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APPLICATION OF ESCHERICHIA COLI. AS A SMART BIO-AGENT TO MAKE SUSTAINABLE FUTURE GENERATION CONCRETE

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Abstract

Concrete is an artificial manmade building material which is obtained by carefully permitting proportioned mixture of cement, sand, stone/brick chips and water. Appropriate adjustments of these ingredients can lead concrete with vast ranges of properties. Although concrete can tolerate compressive forces like natural stone, it is prone to cracking due to tensile forces. Thus, crack formation is a widespread phenomenon in concrete, which allows different kinds of foreign substances and water into the concrete structures and reduces the life span of the infrastructures. The possibility of cracking can increase with time due to the variation in humidity and temperature. Maintaining or repairing concrete-based structures can be very costly. Use of self-healing concrete using microbial agent is demonstrated very beneficially in the present scenario for the construction of durable structures. It is proved to be advantageous for improving the properties of concrete and also for reducing the maintenance costs. In this study 100×100×100 mm cubical concrete specimens were prepared with or without using *Escherichia coli* bacteria and periodically subjected to compressive and split tensile strength test. About 10% and 23% increment in compressive and split tensile strengths were respectively observed after 28 days of curing period due to addition of *E. coli* bacteria in concrete. Later on, UPV and SEM analysis were also performed to evaluate material properties. SEM analysis also confirmed the crystalline structures within the powdered mortar sample. Hence, the use of *E. coli* bacteria in concrete is arranged towards increasing sustainability and decreasing the all-out cost-of-ownership for structures.

Keywords: Self-healing concrete; *Escherichia coli*; MICP mechanism; Mechanical strength; UPV analysis; SEM imaging

1.0 INTRODUCTION

Concrete is a versatile building material and capable of resisting compressive load to a limit. On the contrary, it is a relatively brittle material in which tensile strength is small compared with its compressive strength. Tiny cracks can be developed when the load applied to concrete exceeds the limit. Crack formation in concrete is a common phenomenon. This 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 tiny cracks grow further, they reach the reinforcement steel bar and cause decay of rebar due to corrosion. It is required to decrease the crack width and heal the cracks before further deterioration of the structure. As per Maes et al., (2014) cracks are required to be repaired since those micro cracks can lead to larger cracks reducing the concrete serviceability ultimately. Repairing process imparts complication if cracking occurs in places which are impossible to reach. Thus, the preparation of concrete containing constituents which can densify the inner matrices of cementitious material can improve the durability against cracking. Several traditional improvement systems have been introduced and developing yet, those were developed using the traditional material than the natural biological resources, and the solutions have less conformity to the eco-friendliness because using those techniques require production of fly ash or various pozzolanic materials. (A. Alyousif, 2016, M. Şahmaran et al. 2009)

One successful approach to deal with the issue is to search for materials which can densify the inner micro-structure as well as seal the tiny cracks automatically and also ensures higher strength gain compared to traditional approaches. An effort in this regard is development of microbiologically induced "Bacterial Concrete". Bacterial or Microbial concrete is defined as the capability of concrete to make inner structure more compact by the C-S-H gel formation in the matrices and imparting higher mechanical strength than the traditional concrete. It is also known as "Self-healing Concrete". Although concrete is supposed to have autogenous healing capacity due to hydration of cementing materials or carbonation of $Ca(OH)_2$, only limited crack widths can be controlled through autogenous healing process in presence of moisture and absence of tensile forces. It is implied that in presence of moisture crack widths up to 0.02-0.138 mm could completely and up to 0.15 mm could partially be healed through autogenous healing process (Yang et al., (2011); Snoeck, (2014)). Hence, concrete should be modified so as to develop capability like self-healing. Autonomous healing or self-healing systems typically use agents that are directly added directly into the concrete matrix or introduced in form of an encapsulation. Their activation is caused by the cracking itself. If the agent is introduced into the matrix unprotected, its proficiency must lie dormant until cracking occurs. When an agent is encapsulated, the capsules need to be resistant to mixing, which prevents them from colliding with aggregates. Additionally, the healing substance needs to be mobile to provide a sufficient release. It is estimated that autonomous healing system could repair crack widths up to 0.5 mm. Moreover, as per Zabanoot, et al., (2020), if encapsulated agents are introduced in concrete matrix, crack width up to 0.97 mm could effectively be sealed.

Escherichia coli (E. coli) bacteria in Luria-Bertani (LB) media was used as bio-agent in this form of study and the culture was directly added to concrete matrix. An optimum culture density (OD_{600} 0.5±0.1) was strictly maintained throughout the study. The primary aim of this investigation was divided into three distinct categories:

1. Evaluate mechanical properties of *E. coli* induced concrete
2. Find out the effectiveness of such concrete in terms of developing dense microstructure.
3. An approach to understand the suitability of such bio-agent to develop self-healing concrete by Scanning Electron Microscopy (SEM) approach.

