data points are merged together and plotted as shown in Figure 10. Plotting of test results shows that compressive strength is linearly correlated with UPV. The coefficient of determination $R^2$ of general relationships was 0.88 for both C25 and C35 concrete mix proportions. This represents that the variation in compressive strength with UPV is accounted well by linear relationship.

4.6. Prediction Model for Compressive Strength. A nonlinear regression analysis with 95% confidence level was carried out to determine the strength of concrete specimens for various ages and for different ratios of water to bacterial media.

The empirical relation found from the analysis is as follows:

$$ z = a + bx + c \ln (y) + d \ln (y) + e \ln (y)^2, \quad (9) $$

where $z$ is compressive strength in MPa, $x$ is the ratio of water to bacterial media, and $y$ is the age of the specimen in days. Since there are two different types of mix design, the analysis is carried out for two sets of data separately and the coefficients are $a = 2.84, b = 7, c = 9.18, d = -0.395, e = -0.754$ for 25 MPa concrete and $a = 2.25, b = 12.03, c = 13.4, d = -1.16, e = -1.11$ for 35 MPa concrete. The percent of error is found to be 1.6%, which is clearly below 5%. The actual vs predicted strengths for both cases are shown in Figure 11. Figure 12 shows the response surface for both cases, which has been developed using MATLAB software.
Figure 7: Variation of water absorption test results for various curing periods for 25 MPa concrete and 35 MPa concrete.
Figure 8: SEM imaging of conventional concrete. (a) 25 MPa plain concrete. (b) 35 MPa plain concrete.

Figure 9: SEM imaging of E coli-induced concrete. (a) 25 MPa microbial concrete. (b) 35 MPa microbial concrete.

Figure 10: Relationship between compressive strength and UPV.
The goodness of fit of the model can be accessed by accessing the coefficient of determination (COD), that is, \( R^2 \) value.

If a data set has \( n \) values marked \( y_1, \ldots, y_n \) (collectively known as \( y_i \)) and each of them is associated with a predicted value \( f_1, \ldots, f_n \) (known as \( f_i \)), then the residual can be written as

\[
 r_i = y_i - f_i, \tag{10}
\]

The mean of the observed data \( \bar{y} \) is

\[
 \bar{y} = \frac{1}{n} \sum_{i=1}^{n} y_i, \tag{11}
\]

Then, \( R^2 \) value can be obtained using the following formula:

\[
 R^2 = 1 - \frac{\sum (y_i - f_i)^2}{\sum (y_i - \bar{y})^2}. \tag{12}
\]

The COD value is found to be 0.99 for the fit, and a value close to 1 indicates the higher efficiency and low discrepancy of the model from the actual.

Residual sum of squares (RSS) is another measure for the good of a regression curve. It is the sum of the squared estimates of errors [21] or the difference between the actual data and an estimation model as expressed in (13). A small RSS refers to the tight fit of the model.

\[
 RSS = \sum_i (y_i - f_i)^2. \tag{13}
\]

For the current prediction model, the average value of RSS (5.4) confirms that the model is effective in predicting the compressive strength.

### 5. Conclusions

The main aim of this study was to study the properties of microbial concrete using \( E. \ coli \) bacteria in the concrete mixture, which has self-healing capacity and hence can be a good solution for durable concrete.

The inclusion of bacterial cultures did not show any adverse effect on the crushing compressive strength as they were found higher than normal concrete in the current study for the investigated parameters. In terms of the optimum ratio of water to bacterial culture media, it is found that the higher the amount of bacterial dose, the better the performance and 50% bacterial culture media exhibits better results than 25% bacterial culture.
On the other hand, the nondestructive UPV test also indicates that microbial groups are more compact than normal concrete, which means the microorganism was effective in producing dense concrete structures. The results obtained from water absorption test also supported this finding, which shows that the use of microorganisms in concrete reduces the absorption of the material, which means less porosity and hence denser concrete structures. The calcium carbonate formed due to chemical action precipitated in the voids making the surface more compact, and hence, this also improves the stability of the structure since the liquid and ion absorption causing reinforcement corrosion were prevented.

The SEM test also exhibited less voids in the microstructure for concrete with high bacterial culture media. A linear relationship is established between the compressive strength found from destructive test and the velocity found from nondestructive UPV test. From the results of the two proportions of bacterial injections, it can be said that the use of Escherichia coli (50% bacterial culture) having OD600 0.5 ± 0.1 has performed better and the use of this ratio will facilitate the production of durable concrete, which in turn can minimize the cost as it eliminates the need for casting new concrete. The investigational data for strength are supported through a prediction model and the fit of the model is found to be acceptable for practical purposes.

Data Availability

The data will be available upon request from the authors.

Disclosure

The abstract of this manuscript was presented at ACI concrete competition project 2021.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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