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An Experimental Study of the Physio-Mechanical and Microstructural Performances of Escherichia Coli Bacteria-Based Bio-Concrete

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Abstract: A balanced mixture of cement, sand, stone or brick chips, and water is carefully allowed to form concrete, a man-made building material. These elements can be adjusted appropriately to produce concrete with a variety of qualities. Although concrete may endure compressive forces, like natural stone, tensile forces can cause it to crack. As a result, crack formation is a frequent occurrence in concrete, allowing various foreign chemicals and water to enter the structures and shortening their life span. The likelihood of cracking grows with time due to variations in humidity and temperature. It can be exceedingly expensive to maintain or repair concrete construction items. The use of bio-concrete for the construction of durable structures has shown to be quite advantageous in this perspective. It is beneficial for improving the properties of concrete as well as lowering maintenance costs. In this investigation, concrete samples measuring $100 \times 100 \times 100$ mm were made and periodically tested for compressive and split tensile strength testing. Following a 28-day curing period, the concrete treated with *Escherichia coli* bacteria had compressive and split tensile strengths that were 10% and 23% higher than identical bacteria-free. The non-destructive test on cylindrical samples was then conducted to evaluate the material qualities. The mortar samples of crystalline structures were also validated by SEM examination. In order to properly and reliably anticipate the strength of concrete, the RSM model was also formulated.

Keywords: Bio-concrete, *escherichia coli*, MICP mechanism, mechanical strength, UPV analysis, SEM imaging

1. Introduction

Concrete is a flexible construction material that can withstand compressive loads up to a certain level. On the contrary, it is a material that is relatively brittle and has a low tensile strength compared to its compressive strength. Tiny cracks can be developed when the load applied to concrete exceeds the limit. Therefore, micro-cracks are inherently present in concrete. Cracking can also take place during curing as heat is liberated. Water and gases

containing harmful substances can seep through these cracks, which widen the gaps. If these microscopic cracks grow further, they will eventually reach the reinforcement steel bar level and cause the decay of the rebar due to corrosion. More than 0.8 mm wide cracks are reportedly challenging to repair (Luo et al., 2015). Controlling the crack width and healing the cracks as immediately as possible is essential. Repairing those cracks is always necessary since micro-cracks can result in huge openings, reducing the concrete's serviceability limit (Maes et al., 2014). Repairing cracks in difficult-to-reach areas can be difficult. As a result, preparing concrete with constituents that can densify the inner matrices of cementitious material can improve cracking resistance. Numerous long-established improvement systems are introduced to facilitate this in concrete, but these systems can not be fully utilized because they can be particularly expensive, and the solutions are not long-lasting.

One successful approach to dealing with the problem is to look for materials that can densify the inner microstructure as well as automatically seal the tiny cracks while also ensuring a higher strength gain than traditional approaches. The development of microbiologically induced "Bio-concrete" is one such effort. This biotechnological method can effectively make the inner structure more compact by forming CSH gel in the matrices and demonstrating excellent results in repairing cracks in the early stages of crack formation (Anne et al., 2010).

Despite the fact that concrete is supposed to have the ability to repair itself because of the hydration of cementing materials or carbonation of $Ca(OH)_2$, only limited crack widths can be controlled through the autogenous healing process when moisture is present. According to some research, the autogenous healing process could partially heal cracks of 0.15 mm in width and completely heal cracks of 0.05 mm in width when moisture is present (Snoeck et al., 2014). As a result, concrete should be modified to have autonomous healing. An autonomous healing system could repair crack widths of up to 0.5 mm, according to reports (Wang et al., 2014). The most of autonomous healing systems use bio-agents that are either encapsulated or applied externally to the concrete matrix. The activation of the healing agent is caused by the cracking itself. If the agent is introduced into the matrix as encapsulated, its proficiency must be dormant until cracking occurs. To achieve optimal release when the agent is encapsulated, the healing materials must be mobile, and the capsules must be able to withstand mixing and colliding with aggregates. Direct application has been shown to heal cracks up to 27.2 mm wide (Achal et al., 2013). When added to the concrete matrix, encapsulated agents could effectively seal cracks as wide as 0.97 mm (Zabanoot et al., 2020).

Escherichia coli bacteria in the Luria-Bertani (LB) media was used as a bio-agent in this form of study and the culture was directly added to the concrete matrix. An optimum culture density (OD_{600} 0.5±0.1) was strictly maintained throughout the study. The following four distinct categories of tests describe the research exploration's main goal.

a) To assess the mechanical strengths of *Escherichia coli*-based bio-concrete
b) To find out the effectiveness of such concrete in terms of developing dense microstructure
c) To understand the suitability of such bio-agent to develop bio-concrete by Scanning Electron Microscopy (SEM) approach
d) To develop an RSM model for predicting concrete strength and checking marginal errors and deviations using RMSE

Evaluation of the rheological characteristics of concrete specimens is done using the slump cone test. The mechanical properties of such new concrete were evaluated using compressive and split tensile strength tests. SEM analyses were carried out to determine whether such bacteria were suitable for the development of bio-concrete. Finally, the density of the bio-concrete samples was compared to the control concrete using ultrasonic pulse velocity (UPV) analysis.