Assessment of the mechanical properties of such types of concrete other than the conventional types have also been conducted via Compressive strength and Tensile strength tests. To understand the suitability of such bacteria to develop self-healing concrete, SEM analysis were done. Ultrasonic Pulse Velocity (UPV) analysis was conducted to assess the density of the concrete samples compared to traditional concrete.

From this experimental research study, it was finally concluded that E. coli bio-agent possesses huge ability and possibilities to generate a new way in sustainable concrete development trough self-healing and strength gaining. But less attention towards such bio-agent was also a question that needed to be eradicate by doing extensive research.

2.0 LITERATURE ANALYSIS

When water is added to cement matrix, usually a high alkaline environment is created, and the pH value rises up to 13. This environment becomes inhospitable for most of the living microorganisms. But "alkaliphilic" types of microorganisms could withstand at that 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. It was estimated that more than 98% of the urea that was hydrolyzed could originate from production during incubation (Pedersen et al., 1993). Bacteria can convert this urea into ammonia and carbonate. If sufficient concentration of calcium ion and carbonate present in solution, eventually limestone is produced at the microbial cell wall Qian et al., (2010).

Different urease producing bacteria were investigated in recent years. But most of the research works were concentrated on discovering bacteria of "bacillus" genus which could effectively produce limestone. However, several researchers broke the trend and looked for other genus of bacteria if they could actively take part in urease activity. Zambare et al. (2020) conducted a research on ureolysis-driven microbiologically induced calcite precipitation by single E. coli. MJK2 cells and found calcium carbonate precipitation on all concentrations if MJK2 cells and urea present there together. In their research using single E. coli. MJK2 cells it was also exhibited a dense interior structure with a grainy exterior when examined through electron microscopy This literature study will solely be concentrated on *Escherichia coli* induced concrete.

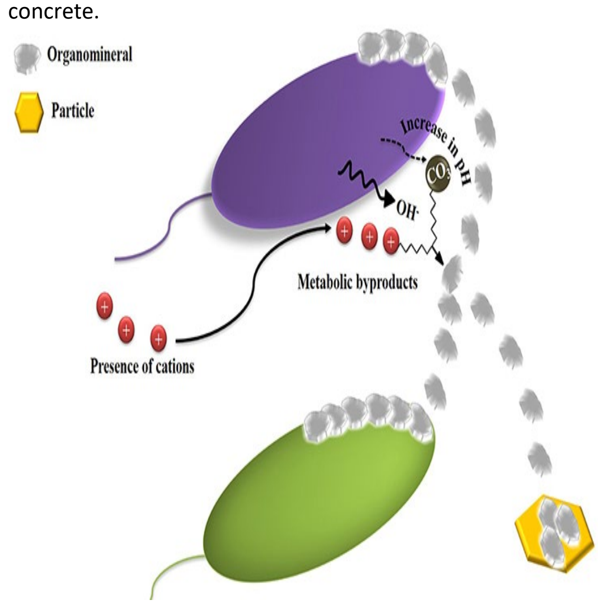

Figure 1: Calcite precipitation onto the biological surfaces (Weiner et al., 2003)

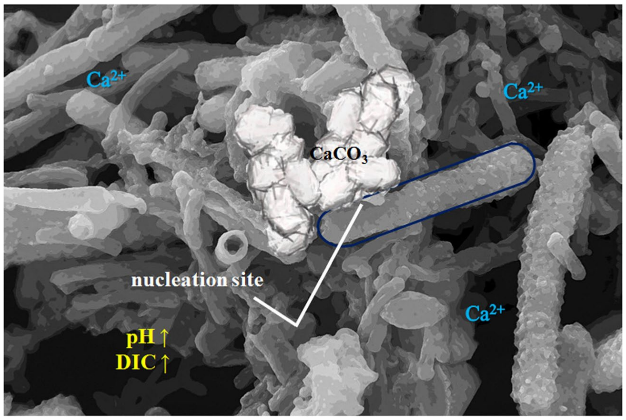

Figure 2: Microbiologically induced calcium carbonate precipitation in Scanning Electron Microscopy (Lee et al., 2018)

Vijay et al., (2019) prepared 150 mm cubical concrete specimens for 3 different *E. coli* percentages (5%, 10% and 15%). Maximum increment in compressive strength was around 6% which was accounted for 15% bacterial percentage. Together with 5% and 10%, Ansari and her team Ansari et al., (2019) had also investigated the 2.5% *E. coli* percentage. Their experiment also justified the test results of Vijay et al. and maximum increment in compressive strength was found for the maximum bacterial percentage (10%). Sarkar and his team Sarkar et al., (2015) had used transformed *E. coli* strain directly into cement mortar and investigated the performances. They reported that around 30% increment in compressive strength and 5% increment in UPV values were observed due to addition of 10^5 *E. coli* bacterial cells per ml of water.

