

The Utilization of *Bacillus Subtilis* Bacteria to Improve the Mechanical Properties of Concrete

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ABSTRACT The utilization of concrete as a building material is well-known worldwide and increasing continuously due to its sustainability, low maintenance cost, durability, performance, etc. The ingredients of concrete, its constructional methodology, and exposure conditions have been observed to be moderating and improving daily but the focus of this research is on the laboratory investigation of Bacterial Concrete which is the technology established on the application of the mineral producing microbes like *Bacillus subtilis* which have the properties of bio-calcification and the ability to precipitate CaCO₃ effectively inside concrete structures. This CaCO₃ precipitation is able to fill the pores and cracks internally and this subsequently makes the structure to become more compact. Nutrient Broth (NB) media was employed for the growth and spore formation of *Bacillus subtilis* bacteria in this experimental study and four different bacterial culture densities including 0.107, 0.2, 0.637, and 1.221 were estimated at OD₆₀₀ and directly added to the concrete matrix using the previously fixed water to culture ratio of 0.5:0.5. Moreover, 100 mm cubical concrete specimens were cast, subjected to compressive and tensile strength tests for different curing ages, and finally compared with Conventional Concrete with OD₆₀₀=0. A significant increase was observed in the mechanical strengths due to the addition of *Bacillus subtilis* bacteria in concretes with a culture density of 0.637. Furthermore, cylindrical concrete specimens with 100 mm diameter and 200 mm height were prepared for Ultrasonic Pulse Velocity (UPV) analysis and the results showed specimens prepared with culture density of 0.637 have higher pulse velocity than other microbial groups. A UPV vs. compressive strength relationship curve was, however, later proposed for different strengths of concrete.

KEYWORDS Bacterial Concrete; MICP Technique; *Bacillus subtilis*; Nutrient Broth Media; Optical Density.

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1 INTRODUCTION

Concrete is a structure material essentially utilized in every aspect of structural buildings. It is important due to the preference for durable and high-performance structures in the construction industry, thereby, leading to numerous attempts in the past decades to ensure higher durability of structures. Several researchers desire to find supplementary cementing materials (SCMs) to partially replace the cement content and at the same time boost its properties. Therefore, this investigation was exclusively focused on traditional “cement-based concrete.”

The generation of cracks in concrete is not a brand-new topic but the provision of the appropriate strategies to remedy the condition has always been a subject of great concern. This is, however, due to the frequent influence of cracks on the functionality of structures by

serving as the pathways for moisture to enter which causes the corrosion of embedded rebar inside the concrete and, ultimately, a decrease in strength and durability and eventually the decay of the structure (Schlangen et al., 2009).

Conventional repair techniques are mostly faced with issues related to the inability to access the locations of structures in need of maintenance. The repair of cracks through manual checks has also been observed to be laborious and economically demanding and this led to the possibility of finding a “self-healing material” with one of the most studied concepts being the “biogenic self-healing concrete.” This technique focuses on increasing sustainability and diminishing the complete repairing expense of concrete structures and it has been shown by experimental studies to have the ability of sealing near completely crack widths up to 0.46

mm (Qian et al., 2019; Wiktor et al., 2011). A healing agent i.e. bacteria with suitable organic nutrients is usually incorporated inside the concrete to precipitate calcium carbonate when it comes in contact with water and eventually solidifies the cracks (Anderson et al., 1992). This bio-mineralization process is called Microbiologically Induced Calcite Precipitation (MICP) and this self-healing concrete technology is better than numerous ordinary advancements due to its eco-friendly nature and self-healing abilities (Elżbieta Stanaszek-Tomal, 2020).

The primary objective of this research was to assess the strength variation of several concrete groups with or without using self-healing agents after which a UPV analysis was conducted to identify the denser group. This laboratory investigation attempted to relate the compressive strength with culture density and also to discover the ideal bacterial concentration in concrete.

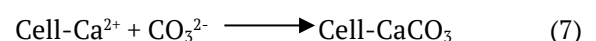
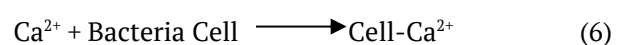
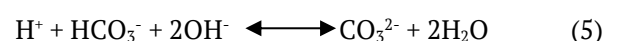
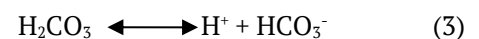
2 SURVIVAL OF BACTERIA IN CONCRETE

The exploration was initiated by determining the microbes with the ability to stay in an extraordinary alkaline condition. This is necessary because cement and water generally fuse at a pH estimation of more than 13 (Behnood et al., 2016), and at the point of combination, an antagonistic situation is usually formed which normally leads to the passage of most microbes at pH estimation of 10 or above. Meanwhile, over 1,500 papers have been distributed on several perspectives on alkaliphiles and alkaliphilic since their discovery and were found to be extraordinary microbes with incredible potential for microbiology and biotechnological abuse. The most consideration was placed on the creation of extracellular enzymes and their genetic examination with the ability to establish a replacement relationship between the fields of civil engineering and microbiology. These bio-spores have the ability to stay intact for up to 200 years in concrete while anticipating a vastly improved condition to germinate and later get actuated during cracking as food becomes accessible and water saturates the structure (Elżbieta Stanaszek-Tomal, 2020).