2. Literature Review

As soon as water is added to the cement matrix, a highly alkaline environment is usually created, and the pH value rises to 13. Most of the living microorganisms become inhospitable in this hostile environment. However, "alkaliphilic" types of microorganisms can withstand this condition. When cracks form in concrete, moisture enters through these cracks and awakens the bacterial spores to action. By producing limestone, it commences the process of healing. This bio-calcification is known as "Microbiologically Induced Calcite Precipitation (MICP)". Several metabolic pathways are involved in achieving MICP. Among all metabolic pathways, "enzymatic hydrolysis of urea" is mostly discussed. Bacterial species that can produce urea are usually found in marine sediments or organic matter. The incubation process produced more than 98% of the urea that was hydrolyzed (Pedersen et al., 1993). Bacteria can break down urea to produce ammonia and carbonate. If the solution contains a sufficient concentration of calcium ions and carbonate, limestone is eventually produced at the microbial cell wall (Qian et al., 2010). Figure 1 depicts a schematic diagram of metabolic byproducts.

In recent years, various urease-producing bacteria have been studied. However, the majority of the research efforts were focused on discovering bacteria of the "*Bacillus*" genus that could effectively produce limestone. Several researchers deviated from the trend and looked for other bacteria genera that could actively participate in urease activity. However, this literature study will focus solely on the "*Escherichia*" genus of bacteria.

Vijay and his team (Vijay et al., 2019) prepared 150 mm cubical concrete specimens for 3 different *Escherichia coli* percentages (5%, 10%, and 15%). The maximum increment in compressive strength was around 6% which accounted for a 15% bacterial percentage. Together with 5% and 10%, Ansari and her team (Ansari et al., 2019) also investigated the 2.5% *Escherichia coli* percentage. Their study also supported the findings of Vijay et al., with a maximum increase in compressive strength for the highest bacterial percentage (10%). A transformed *Escherichia coli* strain that was directly incorporated into cement mortar was the subject of an investigation by Sarkar and his team (Sarkar et al., 2015). They reported that adding 10^5 *Escherichia coli* bacterial cells per ml of water resulted in a 30% and 5% increment in compressive strength and pulse velocity respectively.
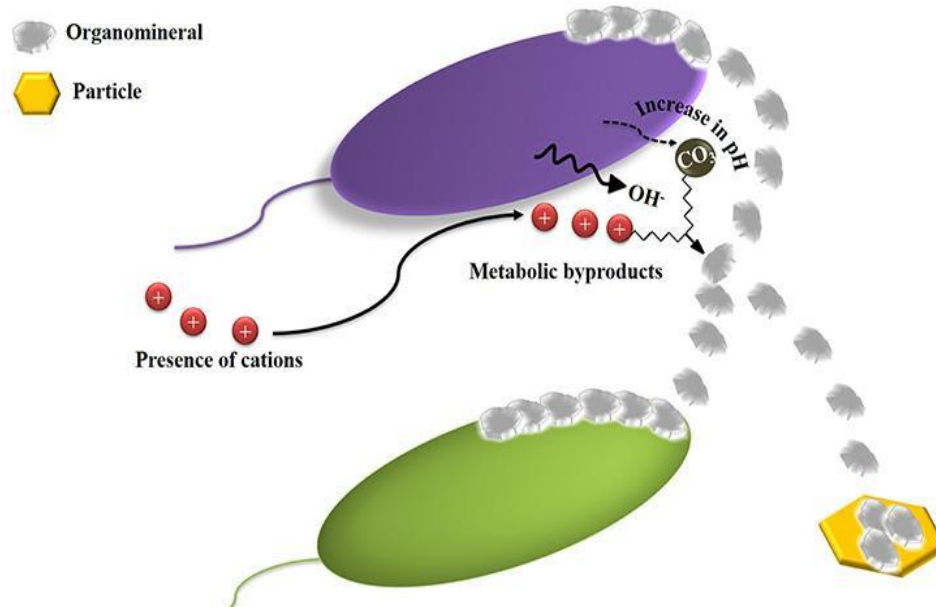

Fig. 1 - Schematic diagram of biologically induced precipitation (Weiner et al., 2003)

3. Methodology

3.1 Materials and Properties

The bio-concrete mix consists of CEM I of strength class 42.5 N, Sylhet sand (Fineness Modulus 2.51), 5-15 mm coarse aggregate, and *Escherichia coli* culture. Luria-Bertani (LB) media was chosen for the cultivation of the bacteria as shown in Figure 2. The recipe for preparing *Escherichia coli* culture is summarized in Table 1.

Table 2 shows the three control mixtures that were used. The ratio of water-to-binder (WB) for concrete specimens was 0.592 (Series I and VI). To investigate the microstructural changes, cement mortar samples (1:4) were also prepared (Series VIII). The samples were cast and stored in the lab for 24 hours before demolding. Following that, the samples were demolded and cured in water for 27 days maintaining 20°C. The compressive strengths of 100 mm cubical samples were measured periodically. The mix design of control specimens is shown in Table 2. Series 1 concrete had an average compressive strength of 21.1 MPa.

