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Assessment on Strength Characteristics of Microbial Concrete by using Bacillus Subtilis as Self-healing Agent: A Critical Review

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Abstract: Concrete is one of the most widely used construction materials by mankind and it is the main material used for the infrastructure development of every country. Microbial concrete, as the name suggest is an improvisation provided to cement using living microbes which are capable of doing so. Using microbes such as Bacillus subtilis which has the properties of bio-calcification can secrete calcium carbonate as an extra cellular product. Thus, filling the pores and the cracks internally, it makes the structure more compact and resistive to seepage. In this experimental investigation, the performance of microbial concrete exposed to plain water. Concrete specimens of 100 mm cubical size were cast and cured for 7, 14, 28, 60, 90 days in plain water with and without using bacterial water. Using a spectrophotometer to measure the optical density at 600 nm (OD_{600}) of a bacterial culture to monitor bacterial growth has always been a central technique in microbiology. Concrete specimens having OD_{600} 0.107, 0.20, 0.637 and 1.221 have been studied in plain water. The specimens were taken out periodically and subjected to compressive & tensile strength tests. From the investigation, it has been revealed that microbial concrete having OD_{600} 0.637 shows better resistance against strength deterioration under all curing conditions and curing ages. Concrete specimens were also subjected to Ultrasonic Pulse Velocity (UPV) test. From this test, it has been observed that specimens having OD_{600} 0.637 shows better velocity. The higher pulse velocity can therefore be used to assess the quality and uniformity of the material

Keywords: OPC, microbial concrete, bacillus subtilis, pptical density, MICP

1. Introduction

Concrete will continue to be the most important building material for infrastructure but most concrete structures are prone to cracking. Tiny cracks on the surface of the concrete make the whole structure vulnerable because water seeps in to degrade the concrete and corrode the steel reinforcement, greatly reducing the lifespan of a structure. Structures built in a high-water environment, such as underground basements and marine structures, are particularly vulnerable to corrosion of steel reinforcement. Motorway bridges are also vulnerable because salts used to de-ice the roads penetrate into the cracks in the structures and can accelerate the corrosion of civil engineering structures. Tensile forces can lead to cracks and these can occur relatively soon after the structure is built. Repair of conventional concrete structures usually involves applying a concrete mortar which is bonded to the damaged surface. Sometimes, the mortar needs to be keyed into the existing structure with metal pins to ensure that it does not fall away. Repairs can be particularly time consuming

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and expensive because it is often very difficult to gain access to the structure to make repairs, especially if they are basements or at a great height.

An advance technique has been developed in last decades in remediating cracks and fissures automatically in concrete by utilizing Microbiologically Induced Calcite or Calcium Carbonate ($CaCO3$) Precipitation (MICP) which will ultimately increase the durability of concrete structure. Microbial concrete technology has been proved to be better than many conventional technologies because of its eco-friendly nature, self-healing abilities and increase in durability of various building materials. The main aim of the investigation is to evaluate the mechanical strength of concrete using various compositions and comparison with these with plain concrete. Later on, UPV tests are also done to identify the denser concrete group

2. Microbial Concrete

2.1 Reasons behind MICP Technique

Different mechanisms of bacterial involvement in calcification have been proposed and they have been a matter of controversy throughout the last century. It is generally accepted that this microbial activity can be influenced by environmental physical-chemical parameters, and it is correlated to both metabolic activities and cell surface structures. Metabolic activities of heterotrophic bacteria are considered, by some authors, to be the most relevant mechanisms in calcium carbonate precipitation. In general, metabolic pathways able to increase the environmental pH toward alkalinity can, in the presence of calcium ions, foster calcium carbonate precipitation. Bacterial surfaces also play an important role in calcium precipitation. Due to the presence of several negatively charged groups, at a neutral pH, positively charged metal ions could be bound on bacterial surfaces, favoring heterogeneous nucleation. Commonly, carbonate precipitates develop on the external surface of bacterial cells by successive stratification and bacteria can be embedded in growing carbonate crystals. However, the actual role played by bacteria in calcium mineralization is still debated.