The different types of metabolic activity prompting CaCO_3 precipitation are known as the typical bacterial metabolic pathways which increase the concentration of carbonate ion and an approach to accomplish MICP in concrete is through enzymatic hydrolysis of urea. This process involves the conversion of urea into ammonia and carbon-di-oxide and the ammonia increases the pH of the environment which finally leads to the combination and deposition of calcium and carbonates along the cell surface (Wang et al., 2016) as illustrated in Figure 1 (Castro-Alonso et al., 2019).

The healing process starts at the occurrence of cracking and this involves the entrance of water into the cracks to release nutrients embedded in the crack zone for the bacterial spores to germinate and recover the ureolytic activity. This urease-mediated process usually leads to the reaction of urea and water to produce CO_2 and ammonia.

Bio-mineralization is usually achieved by microbes on the cell surface already charged with anions with the divalent cations such as Ca^{2+} or Mg^{2+} secured at an unbiased pH to make them the ideal nucleation destinations for CaCO_3 deposition. Meanwhile, the calcium ions join more frequently than magnesium ions since they have more grounded ionic selectivity. The restricting cations, Ca^{2+} or Mg^{2+} , also respond with the anion, CO_3^{2-} , to frame insoluble calcium carbonate. Therefore, the bacterial cells have a significant influence on the MICP due to their effect on the encircled mineral despite being used as nucleation destinations. Equations 1-7 show the general response of enzymatic urea hydrolysis (De Belie, 2010).



Bacterial genus like “*Bacillus*” has the special ability to produce filler products with binding properties (Saifee et al., 2015; Elżbieta Stanaszek-Tomal, 2020). Some species are also able to make urease enzymes in order to extract calcite precipitates to ensure the pores or micro-cracks are easily filled up and increase the density of the concrete matrix. The examples of the species applicable in bacterial concrete preparation are stated as follows.

- *Bacillus pasteurii*
- *Bacillus cohnii*
- *Bacillus subtilis*
- *Bacillus sphaericus*
- *Bacillus megaterium*
- *Bacillus pseudofirmus*
- *Bacillus balodurans*
- *Bacillus cereus*
- *Bacillus flexus*

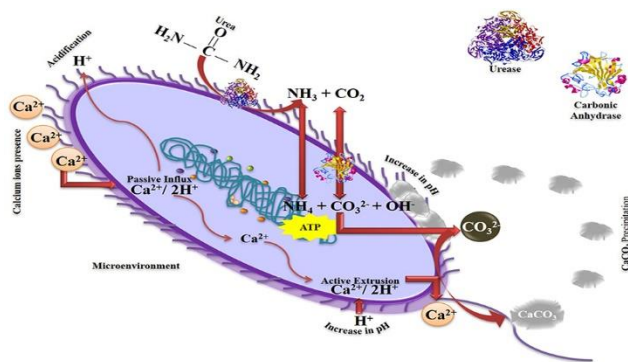


Figure 1. Ureolytic Carbonate Precipitation at the Microbial Cell Wall

3 MATERIALS AND METHODS

Different grades of concrete were prepared for different bacterial concentrations, subjected to mechanical and pulse velocity tests, and finally compared with the results of conventional concrete. The materials used in this laboratory investigation are discussed as follows.

The Ordinary Portland Cement (OPC) (CEM II) with strength class 42.5 N (EN 196:1) was used in casting the specimens and the initial and final setting times were 150-180 and 190-230 minutes respectively while the specific gravity was found to be 3.15 in line with the ASTM C188 guidelines. Moreover, locally available “Sylhet sand” was used as fine aggregate based on ASTM C778

standard and some of its physical properties are presented in Table 1.

Table 1. Physical properties of fine aggregate

Field moisture content (%)	1.12
Absorption capacity (%)	1.45
Bulk specific gravity (SSD)	2.55
Fineness modulus (FM)	2.57

The “crushed stones” were used as coarse aggregate based on ASTM C33 requirements with a maximum size of 19 mm and maximum nominal size of 12.5 mm and other properties summarized in Table 2.