Table 1 - Recipe for preparing Escherichia coli culture

Media	Tryptone (gml⁻¹)	Yeast extract (gml⁻¹)	NaCl (gml⁻¹)	Incubation period (hr.)	Incubation temperature (°C)	OD$_{600}$
LB	10	05	10	24	37	0.5±0.1

Table 2 - Control specimens (28 days cube strength and pulse velocity)

Series	WB	Cement (kgm⁻³)	Sand (kgm⁻³)	CA (kgm⁻³)	Compressive strength (MPa)	UPV (ms⁻¹)
I	0.592	321	823	1088	21.1	
VI	0.592	321	823	1088		3230
VIII	0.5	383	1700			

3.2 Slump Cone Test

Slump cone tests were done to learn more about the rheological characteristics of concrete specimens. The test was conducted according to ASTM C143 specifications. To fill the slump cone, three equivalent layers of concrete were used. Each layer was prepared with 33% of the total concrete volume and rodded with a round-ended tamping rod. In a consistent pattern, each layer received 25 strokes. The top point of the slump cone was identified and leveled after the final layer was completed by removing excess cement mortar from the equipment's side. To prevent concrete from leaking from the base, the slump cone's hooks were removed and the cone was clamped with the attached handles. Once the base was free of the block, the slump cone was lifted. This task took only five seconds to complete. The slumped concrete's top surface was then estimated and compared to the height of the 300 mm concrete slump cone. The recorded concrete slump value was the difference in height between the base and the material after slumping. The concrete slump test is depicted in Figure 3.

Fig. 2 - Prepared *Escherichia coli* culture   **Fig. 3 - Slump test of concrete (True slump)**

3.3 Mechanical Strength Test

Tests on concrete specimens measuring 100 mm in cubical shape included split and compressive strength tests. The dried samples were directly placed inside the testing machine under a circular bearing block for the compressive strength test. But a special steel mold (350×250×264 mm) was used to hold the specimens for the split tensile strength test. The specimens were placed at the center location and a constant rate of loading was maintained. The strength test is depicted in Figure 4.

3.4 Ultrasonic Pulse Velocity Test

To assess material homogeneity, 100×200 mm cylindrical concrete samples were utilized for ultrasonic pulse velocity (UPV) analysis. It is possible to evaluate the strength or distinguish heterogeneous areas in concrete by using propagation varieties of ultrasonic speed waves. Each cylindrical sample had a pulse transmitter attached to one side. The ultrasonic speed wave was received by a receiver placed on the other side of the sample, and the pulse velocity was eventually calculated. Table 3 summarizes the chart used to categorize concrete groups using UPV analysis.

Table 3 - Categorization of concrete group based on UPV values (Saint-Pierre, 2016)

Pulse velocity (ms^{-1})	> 4,500	3,600-4,500	3,000-3,600	2,100-3,000	1,800-2,100
Concrete Group	Excellent	Good	Medium	Poor	Very Poor

3.5 Scanning Electron Microscope Test

To investigate the microstructural changes caused by *Escherichia coli* bacteria in concrete, SEM analysis was performed. Cement mortar powder samples were collected from the cubes' cores. Following that, the samples were examined under various magnification levels to examine the microstructure of each sample after 28 days. A summary of bio-concrete specimens is shown in Table 4.

Table 4 - Bio-concrete specimens

Series	WB	Dimension (mm)	Culture		Water	
			kgm^{-3} by mass	% by mass	kgm^{-3} by mass	% by mass
II	0.592	100×100×100	30.5	25	91.5	75
III	0.592	100×100×100	61.0	50	61.0	50
IV	0.592	100×100×100	91.5	75	30.5	25
V	0.592	100×100×100	122.0	100	0	0
VII	0.592	Ø100×200	122.0	100	0	0
IX	0.5	Powder	122.0	100	0	0

3.6 Data Modeling

Using the program Minitab 2019, Response Surface Methodology (RSM) data modeling was carried out. Data modeling was used to examine more thoroughly how the curing period and pulse velocity influence the concrete compressive strength. Additionally, based on these two variables, RSM was utilized to develop a concrete compressive strength prediction model. Instead of using linear and quadratic terms of variables in traditional mathematical expressions, RSM examines the interaction impact of variables for more in-depth modeling. Furthermore, it may establish the spectrum of two variables using a surface plot and show the impact of each variable in great detail using the main effect plot. The ability of the models to explain variance in the dependent variables was evaluated using the regression coefficient (R^2) and adjusted regression coefficient (R^2 adj) values. The Root Mean Square Error (RMSE), which gauges the discrepancy between expected and actual experimental data, was also used to assess the model's correctness.