2.2 Tables Types of Bacteria in Concrete

1. Bacillus pasteurii
2. Bacillus cohnii
3. Bacillus subtilis (Used in this study)
4. Escherichia coli
5. Bacillus sphaericus
6. Bacillus pseudifirmus
7. Bacillus balodurans

2.3 Survival of Bacteria in Concrete

The starting point of the research was to find bacteria capable of surviving in an extreme alkaline environment. Cement and water has a pH value of more than 13. When mixed together, usually a hostile environment is created. Most organism die in environment with a pH value of 10 or above. The search concentrated on microbes that thrive in alkaline environments which can be found in natural environments, such as alkali lakes in Russia, Carbonate rich soils in desert areas of Spain and soda lakes in Egypt. Samples of endolithic bacteria (Bacteria that can live inside stones) were collected along with bacteria found in sediments in the lakes. Strains of the bacteria genus Bacillus were found to thrive in this high alkaline environment. Different types of bacteria's are incorporated into a small blocks of concrete. Each concretes block would be lefts to two months to set hard.

Then the block would be pulverized and the remains tested to see whether the bacteria had survived. It was found that the only group of bacteria that were able to survive was the ones that produced spores comparable to plant seeds. Such spores have extremely thick cell walls that enable them to remain intact for up to 200 years while waiting for a better environment to germinate. Further it would become activated at the time of cracking, food is available and water seeps into the structure. This induces low pH value of highly alkaline concrete of the range (pH 10 to 11.5).

3. Materials

3.1 Bacteria

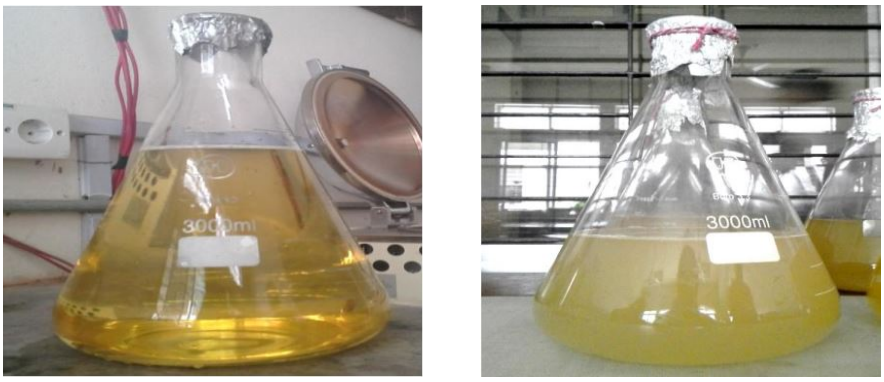
Bacillus subtilis strain 121 has been used in the following study. It was obtained from Micro-biology Department, Chittagong University. 'Bacillus subtilis' is a model laboratory bacterium which can produce calcite precipitates on suitable media supplemented with a calcium source. It is gram positive, ellipsoidal or cylindrical in size and has optimum growth temperature at 28°C to 30°C. Media used was nutrient broth for B. subtilis growth. Four different OD600 has been used to investigate the performances and optimum cell concentration of bacteria.

3.2 Cement

Ordinary Portland Cement (OPC) ASTM Type-1, conforming to ASTM C-150 was used as binding material. Its physical properties and chemical compositions are given in Table 1.

3.3 Aggregate

Locally available natural sand passing through 4.75 mm sieve and retained on 0.075 mm sieve was used as fine aggregate. The coarse aggregate was crushed stone with a maximum nominal size of 12.5 mm. The properties of aggregates are given in Table 2.



(a) **(b)**

Fig. 1 - (a) Prepared nutrient broth media; (b) Cultured bacterial sample

3.4 Water

Water confirming to the requirements of IS456-2000 was taken with pH value 7 and at zero turbidity.

Table 1 - Physical properties and chemical composition of OPC.