Table 2. Physical properties of coarse aggregate

Field moisture content (%)	0.57
Absorption capacity (%)	0.60
Bulk specific gravity (SSD)	2.59
Dry rodded unit weight (Kgm ⁻³)	2.57

The *Bacillus subtilis* strain was utilized in the accompanying study and found to be a modeled lab bacterium with the ability to produce CaCO₃ as an extracellular product when a calcium-enriched media is present. Its optimum growth temperature is from 28°C to 30°C. Moreover, the Nutrient Broth (NB) media was first prepared at the Microbiology Department of the University of Chittagong according to the recommendations of APHA using the recipes summarized in Table 3 and later autoclaved. This was followed by the introduction of the *Bacillus subtilis* strain into the culture media and kept in a refrigerator for the germination of the spores. Furthermore, four distinctive optical densities (OD₆₀₀) were utilized to examine the exhibitions and ideal cell convergence of microorganisms based on the direct proportionality of the optical density to the cell number i.e. the concentration of the media (Stevenson et al., 2016). A spectrophotometer was finally used to estimate the absorbance of the cell suspension. However, the bacterial sample prepared is shown in Figure 2.

Table 3. Recipe for NB media

Beef Extract	5 gm/liter
Peptone	3 gm/liter
NaCl	Slight amount

The bacterial culture containing spores and calcium lactate was “directly” applied to the concrete mix with the water to culture ratio maintained at 0.5:0.5 (Manikandan et al., 2015). Concrete Mix 1 represents the control specimens while Mixes 2, 3, 4, and 5 indicate the bacterial concrete groups, and the optical densities of the groups at 600 nm wavelength were found to be 0.107, 0.2, 0.637, and 1.221. Table 4 also shows the cell concentration of *B. subtilis* was higher at Mix 5 than other bacterial groups.

Table 4. Culture density (OD_{600}) of different concrete mix

Mix 1 (M1)	0
Mix 2 (M2)	0.107
Mix 3 (M3)	0.2
Mix 4 (M4)	0.637
Mix 5 (M5)	1.221

The portable water used in mixing concrete and curing the specimens was in line with the ASTM C1602 guidelines such that its pH value is not less than 6.



Figure 2. Cultured Bacterial Sample

4 VARIABLES

The performances of the bacterial concrete were evaluated through the selection of three arbitrary design strengths which are 20 MPa, 30 MPa, and 40 MPa while five curing periods, 7, 14, 28, 60, and 120 days, were periodically selected to test the specimens in plain water.

The concrete specimens were prepared according to ASTM C92 guideline using 100 mm cube to evaluate the mechanical strength of the concrete after which cylindrical specimens with 100 mm diameter and 200 mm height were cast for UPV analysis. All the specimens were cast in the Engineering Materials Laboratory of CUET and stored at room temperature for 24 hours after which they were removed from the mold and exposed to different curing periods in plain water.

The concrete mix design was produced in line with the ACI 211 standard procedure. Moreover, the ratio for 20 MPa design strength on the 28th day was derived to be 1.0:2.57:2.71 for a water-cement ratio of 0.592 by mass, conventional concrete, and water-cement ratio of 0.296. The bacterial culture was 0.296 for bacterial concrete by mass and this means 50% of water and 50% of a bacterial culture by mass were used in preparing bacterial concrete instead of 100% water (Manikandan et al., 2015). Furthermore, 30 MPa and 40 MPa concrete were also prepared to monitor the mechanical strengths of bacterial concrete. The summary of the mix designs is presented in Table 5.

Table 5. Summary of Mix Design

Design Strength	Cement	Fine Aggregate	Coarse Aggregate	Conventional Concrete	Bacterial Concrete	
				Water to Cement Ratio	Water to Cement Ratio	Culture to Cement Ratio
20 MPa	1.0	2.57	2.71	0.592	0.296	0.296
30 MPa	1.0	1.68	2.04	0.446	0.223	0.223
40 MPa	1.0	1.28	1.73	0.380	0.190	0.190

5 RESULTS AND DISCUSSION

The compressive strength was tested according to ASTM C39 guidelines and the results are summarized in both graphical and tabular form

with the graphical representations presented in Figures 3-7.