4. Results and Discussion

4.1 Slump Cone Test Analysis

Concrete workability as assessed by the slump cone test is shown in Figure 4. The control specimen had a slump of 70 mm. However, as *Escherichia coli* cultures were employed to replace the water content, workability significantly declined. The graph demonstrates that adding bacterial culture instead of water somewhat reduces the workability of concrete. When 100% culture was substituted for water, the slump value of the control concrete, which was 70 mm, decreased to 60 mm. Previous researchers also made a similar observation (Sekhar et al., 2017).
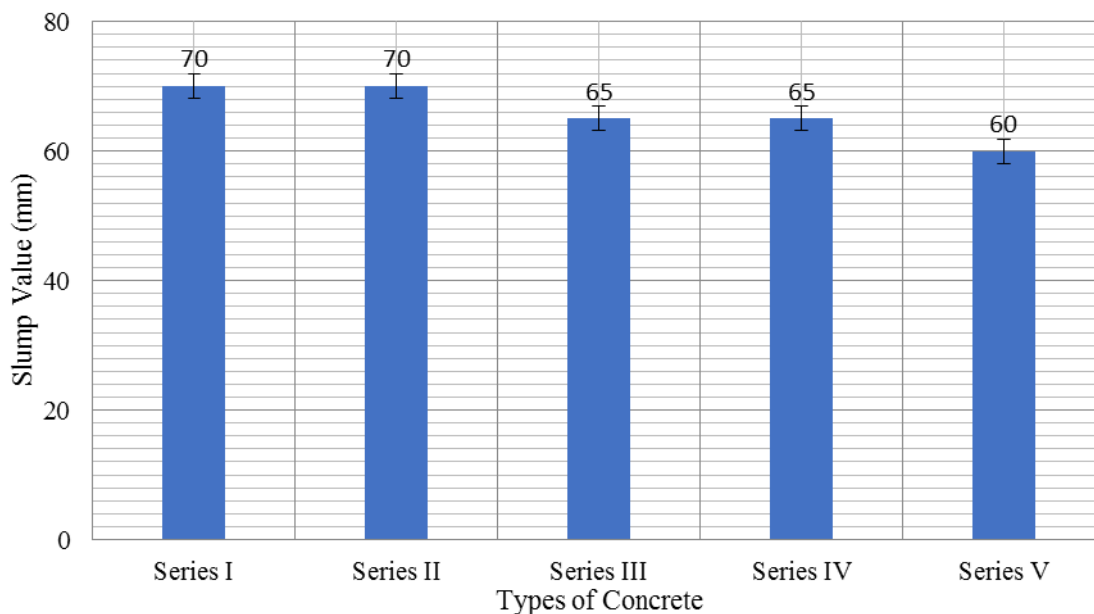

Fig. 4 - Slump test results of different concrete groups

4.2 Mechanical Strength Analysis

Mechanical strength test results are critically analyzed and summarized in graphical form. The test results for control and *Escherichia coli*-induced concrete are shown in Figures 5 to 8 which include compressive, split tensile, and UPV results. The use of the highest *Escherichia coli* culture in concrete had a remarkable effect on the mechanical strength and compacity of the concrete. Table 5 summarizes the compressive, split tensile, and UPV test results of Series V and VII at 28 days of curing.

Table 5 - Compressive, split tensile, and UPV test results of Series V and VII (28 days)

Series	Culture		Water		Compressive strength (MPa)	Split Tensile Strength (MPa)	UPV (ms^{-1})
	kgm^{-3} by mass	% by mass	kgm^{-3} by mass	% by mass			
V	122	100	0	0	23.1	2.7	
VII	122	100	0	0			3420