SL. No	Characteristics	Value
1	Blaine's Specific surface (cm^2/gm)	2900
2	Normal Consistency	26%
3	Soundness by Le Chatelier's Test (mm)	4.5 mm
4	Specific gravity	3.15
5	Setting Time (a) Initial (min) (b) Final (min)	 70 175
6	Compressive Strength (a) 3 Days (MPa) (b) 7 Days (MPa) (c) 28 Days (MPa)	 16.2 21.2 31.4
7	Calcium Oxide (CaO)	64%
8	Silicon Dioxide (SiO_2)	21%
9	Aluminum Oxide (Al_2O_3)	6%
10	Ferric Oxide (Fe_2O_3)	3.5%
11	Magnesium Oxide (MgO)	1.2%
12	Sulfur Trioxide (SO_3)	2.5%
13	Loss on ignition	1.2%
14	Insoluble matter	0.6%
14	Insoluble matter	0.6%

4. Variables

4.1 Concrete Quality

Three different grades of microbial concrete having OD600 0.107, 0.20, 0.637, 1.221 were used. OPC concrete was cast for comparing its properties with that of microbial concrete.

4.2 Exposure Period

Specimens were tested periodically after the specified curing periods of 7, 14 and 28, 60, 120 days in plain water.

4.3 Size of Specimens

100 mm x 100 mm x 100 mm cube specimens were prepared following ASTM standard procedure. Also cylindrical specimens of 100 mm dia and 200 mm high were used following ASTM standard procedure.

Table 2 - Physical properties of aggregate.

Properties	Coarse Aggregate	Fine Aggregate
Specific Gravity	2.59	2.55
Unit Weight	1560 Kg/m^3	1580 Kg/m^3
Fineness Modulus	6.77	2.57
Absorption Capacity	0.6%	1.45%
Moisture Content	0.57%	1.12%

4.4 Curing Environment

A total of 450 concrete specimens were cast in the laboratory. After casting, the specimens were kept at 27°C temperature and 90% relative humidity for 24 hours. After demoulding, all the specimens were cured in plain water for different curing ages at room temperature.

Table 3 - Properties of microbial water. Absorbance reading: T60 UV-VIS Spectrophotometer @ RT. Absorbance at 600 nm wave length

Group	Control	Bacterial Treatment	Optical Density
A	0.089	0.196	0.107
B		0.289	0.20
C		0.762	0.637
D		1.31	1.221

4.5 Mix Proportion

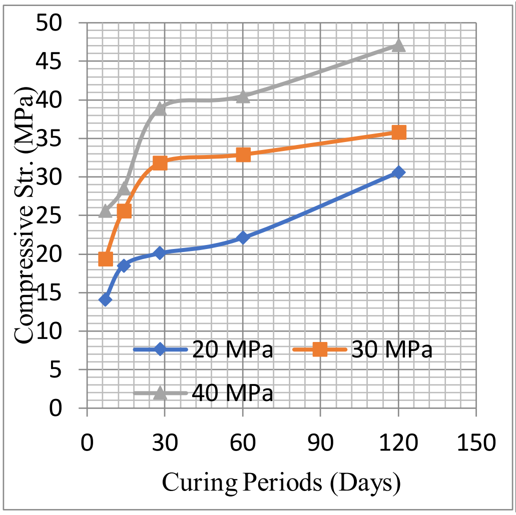
Concrete was designed on the basis of material properties. For a mix design of 20 MPa concrete the ratio of cement, fine aggregate, coarse aggregate was derived to be 1.0: 2.57: 2.71 with water cement ratio of 0.592 by mass. For 30 MPa concrete the ratio was derived to be 1.0: 1.68: 2.04 with water cement ratio of 0.445 and for 40 MPa concrete it was 1.0: 1.28: 1.73 with water cement ratio of 0.38 by mass. 100% water by mass was used for conventional concrete and a water cement ratio of 50% and microbial culture of 50% for microbial concrete by mass was used.

Fig. 2 - Microbial concrete

5. Results and Discussion

5.1 Compressive Strength Test

The results of the test specimens have been critically analyzed and presented in both graphical and tabular form. Figures 3 to 7 present the experimental results of compressive strength of microbial concrete for different OD600 and curing periods. These figures clearly demonstrate that concrete specimens with OD600 0.637 gives lower strength deterioration for all curing periods.