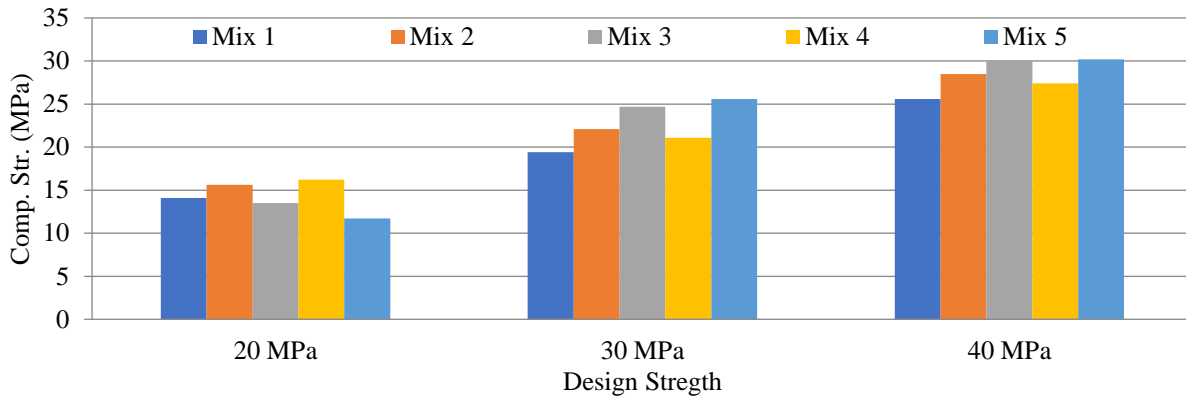


Figure 3. Compressive strength vs. Optical density (7 days)

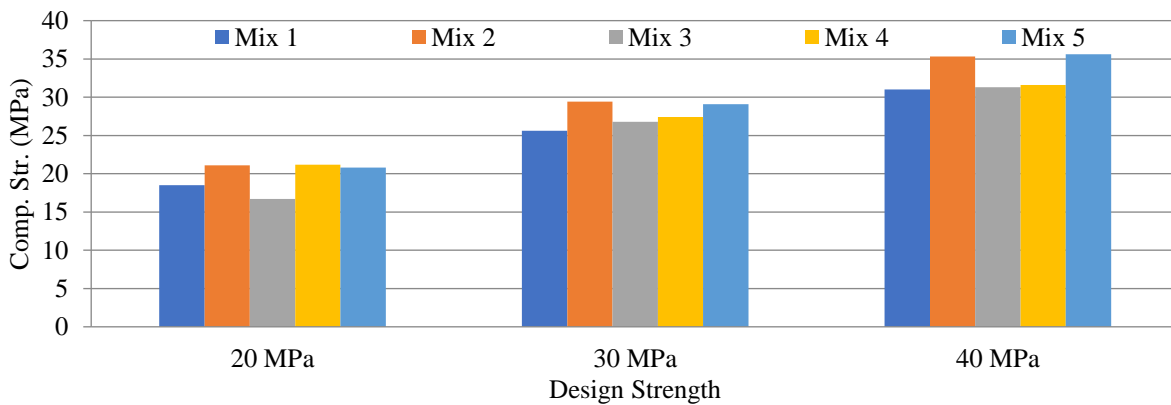


Figure 4. Compressive strength vs. Optical density (14 days)

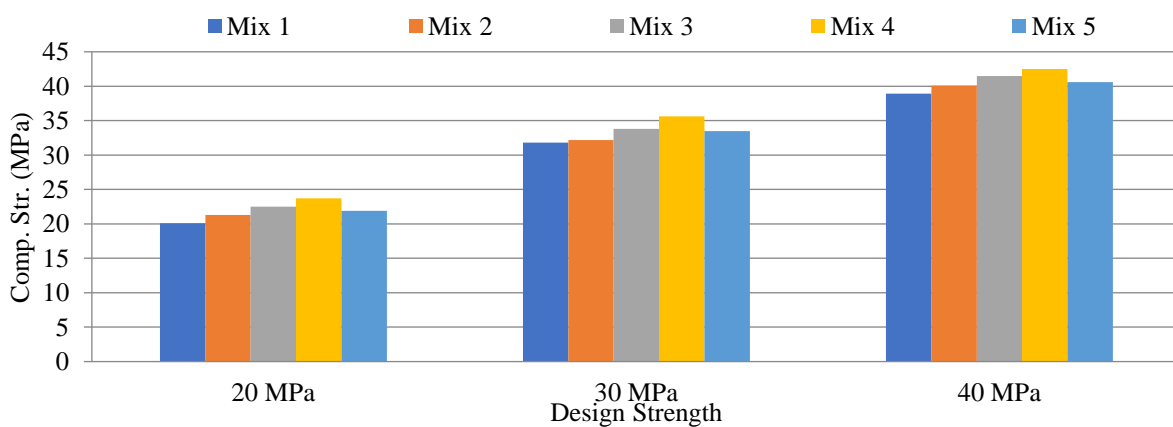


Figure 5. Compressive strength vs. Optical density (28 days)