The compressive strengths of Series I concrete were 14.4, 16.2, 21.1, 23.1, 24.9, and 26.2 MPa respectively for 7, 14, 28, 60, 90, and 180 days whereas the corresponding values of Series V concrete were 15.1, 18.6, 23.1, 25.1, 27.2 and 29.5 MPa (Figure 5). The gain in strength can easily be visualized from relative strength data. Figure 6 depicts the compressive strength gain for various curing periods. The maximum gain in compressive strengths (Series V) made with *Escherichia coli* bio-agent was 4.9%, 14.8%, 9.5%, 8.7%, 9.2%, and 12.6% for respective curing days. It can be easily visualized that the corresponding strength increment after 14 days of curing was much higher. This was primarily due to the deposition of $CaCO_3$ inside concrete matrix.
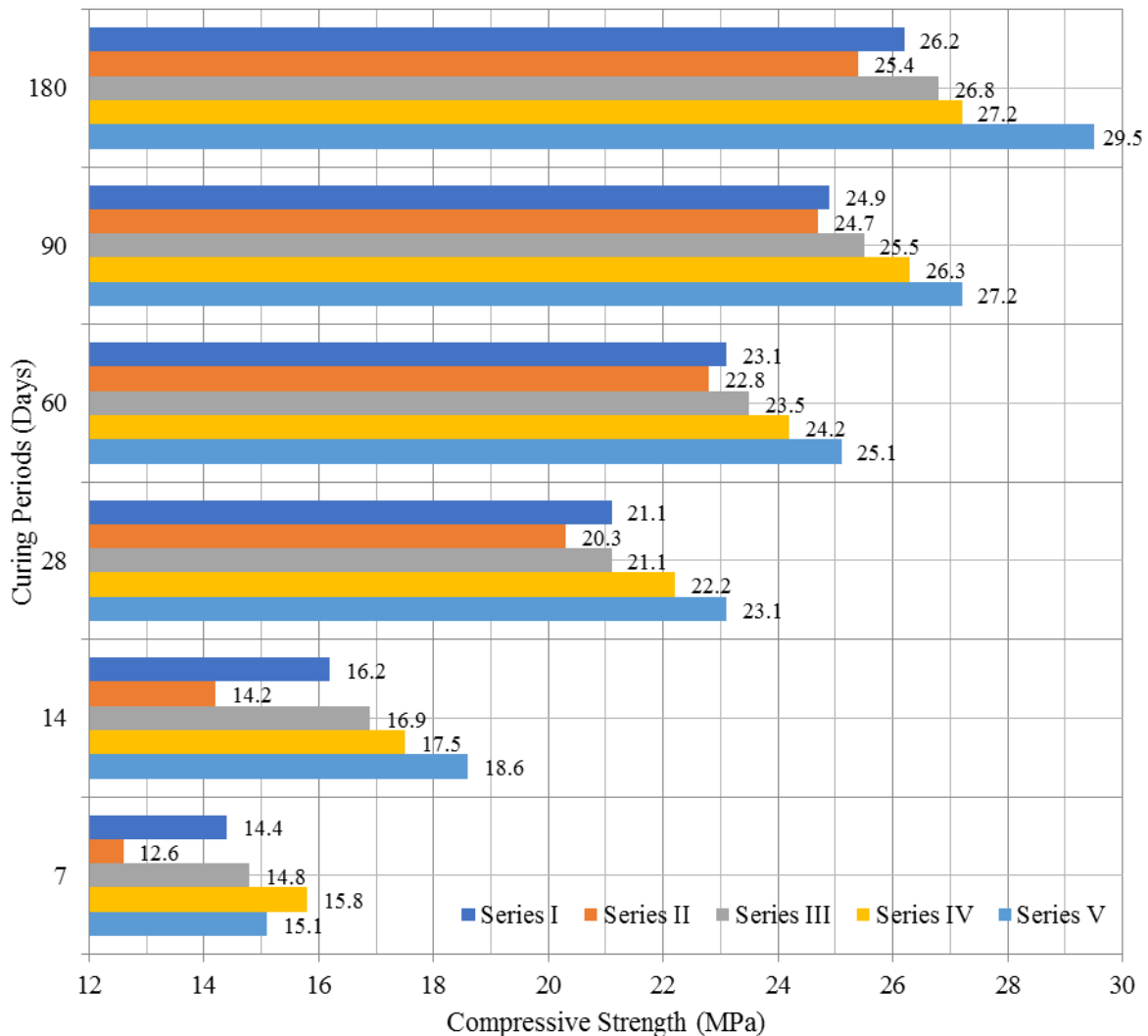
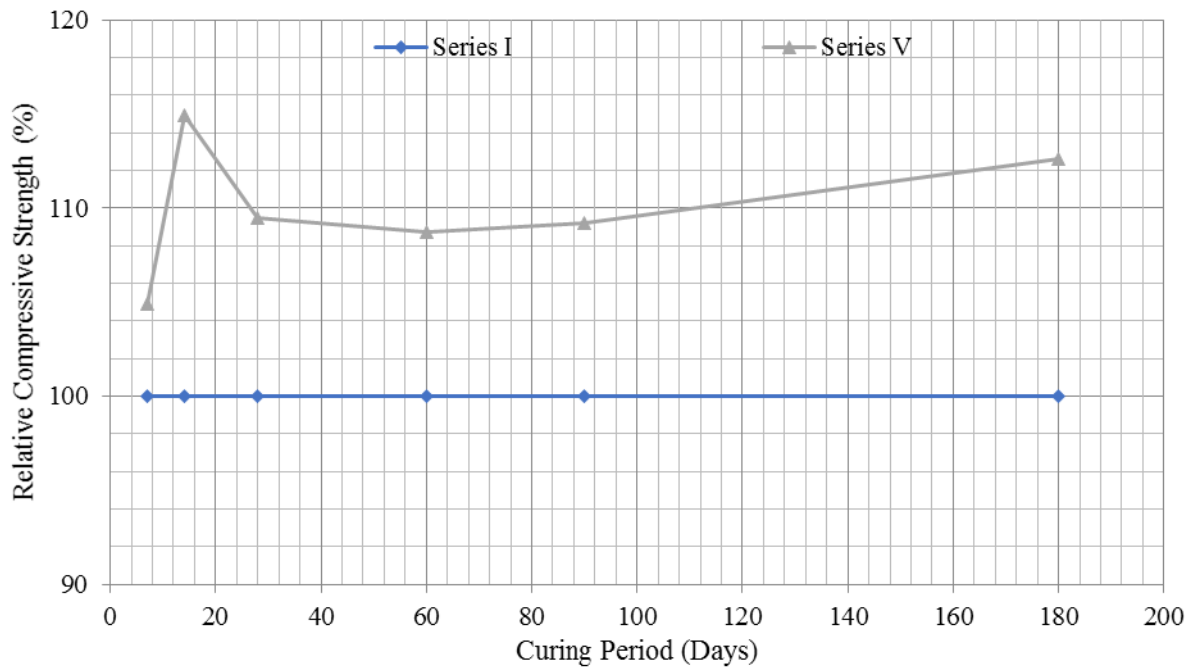**Fig. 5 - Compressive strengths of different concrete specimens**

Fig. 6 - Maximum improvement (%) in compressive strength (Series V)