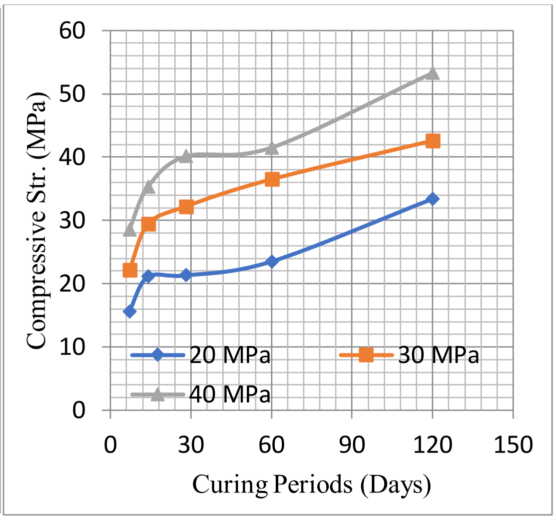


Fig.3 - OD = 0

Fig. 4 - OD = 0.107

For OPC of 20 MPa designed concrete the compressive strength for 28 days curing period is 20.1 MPa whereas the corresponding values are 21.3 MPa, 22.5 MPa, 23.7 MPa and 21.9 MPa for microbial concrete of OD_{600} 0.107, 0.2, 0.637 and 1.221.

For OPC of 30 MPa designed concrete the compressive strength for 28 days curing period is 31.8 MPa whereas the corresponding values are 32.2 MPa, 33.8 MPa, 35.6 MPa and 33.5 MPa for microbial concrete of OD600 0.107, 0.2, 0.637 and 1.221.

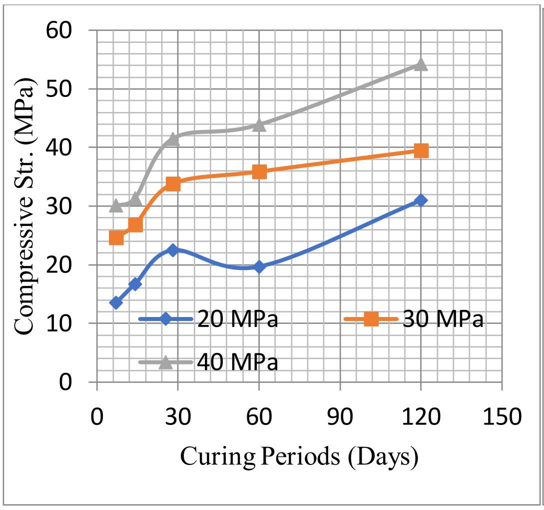


Fig. 5 - OD = 0.2

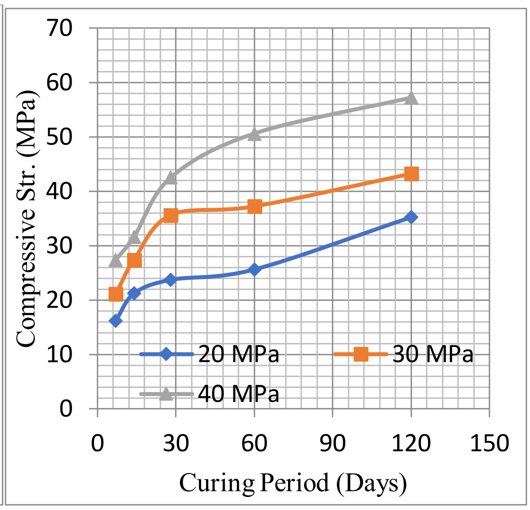


Fig. 6 - OD = 0.637

For OPC of 40 MPa designed concrete the compressive strength for 28 days curing period is 38.9 MPa whereas the corresponding values are 40.1 MPa, 41.5 MPa, 42.5 MPa and 40.6 MPa for microbial concrete of OD_{600} 0.107, 0.2, 0.637 and 1.221.

Table 4 demonstrates the increase in strength for all microbial groups. It has been observed that concrete specimen with OD_{600} 0.637 shows maximum increase in strength. The resistance against strength deterioration of microbial concrete is primarily due to the precipitation of $CaCO_3$ on the surface of concrete structure.