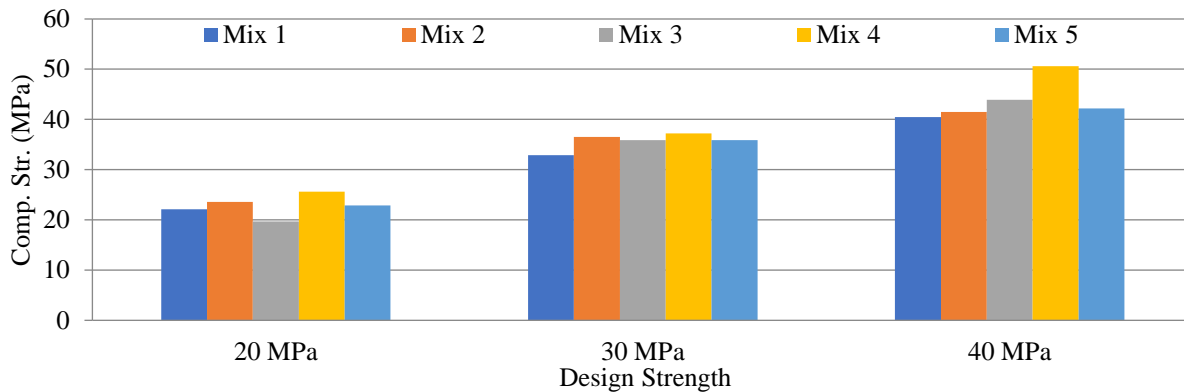


Figure 6. Compressive strength vs. Optical density (60 days)

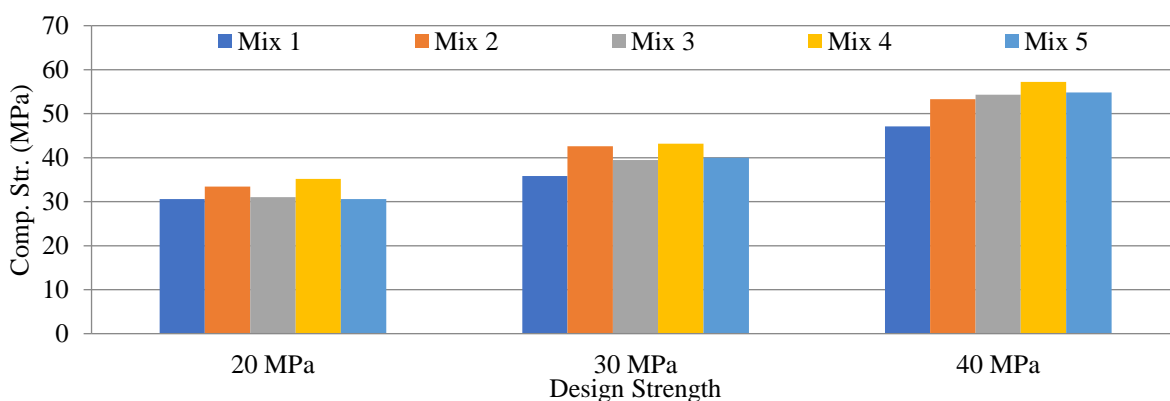


Figure 7. Compressive strength vs. Optical density (120 days)

Figures 3-7 clearly demonstrate the variation in compressive strengths with optical densities for different curing periods. The compressive strengths of the control specimen or Mix 1 at the age of 28 days were found to be 20.1, 31.8, and 38.9 MPa respectively for the designed strengths. The increment in the values was observed for all bacterial groups due to the addition of *Bacillus subtilis* bacteria with the maximum recorded in Mix 4 as 23.7, 35.6, and 42.5 MPa respectively.

The compressive strengths of Mix 1 at the age of 120 days were discovered to be 30.6, 35.8, and 47.1 MPa, and Mix 4 was found to be more effective than the others after the specified curing period with the values recorded to be 35.2, 43.2, and 57.2 MPa respectively for the designed strengths.

The increase in strengths at the 28th day for different culture densities is presented in Table 6 and Mix 4 with $OD_{600}=0.637$ is clearly observed to

show a better increase for all the designed strengths. This is probably associated with the development of more noteworthy mineral depositions in the internal structures of concrete (Ehrlich, 1999). Similar observations were also found by researchers including Niveditha (Niveditha et al., 2016) and Srinivasa Reddy (Reddy et al., 2012).

Table 6. Increased in Compressive Strength (28 days)

Bacterial Concrete	20.1 MPa	31.8 MPa	38.9 MPa
Mix 2	5.97%	1.26%	3.10%
Mix 3	11.94%	6.29%	6.68%
Mix 4	17.91%	11.95%	9.25%
Mix 5	8.96%	5.95%	4.37%

Plain and bacterial concretes containing different optical densities were used in the split tensile strength test which was conducted with

reference to the ASTM C496 guidelines. It is, however, important to note that Popovivis proposed a curvilinear relationship between the compressive and split tensile strengths using the following formula.

$$f_{sp} = C f_{co}^n \tag{8}$$

The *C* varies from 0.34 to 0.88 while *n* is between 0.71 and 0.74. The relationships between the

split tensile strengths and optical densities for different curing days are, however, presented in Figures 8-12.