3.0 METHODOLOGY

3.1 Materials and Mix Design

Self-healing concrete mix consists of CEM I of strength class 52.5 N, Sylhet sand (Fineness Modulas 2.51), 5-15 mm coarse aggregate and *E. coli* bacteria culture was used for this study. Luria-Bertani (LB) media was used for growth and maintenance of *E. coli* bacteria (Figure 3). Recipe for preparing *E. coli* culture is summarized in Table 1.

One self-healing concrete mix was adopted, as listed in Table 2. Water to binder (W/B) ratio was found as 0.592 from the mix design. The samples were cast and kept in lab conditions for 24 hours before demolding. Afterwards, the samples were demolded and cured in water at 20°C for 27 days. Compressive strengths of 100 mm cubical samples were measured periodically. The strength of control specimens are listed in Table 2. Whereas the average compressive strength of Series I is 21.1 Mpa. An RSM analysis has been developed later for this research to predict the concrete compressive strength precisely.

Table 1: Recipe for preparing *E. coli* culture

Media	Tryptone (gm/l)	Yeast extract (gm/l)	NaCl (gm/l)	Incubation period (hr.)	Incubation temperature (°C)	Optical density (OD_{600})
LB	10	05	10	24	37	0.5±0.1

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

(Series-I)	WB	Cement (kgm^{-3})	Sand (kgm^{-3})	CA (kgm^{-3})	Compressive strength (MPa)	Average Compressive strength (MPa)
Sample-1	0.592	321	823	1088	21	21.1
Sample-2	0.592	321	823	1088	20.9	
Sample-3	0.592	321	823	1088	21.4	

3.2 Slump Test

The slump cone was filled with three equivalent layers of concrete. Each layer was prepared with 33% of total concrete volume and rodded by a round ended tamping rod. Each layer got 25 strokes in a uniform way. After finishing the last layer, the top point of the slump cone was identified and leveled by removing excess cement on the side of the equipment. The hooks were eliminated from the slump cone and the cone was clamped with the attached handles to prevent the concrete from leaking from the base. The slump cone was raised in a single, continuous vertical movement with no horizontal or torsional movement after the base was freed from the block, the task was finished in five seconds. The highest point of the slump concrete was then calculated and compared to the underlying height of the 300 mm significant slump cone. The measured concrete slump value was the difference between the base height and the height of the material after it had slumped. The slump found was a true slump (Figure 3). When 100% culture was substituted for water, the slump value of the control concrete decreased. Previous researchers also made a similar observation (Sekhar et al., 2017).

Figure 3 E. coli enriched Luria-Bertani Media

Figure 4: Slump test of concrete (True slump)

4.0 TESTS CONDUCTED

4.1 Mechanical Strength Test

For doing the compressive and split tensile strength test of concrete 100 mm cubical specimens were prepared. The dried specimens were directly placed inside the testing machine under the circularly situated bearing block for compressive strength test result. But an extraordinary steel mold (350×250×264 mm) was used to hold the specimens for split tensile strength test. Afterwards the bearing block was brought down to the outside of the test sample (or mold) and a constant incremental load was applied. The calibration equation for load calculation can be found in Equation 1.

$$Q = 5.928\,P - 16.70 \quad (1)$$

P = Dial reading; Q = Applied load (kN)

Figure 4 and Figure 5 shows the specimens during testing for Compressive strength and Tensile strength test.

4.2 Ultrasonic Pulse Velocity (UPV) Analysis

100×200 mm cylindrical concrete specimens were prepared for ultrasonic pulse velocity analysis to assess the material homogeneity. Longitudinal, transverse, and surface waves are all part of a complicated system of stressed waves. The commencement of the longitudinal waves, which are the fastest to arrive, is detected by the receiving transducer. By using propagation varieties of ultrasonic speed wave, it is feasible to monitor the compactness or compare heterogeneous areas in the concrete. A pulse transmitter was placed at one side of each cylindrical sample. A receiver which was placed on the other side of the sample received the ultrasonic speed wave and eventually pulse velocity could be calculated. Table 3 summarizes the chart to classify concrete group based on UPV analysis. Figure 6 represents the concrete cylinders developed by using *E. coli* bio-agent for UPV testing and Figure 7 represents UPV machine while using to assess the pulse velocity of the cylindrical specimens developed for this study.