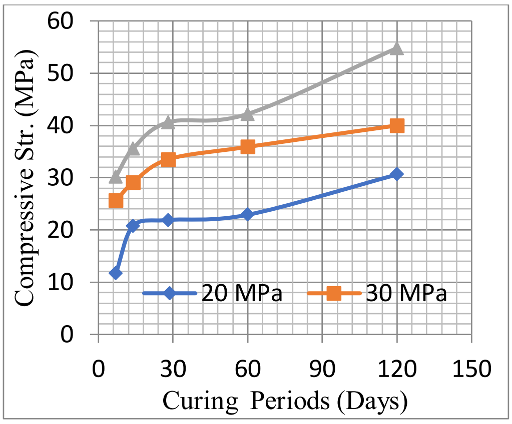


Fig. 7 - OD = 1.221

Table 4 - Strength behavior observations (28 Days curing periods)

Optical Density	Increased in Compressive Strength		
	20 MPa	30 MPa	40 MPa
0.107	5.97%	1.26%	3.1%
0.2	11.94%	6.29%	6.68%
0.637	17.91%	11.95%	9.25%
1.221	8.96%	5.95%	4.37%

5.2 Tensile Strength Test

Plain concrete and microbial concrete containing different optical density were used in split tensile strength. In general, tensile strength of concrete is only about 10% of it compressive strength. The relation of split tensile strength between microbial concrete and control specimen are shown in Figure 8 to 12.

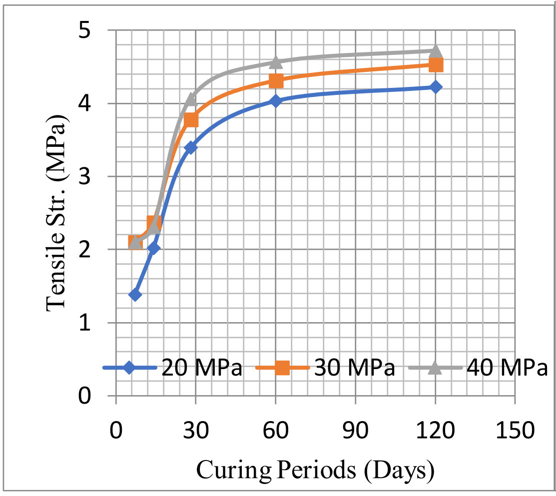


Fig. 8 - OD = 0

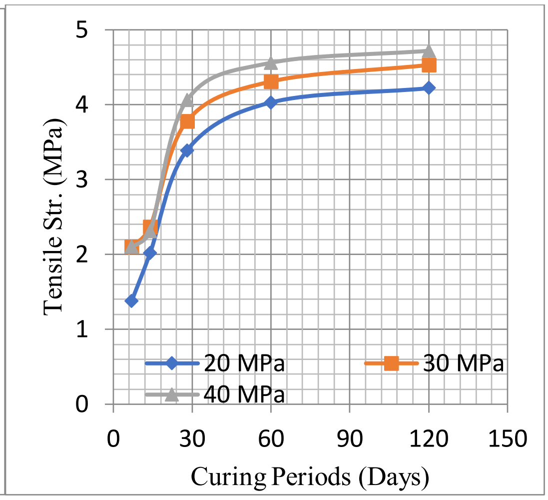


Fig. 9 - OD = 0.107

The percentages of variation of optical density affect the split tensile strength of concrete. When micro organisms are used in concrete it enhances the split tensile strength of concrete. It increases up to 10 - 15% in case of standard grade concrete.