The split tensile strength of bio-concrete increased substantially as bacterial culture was introduced to it. Figure 7 shows the test results at different curing times. The split tensile strengths of Series I concrete were 1.3, 1.5, 2.2, 3.4, 3.9, and 4.3 MPa after 7, 14, 28, 60, 90, and 180 curing days. For the corresponding curing days, the split tensile strengths of Series V specimens were recorded to be 1.7, 2.2, 2.7, 3.7, 4.0, and 4.9 MPa.
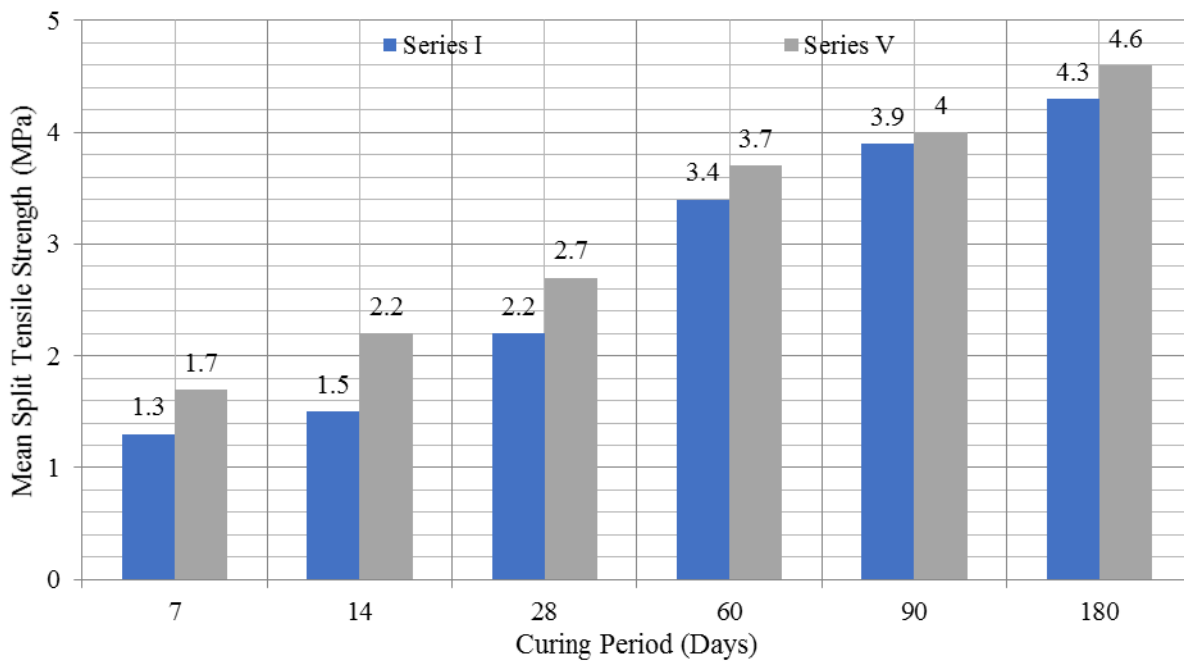
Fig. 7 - Split tensile strengths of concrete specimens

At all curing periods, the use of biotechnological methods based on $CaCO_3$ precipitation improves concrete's mechanical strength. Due to the presence of a highly alkaline environment, the growth of bio-agents is initially slow. $CaCO_3$ may be precipitated on the cell surface and within the concrete during microbial growth. As a result, specimens become less permeable. The bio-agents die or are converted into endospores over time. Hence, the primary reason for the increased mechanical strength of concrete is the $CaCO_3$ deposition inside the concrete (Vijay et al., 2017).

4.3 Ultrasonic Pulse Velocity Analysis

To evaluate the structure's compacity or distinguish heterogeneous areas, an ultrasonic pulse velocity test of Series VI and Series VII was performed. UPV analysis can detect non-homogeneous conditions in concrete specimens. Figure 8 depicts the UPV data of control (Series I) and bio-concrete (Series VII). The pulse velocities of 20 MPa control concrete were found to be 3020, 3110, 3230, 3490, 3550, and 3630 ms^{-1} for the respective curing days of 7, 14, 28, 60, 90, and 180 days. The addition of *Escherichia coli* bacteria to concrete increased pulse velocities, which were 3040, 3180, 3320, 3580, 3660, and 3750 ms^{-1} respectively. These increased pulse velocities indicate that the bio-agents were successful in precipitating $CaCO_3$ within the concrete matrix. However, the initial pulse rate of the control and treated specimens was nearly identical. But, after 14 days of curing, Series VII pulse velocities were found to be significantly higher than control specimens.
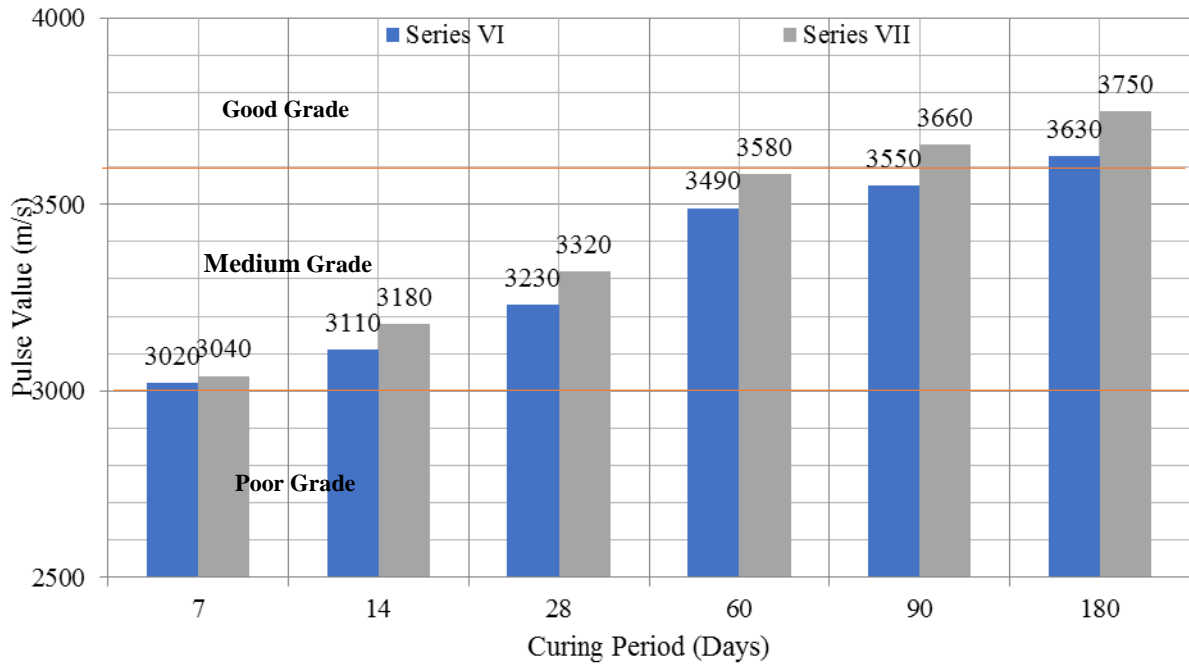