Figure 4: Compressive Strength test

Figure 5: Split Tensile Strength Test

Figure 6: Specimens prepared for UPV testing

Figure 7: UPV Test of cylinder specimen

Table 3: Categorization of concrete group based on UPV values (BS 1881: Part 203 [32])

Pulse velocity (ms^{-1})	> 4,500	3,500-4,500	3,000-3,500	2,000-3,000	<2000
Concrete Group	Excellent	Good	Medium	Poor	Very Poor

Table-3 represents the categorization of concrete specimen as per the British Standard (BS 1881: Part 203 [32]). General principle for pulse velocity to propagate through the concrete acts similarly both on the conventional and E. coli enriched concrete specimen because the the concentration of bacterial culture has little to no impact on the homogeneity on the freshly mixed concrete.

4.3 Scanning Electron Microscope (SEM) Analysis

To investigate the micro-structural changes of E. coli induced concrete scanning electron microscope analysis was done. Powdered samples of cement mortar were collected from the core of the cubes. Afterwards the samples were examined at various magnification levels to contemplate the microstructure of each substantial mixture at 28 days.

Summary of self-healing concrete specimens are shown in Table 4.

Table 4: Self-healing concrete specimens

Series	W/B	Dimension (mm)	*E. coli* culture		Water	
			kgm^{-3}by mass	% by mass	kgm^{-3} by mass	% by mass
II	0.592	100×100×100	122	100	0	0
III	0.592	Ø100×200	122	100	0	0
IV	0.5	Powder	122	100	0	0

5.0 RESULTS AND DISCUSSION

Results are critically analyzed and summarized in graphical form. Figure 8 to Figure 12 show compressive, split tensile and UPV test results of conventional and E. coli induced concrete. It is evident that use of E. coli in concrete had strong influence on increasing mechanical strength and compacity of concrete. Table 5 summarizes the compressive, split tensile and UPV test result for the curing period of 28 days.

Table 5: Compressive, split tensile and UPV test result (28 days)

Series	*E. coli* culture		Water		Compressive strength (MPa)	Split Tensile Strength (MPa)	Pulse velocity (m/s)
	kgm^{-3} by mass	% by mass	kgm^{-3}by mass	% by mass			
I	0	0	190	100	21.1	2.2	3230
II	122	100	0	0	23.1	2.7	
III	122	100	0	0			3420

The compressive strengths of 20 MPa conventional 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 on the other hand the corresponding values of E. coli induced concrete were 15.1, 18.6, 23.1, 25.1, 27.2 and 29.5 MPa. Strength gain can easily be observed from the relative strength data. Figure 8 shows the gain in compressive strength for different curing periods. The Strength gain for 20 MPa concrete made with E. coli strain were 4.9%, 14.8%, 9.5%, 8.7%, 9.2% and 12.6% for respective curing days. In Figure 9, relative strength gain of E. coli induced concrete compared to traditional concrete has been showed.

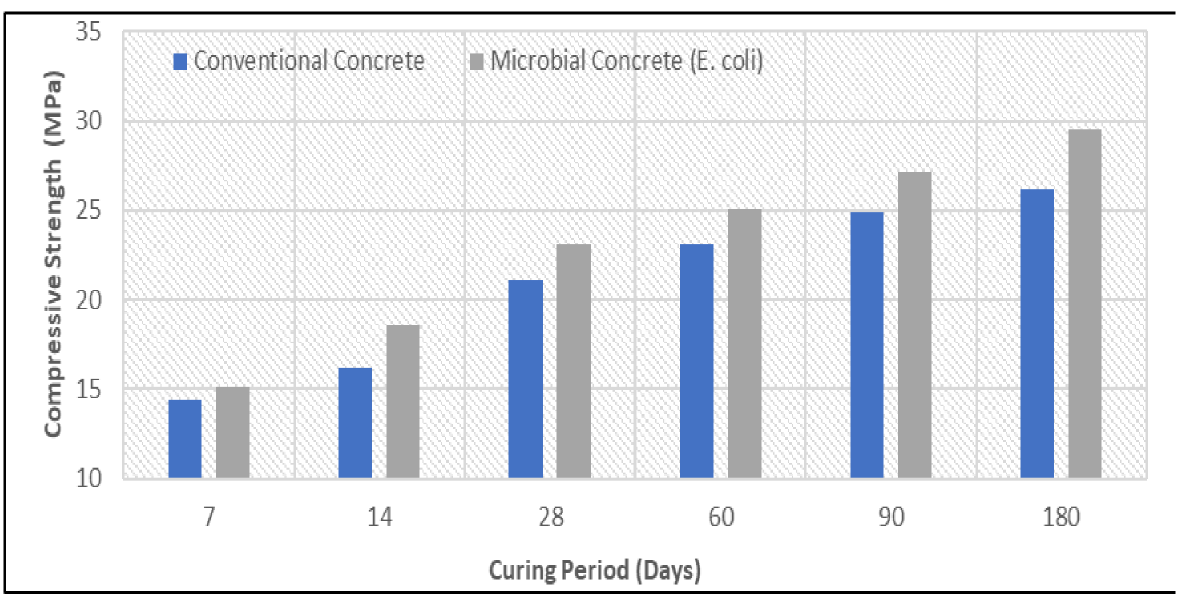

Figure 8: Comparison of Compressive strength in between *E. coli* culture induced and Traditional Samples