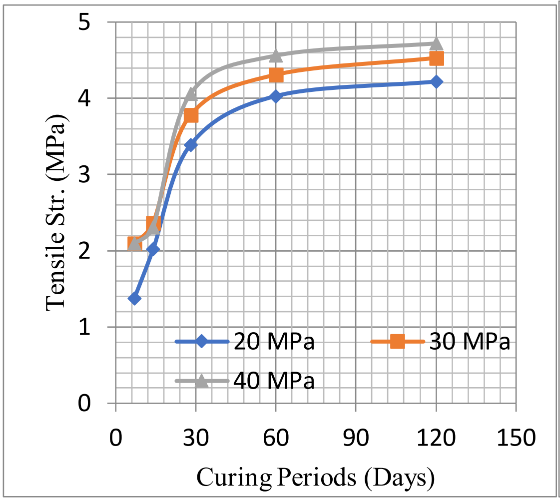


Fig. 10 - OD = 0.2

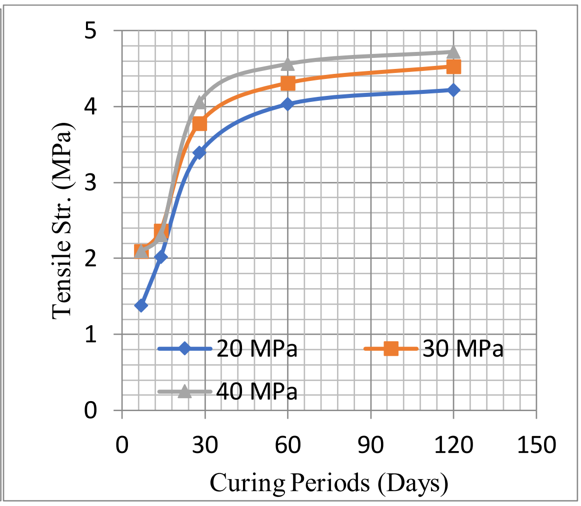


Fig. 11 - OD = 0.637

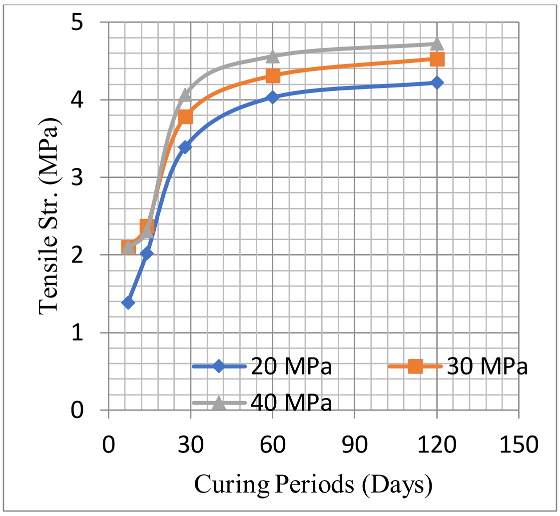
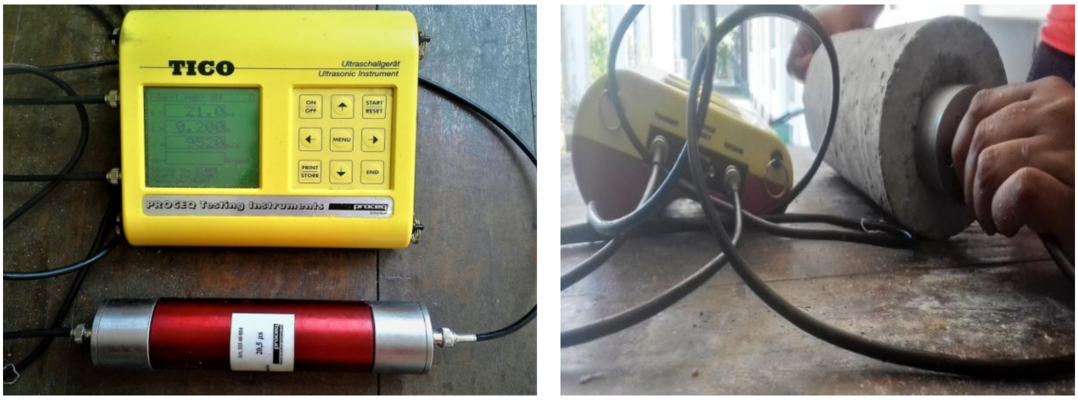


Fig. 12 - OD = 1.221

5.3 Ultrasonic Pulse Velocity (UPV) Test

It is a well-established non- destructive test method which determines the velocity of longitudinal waves through the concrete mass. It consists of measurement of time taken by a pulse to travel a measured distance.

UPV results directly represent the density of the prepared specimens. The higher the value, the lesser will be the voids. This means that concrete specimens with higher pulse velocity will be denser.



(a) **(b)**

Fig. 13 - (a) Ultrasonic measurement; (b) Ultrasonic measurement process.