The split tensile strengths of Mix 1 at 28 days were 3.2, 3.5, and 3.8 MPa respectively for the designed strengths and the maximum increase was observed in Mix 4 with 3.5, 3.9, and 4.2 MPa respectively.

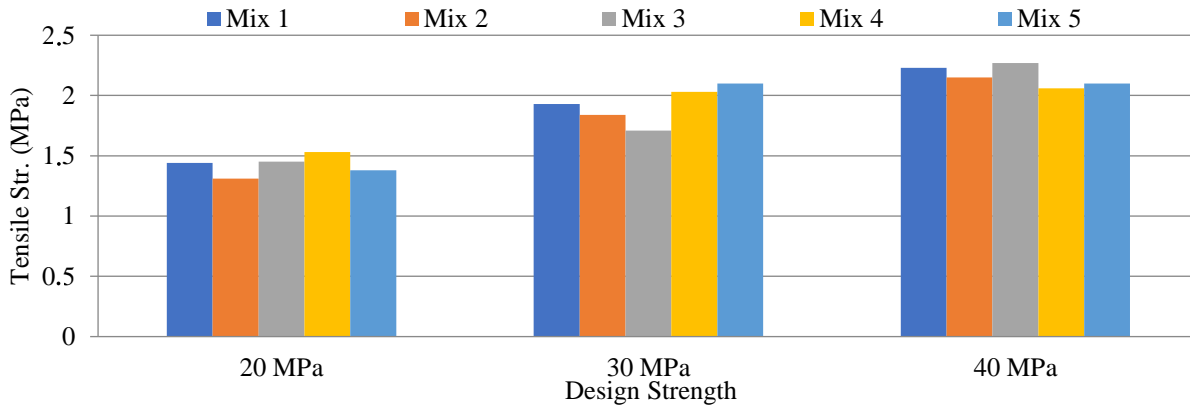


Figure 8. Tensile strength vs. Optical density (7 days)

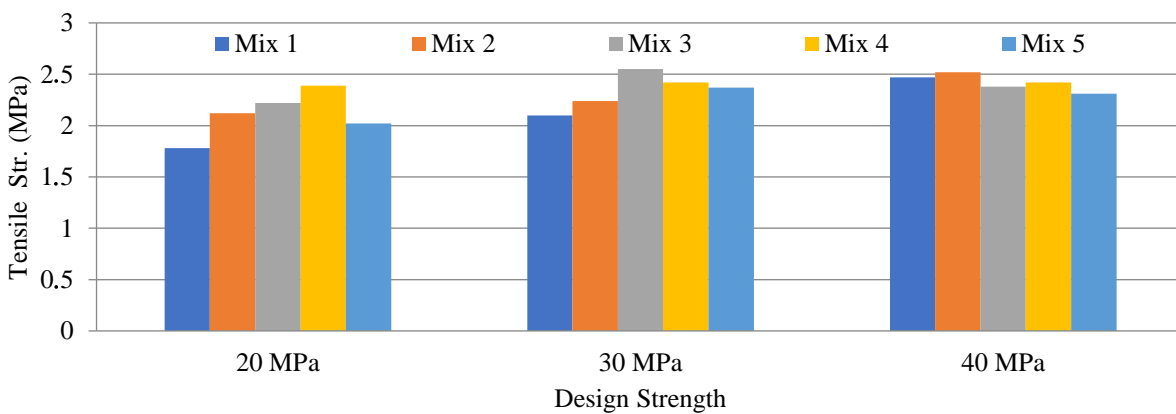


Figure 9. Tensile strength vs. Optical density (14 days)

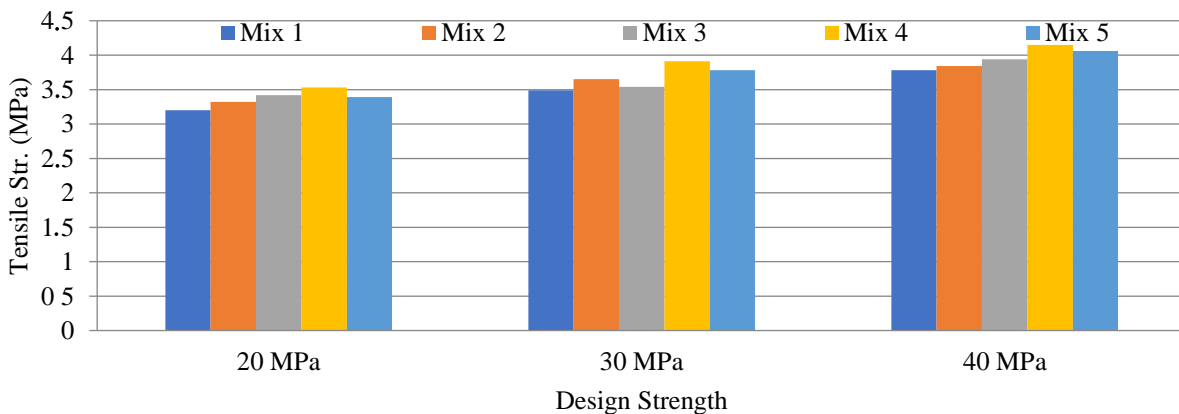


Figure 10. Tensile strength vs. Optical density (28 days)