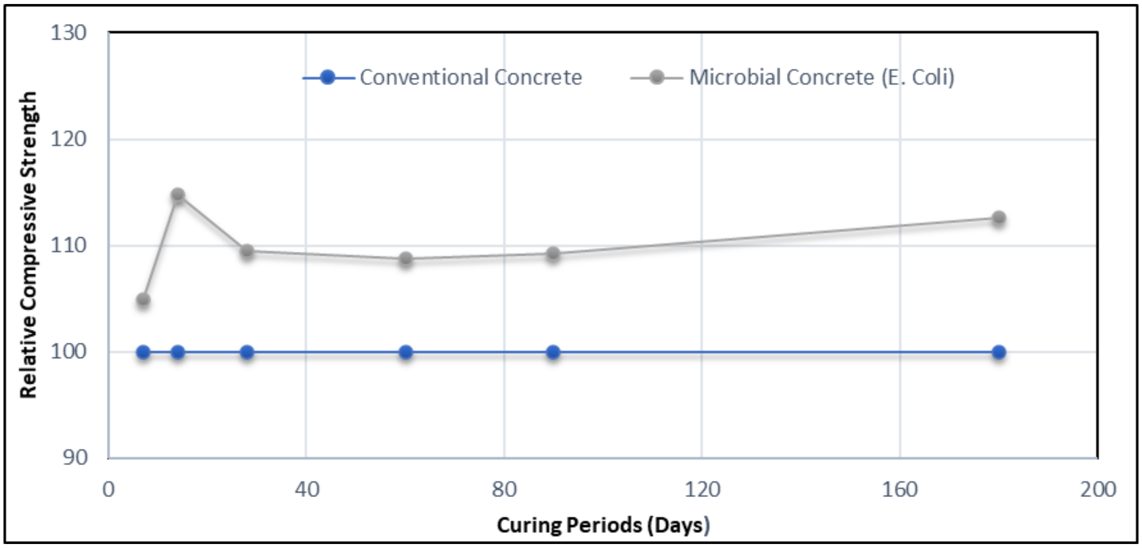

Figure 9: Relative gain in compressive strength of 20 MPa concrete made by using *E. coli* culture compared to Conventional Concrete

Significant proportion of increment in split tensile strength was also observed due to addition of *E. coli* bacteria as micro agent in concrete. Figure 10 shows the split tensile strength of concrete for different curing periods. Split tensile strength of 20 MPa plain concrete were recorded as 1.3, 1.5, 2.2, 3.4, 3.9 and 4.3 MPa at 7, 14, 28, 60, 90 and 180 days of curing. The split tensile strength of *E. coli* induced concrete for corresponding curing days were found as 1.7, 2.2, 2.7, 3.7,4.0 and 4.9 MPa. The gain in strengths were due to the usage Escherichia strain in concrete.

Results of pulse velocity analysis of concrete specimens with and without bacterial culture has been showed in Figure 11. From the graph it can be easily seen that for E. coli induced concrete the pulse velocity was about 200 ms^1 to 400 ms^{-1} higher than the traditional concrete. In this regard it was observed that for higher Compressive Strength the UPV result were also higher. So, an attempt was taken to investigate the relation between Compressive Strength and UPV test.