Fig. 8 - Ultrasonic pulse velocities of concrete specimens

4.4 Scanning Electron Microscope Analysis

To explore the microstructure of different mortar groups, SEM imaging tests were done at 28 days and studied at different magnification levels. Figure 9 and Figure 10 show SEM imaging test results of the control and self-healing mortar groups. It can be easily visualized that the inclusion of *Escherichia coli* bacteria had a positive effect on improving microstructure. CSH gel was well dispersed and well compacted in *Escherichia coli*-induced sample.
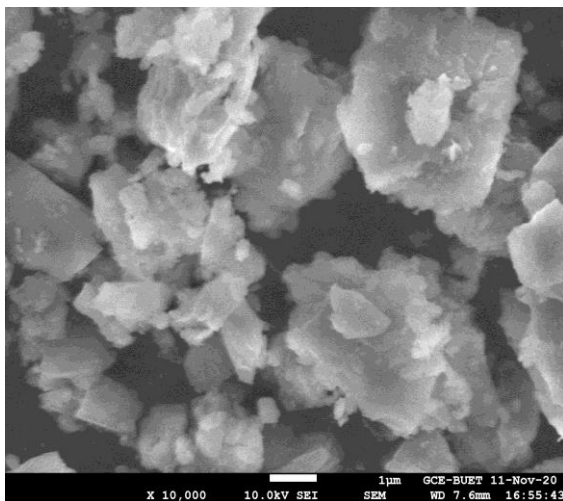

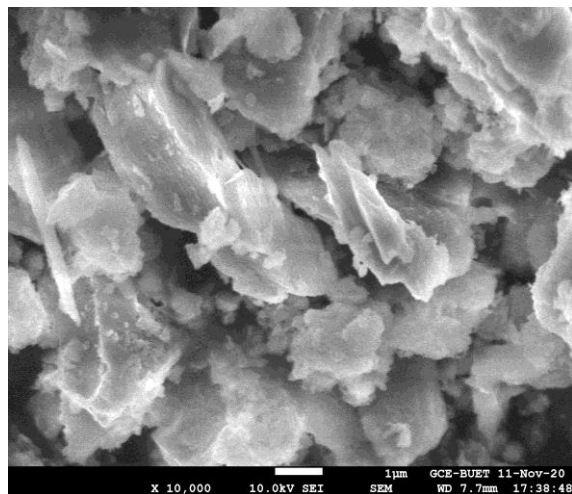**Fig. 9 - SEM test result of Series VIII**

Fig. 10 - SEM test result of Series IX

Different calcite crystals (in the form of CaCO₃) embedded with *Escherichia coli* bacteria were identified through SEM images. These crystals eventually improved the impermeability and durability of mortar samples (Kim et al., 2013). SEM analysis also confirmed that the addition of *Escherichia coli* bacteria increased the strength of the bio-concrete by depositing $CaCO_3$ in the pores. Previous researchers also made similar observations (Chahal et al., 2012).

4.5 RSM Model of Compressive Strength

Series V concrete was found to be the most effective for all curing ages of all concrete groups. So, to predict the concrete compressive strength (CS) of Series V specimens, the Response Surface Methodology (RSM) was employed. The analysis included two independent variables: curing period (CP) and pulse velocity (UPV). Without performing any destructive testing, all curing period and pulse velocity data could be gathered as the model was designed to anticipate the Series V compressive strength. The curing ages of bio-concrete could be selected during the design stage, while pulse values were obtained via non-destructive tests. The RSM analysis is summarized in Table 6. R^2 and adjusted R^2 values for the model are 0.9626 and 0.9377, respectively. The model's RMSE is 1.34924, indicating that it can predict the concrete compressive strength with minimal error. The regression equation is found as follows,

$$CS_{Series\ V}\ (MPa) = -39.6 + 0.0018\ CP + 0.01830\ UPV \qquad (2)$$

Table 6 - Summary of the RSM model

Source	DF	Adj SS	Adj MS	F-value	P-value
Regression	2	140.559	70.2793	38.61	0.007
CP	1	0.015	0.0152	0.01	0.933
UPV	1	31.233	31.2330	17.16	0.026
Error	3	5.461	1.8204		
Total	5	146.020			
RMSE	1.34924				
R²	96.26%				
R² adj	93.77%				