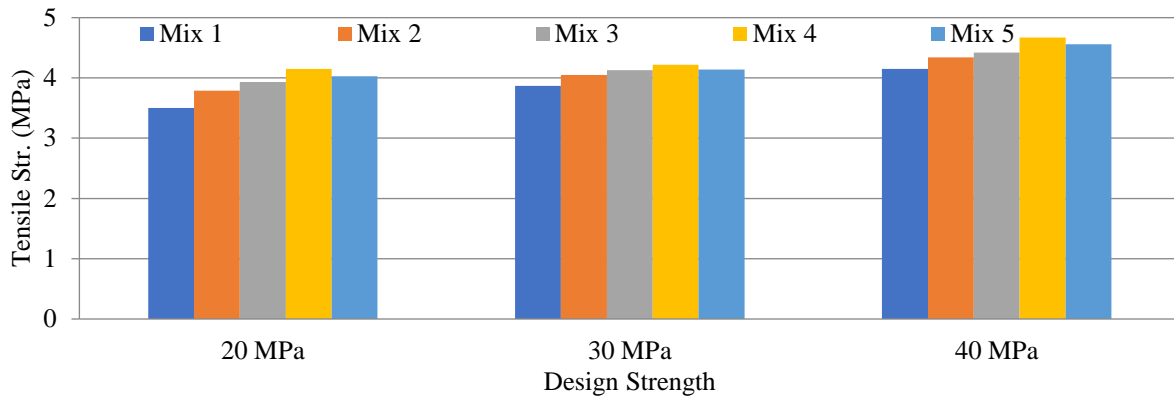


Figure 11. Tensile strength vs. Optical density (60 days)

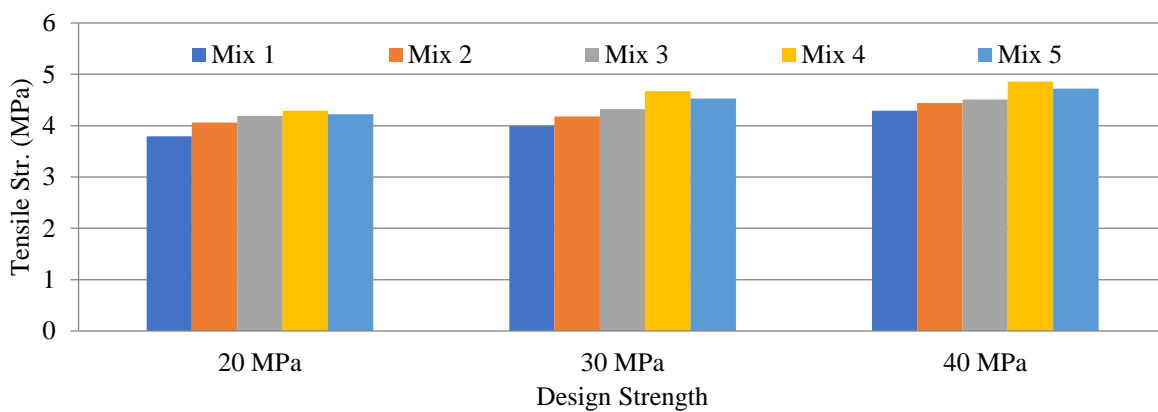


Figure 12. Tensile strength vs. Optical density (120 days)

Significant improvements between 13-18% were also observed in split tensile strength for Mix 4 concrete at 120 days of curing for all grades. The comparative perceptions were additionally found by Niveditha, et al. (2016). Meanwhile, ASTM C597 briefly describes the procedure of UPV analysis as an in-situ, non-destructive test which decides the speed of longitudinal waves through the concrete. It is established on the idea that a pulse wave travels a specific distance through the concrete specimen and the speed of travel is estimated to check the quality of the concrete such that a higher value indicates the presence of lesser voids. This, therefore, means the concrete specimens with higher pulse velocity are denser (Saha et al., 2020).

The pulse velocities of the conventional concrete, Mix 1, were recorded to be 2700, 2790, and 2870 m/s for 20, 30, and 40 MPa respectively while higher speed values were observed for all the bacterial groups. Siva Ram Prasad (Prasad et al., 2020) also found similar observations while determining the quality of concrete. Meanwhile, Mix 4 with $OD_{600}=0.637$ was found to be more effective in improving pulse velocities among all the bacterial groups with the values recorded to be 2890, 2980, and 3120 m/s respectively and this means they are denser than other bacterial groups. The same pattern was also observed with compressive and tensile strengths. The UPV values for different concrete strengths are, however, presented in Figure 13.