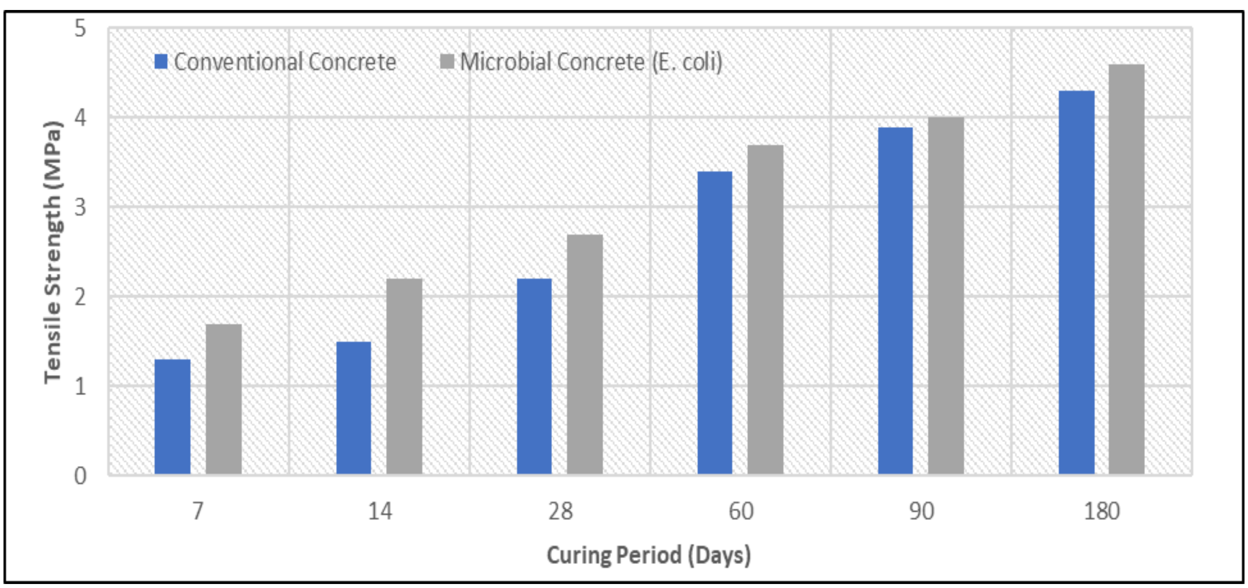

Figure 10: Comparison of Split tensile strength in between *E. coli* culture induced and Traditional Samples

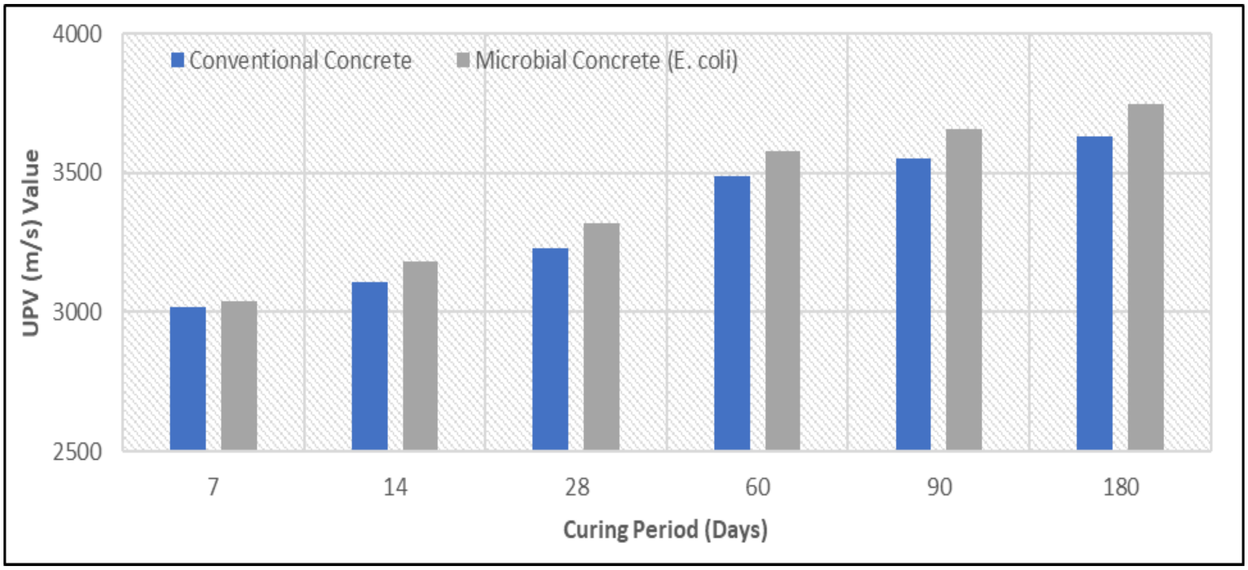

Figure 11: Comparison of Ultrasonic pulse velocities in between *E. coli* culture induced and Traditional Samples