Figure 11 depicts the individual general influences of the curing period and pulse velocity on Series V compressive strength. Figure 12 depicts a surface plot of the curing period and pulse velocity vs. compressive strength. Both independent variables are related to compressive strength in a positive way.
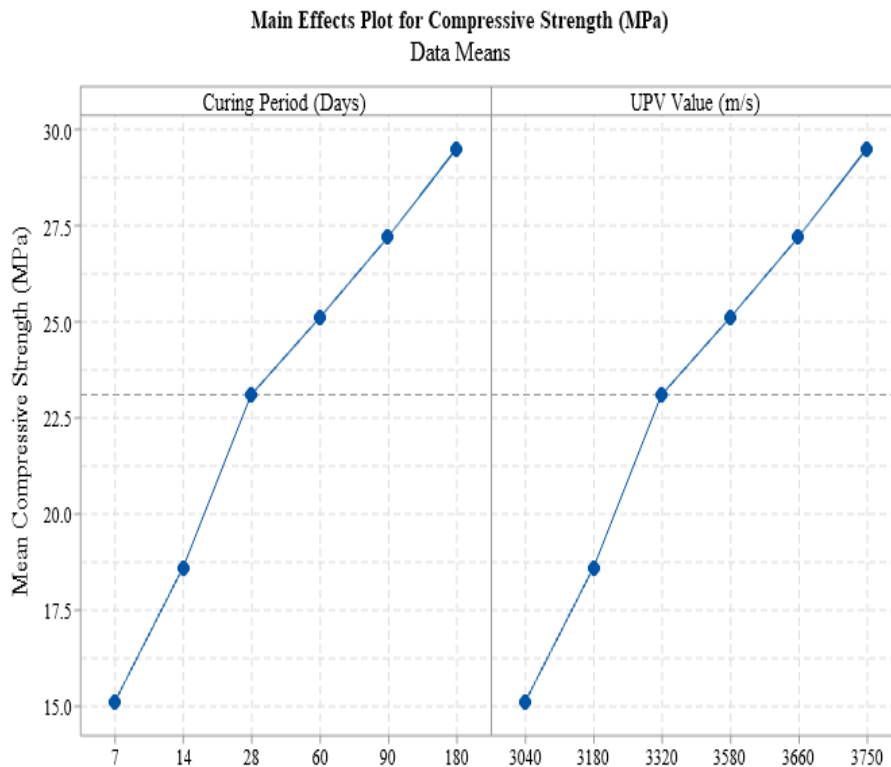

Fig. 11 - Main effect plot for compressive strength (Series V)

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Surface Plot of Compressive Strength (MPa) vs Curing Period (Days), UPV Value (m/s)
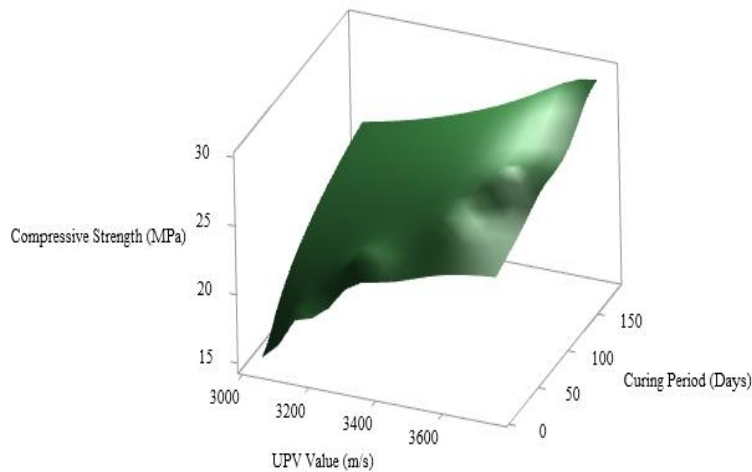

Fig. 12 - Surface plot of compressive strength vs. curing period and UPV (Series V)

4. Conclusions

In this study, using RSM, *Escherichia coli* culture was used to experimentally assess and model the mechanical strength of concrete. The findings of the investigation lead to the following conclusions:

1. The presence of *Escherichia coli* culture in concrete slightly reduces its workability. Due to the calcite deposition in inner structures, the slump value reduced from 70 mm to 60 mm. Low workability, on the other hand, is unfavorable for high-strength concrete. Therefore, the replacement material proportion should be optimized.
2. At 28 days, around 10% and 22% increments in compressive and tensile strength were respectively observed when Series V (100% *Escherichia coli* culture) was used. This is mainly due to the $CaCO_3$ deposition on the cell surface and pores.
3. Non-destructive test (UPV) confirms the homogeneity and integrity of bio-concrete. A relatively good quality grade of concrete can be achieved when bio-culture is used. The pulse velocity ranged from 3040-3750 ms^{-1} due to the inclusion of *Escherichia coli* culture in concrete.
4. Calcite crystals embedded with *Escherichia coli* bacteria can be confirmed through SEM imaging. Micro-structural improvement was noticed after the incorporation of *Escherichia coli* bacteria in concrete.
5. The RSM model accurately predicts the concrete's compressive strength. The regression coefficient for the model is found as 0.9626, which is close to 1. However, the model also evaluated RMSE and found it to be minimal.

Declaration of Competing Interest

The authors affirm that they don't have any apparent conflicts of interest that might have affected the experimental work.

Authors' Contribution Statement

All of the laboratory work required for this research study was performed by Sudipto Nath Priyom. Additionally, he produced the final manuscript and data summaries. On the computer, Md. Fahad Shahriar Zawad and Md. Asifur Rahman entered the sections. During the writing of the paper, G. M. Sadiqul Islam, Md. Moinul Islam, Md. Saiful Islam and Wahhida Shumi provided all the necessary comments.

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