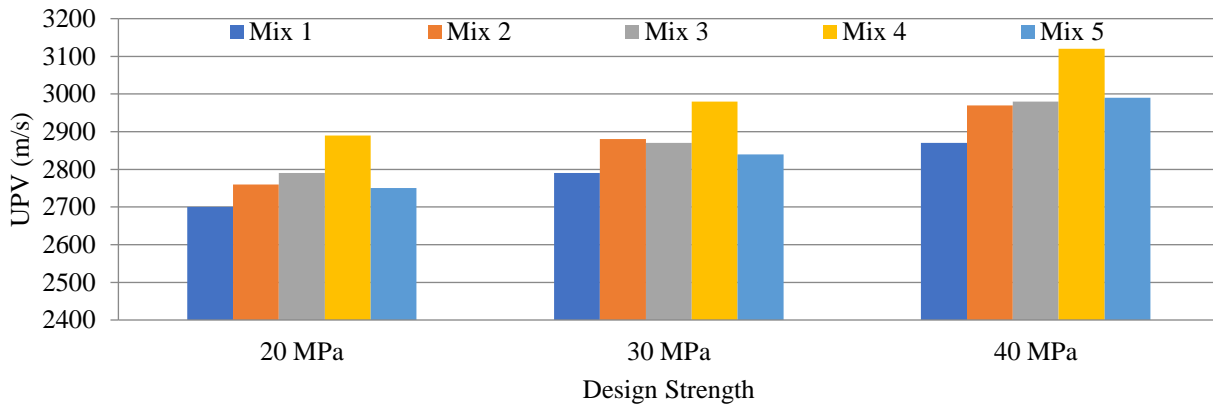


Figure 13. UPV Analysis

Several scientists have tried to determine the correlations between compressive strength and UPV and a non-linear model using the following equation was suggested by Tharmaratnam.

$$F_c = ae^{bV} \quad (9)$$

F_c = Compressive strength (MPa), V = Pulse velocity (kms^{-1}), and a, b = Empirical constants

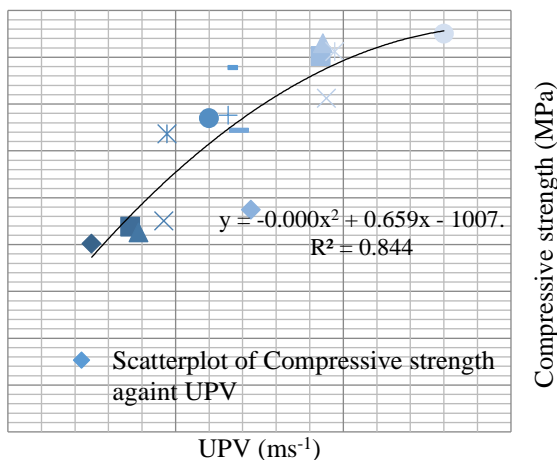


Figure 14. Correlation between compressive strength against UPV

The pulse velocities were plotted against the compressive strengths and a law relating the two variables is developed in Figure 14 which shows a commendable polynomial association between UPV and compressive strength. Moreover, the R^2 was 0.844 and this means 84.4% of the assortment in the assessments of compressive quality was responded to by the polynomial relationship with UPV.

6 CONCLUSION

This experimental investigation focused on the utilization of *Bacillus subtilis* bacteria in concrete and the development of the ideal bacterial culture density in concrete. The results are summarized as follows based on the predetermined number of factors.

- The addition of *Bacillus subtilis* bacteria in concrete significantly increased the compressive strength of the concrete in comparison with the control specimen or Mix 1. Therefore, the optimum culture density was found to be 0.637 at Mix 4 and this was based on its ability to increase the compressive strength by approximately 10-18% at 28 days of curing.
- The maximum effect of up to 12% increment on split tensile strength was also found with the culture density of 0.637 which is at Mix 4.
- The ultrasonic pulse velocities were also improved by 6-9% with the use of $OD_{600}=0.637$ culture density.
- It was observed that the compressive strength increased more with 17.91% for 20 MPa at lower grades of concrete while the gain became relatively significant at later ages of curing.
- Figure 14 shows 84.4% of the variety in the estimations of compressive strength was represented by the polynomial relationship with UPV.

- The specimen with a culture density of 0.637 was discovered to be the best among all bacterial densities to increase the mechanical properties of the concrete. This means an optimum optical density of 0.5 ± 0.1 can be generally selected for further culture preparation and investigations.

DISCLAIMER

The authors declare no conflict of interest.

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