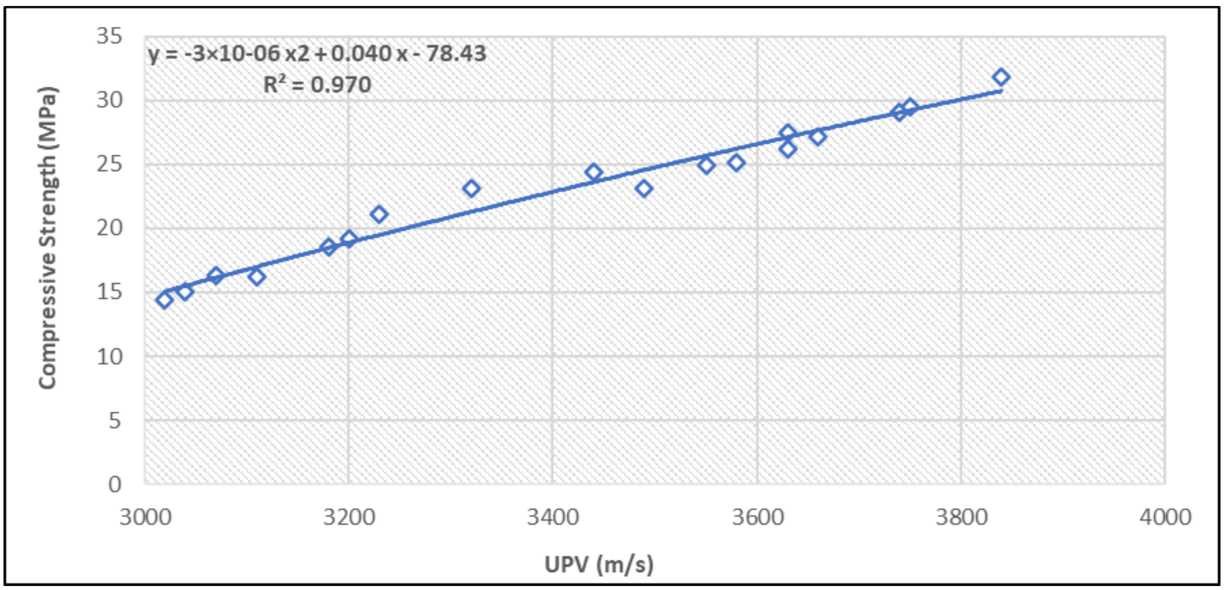

Figure 12: Correlation between 20 MPa concrete and UPV

From the above figure 12 a polynomial relationship can be established between 20 MPa concrete and pulse velocity (ms^{-1}). The correlation formula of the simulation curve can be established through Equation 2.

R = -3×10-06 x2 + 0.040 S - 78.43 (2)
S= Curing period (days); R= UPV Value (ms^{-1})

An adequate correlation between compressive strength and UPV data is indicated by the regression value. It means that 97% of the data from the regression model correspond to the actual observations.

To explore the microstructure of distinct mortar group, SEM imaging analysis were carried out for 28 days and 180 days curing period sample. Figure 13 and Figure 14 show SEM imaging test results of control and microbial mortar group for 28 days curing period. It can be easily visualized that inclusion of E. coli bacteria had a positive effect in improving microstructure. CSH gel was well dispersed and well compacted in *E. coli* induced sample. Figure 15 and Figure 16 show that curing of E. coli enriched concrete for 180 days results in comparatively denser microstructure than the controlled concrete. For 180 days curing period from the SEM image (Figure 16) it can be seen that The CSH gel is more dispersed than the previous 28 days curing sample. This was mainly due to the $CaCO_3$ precipitation by E. coli bacteria for a comparatively longer time in mortar.

The SEM imaging completely indicated the ability of such *E. coli* induced concrete in developing highly impactful self-healing concrete.