It can be found that concrete specimens having OD_{600} have the higher velocity. That means that specimens having OD_{600} are denser than other microbial groups. Compressive and tensile strength results also indicate that specimens having OD_{600} have the better strength values than other microbial groups. UPV values have been represented in Figure 13.

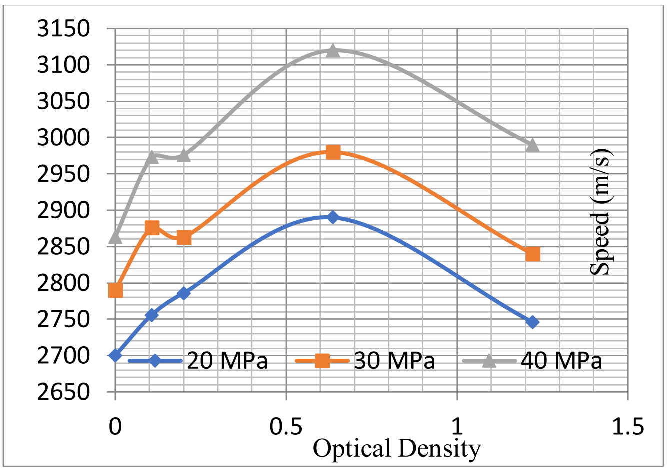


Fig. 14 - UPV analysis.

5.4 Correlation between Compressive Strength against UPV

Many scientists have studied how UPV can be correlated with concrete strength. According to previous research by **Tharmaratnam**, the compressive strength and ultrasonic pulse velocity UPV values are related by the following equation (Non-linear model is suggested):

$$F_c = ae^{bV} \qquad (1)$$

Here,

F_c= Compressive strength
V = Pulse velocity (km/s)
a and b are empirical constants.

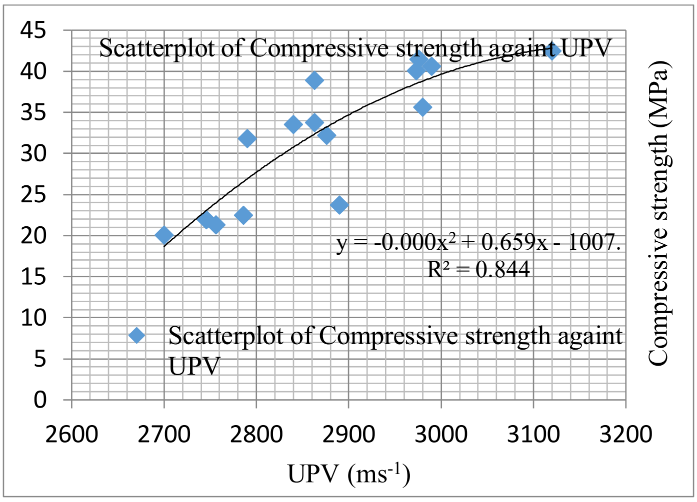


Fig. 15 - Correlation between compressive strength against UPV.

For all results, we found the following law relating compressive strength (F_C in MPa) to UPV (V in m/s):

$$y = -0.000x^2 + 0.659x - 1007$$

$$R^2 = 0.844$$

There was an acceptable polynomial relationship between UPV and compressive strength. Because R^2= 0.844. It can be said that 84.4% of the variation in the values of compressive strength is accounted for by polynomial relationship with UPV.

6. Conclusion

Based on the limited number of test variables and exposure conditions stated above, the following conclusions can be drawn. The study may provide some necessary information related to the use of microbial concretes for the construction of marine onshore / offshore reinforced concrete structures:

- Microbial concrete technology has been proved to be better than many conventional technologies because of its eco-friendly nature, self-healing abilities and increase in durability of various building materials.
- Mix proportion of microbial water with plain water has a significant effect on strength development of microbial concrete. Among the microbial concretes, concrete with OD_{600} 0.637 is found to be most effective in increasing compressive strength. Hence, OD_{600} of 0.5±0.1 can be used as optimum optical density for microbial concrete preparation.
- It can be seen from the above Table 4 that strength increases more in case of lower grade concrete than higher grade concrete. The development of compressive strength for microbial concrete is not significant at the early age of curing. The gain in strength occurs at relatively rapid rate at later ages of curing.

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