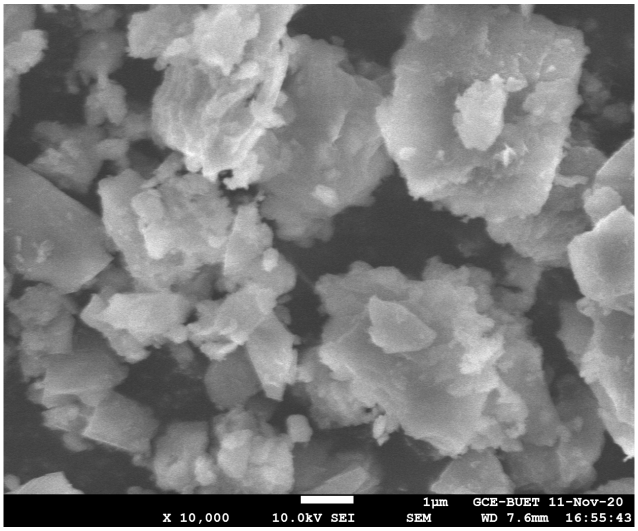

Figure 13: SEM imaging of control sample at 28 days

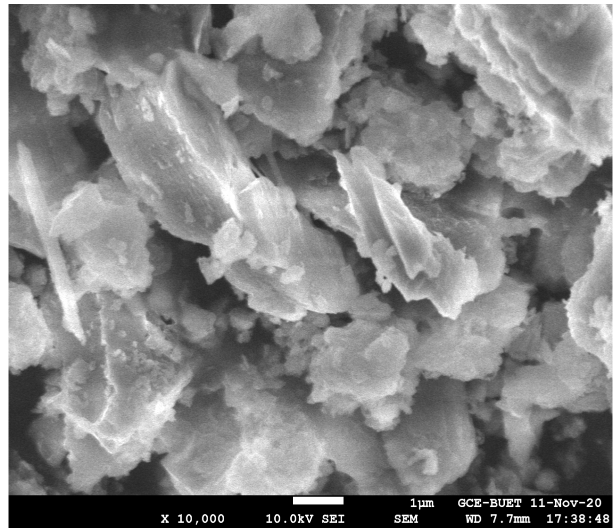

Figure 14: SEM imaging of *E. coli* induced sample at 28 days

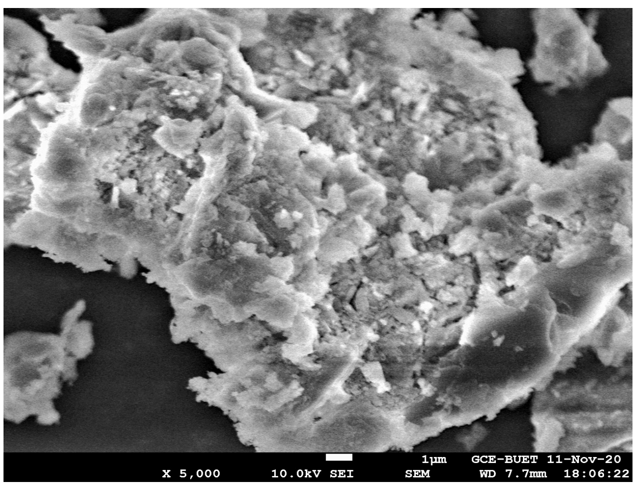

Figure 15: SEM imaging of control sample at 180 days

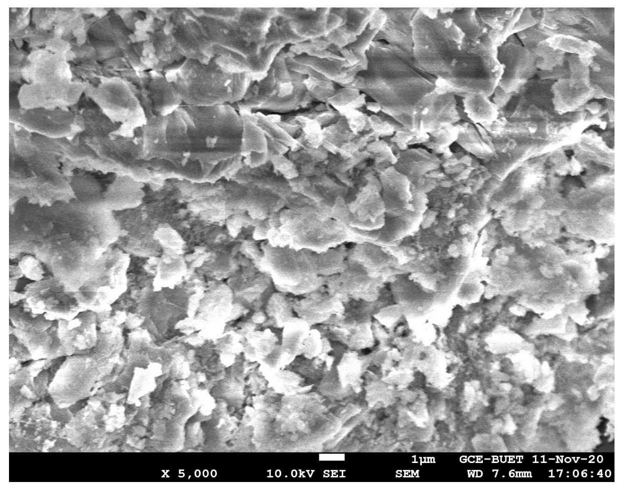

Figure 16: SEM imaging of E. coli induced sample at 180 days

6.0 RSM MODEL FOR COMPRESSIVE STRENGTH

Of all the concrete groups, concrete containing E. coli was discovered to be the most efficient for all curing ages. As a result, the Response Surface Methodology (RSM) was used to forecast the concrete compressive strength (CS) of E. coli enriched specimens. Curing period (CP) and pulse velocity were included as independent variables in the analysis (UPV). Since the model was created to predict the compressive strength, all curing periods and pulse velocities data could be collected without the need for any destructive testing. The design stage allowed for the selection of the bio-curing concrete's ages, and non-destructive tests were used to determine pulse values. Table 6 provides a summary of the RSM analysis. The model's R2 and adjusted R2 scores 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,

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

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				
R^2	96.26%				
R^2 adj	93.77%				

7.0 CONCLUSIONS

Use of *E. coli* bacteria in concrete has a significant impact in improving concrete properties. Water was completely replaced by E. coli culture in this form of study. An optimum optical density OD_{600}=0.5±0.1 was maintained here. Significant improvements in mechanical strength and microstructure were observed. Followings are the major findings of this research study:

(1) Compressive strength increased up to 9.5% and 12.6% respectively after 28 and 180 days of curing period.
(2) Respective increments in split tensile strengths were 22.7% and 6.98%.
(3) Improvements in pulse velocities for E. coli induced concrete were also observed and the values lied in between the range 3040-3750 ms^{-1}.
(4) Finally, SEM analysis tests were also done to confirm the effect the E. coli bacteria in concrete. It can easily be visualized the significant micro-structural improvement and densification of the cementitious matrices after addition of E. 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.

List of Abbreviations:

OD_{600}: Optical density at 600 nm wavelength
MICP: Microbiologically Induced Calcite Precipitation
UPV: Ultrasonic Pulse Velocity
SEM: Scanning Electron Microscope

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