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Influence of Pseudomonas Putida Bacteria on the Strength Characteristics of Concrete Incorporating G.G.B.S.

Sanket Naik ¹, Sujay Deshpande ²

¹Post-Graduate Student, ²Assistant Professor, Department of Civil Engineering, KLS Gogte Institute of Technology, Belagavi-590008

Abstract: *The cracks in concrete are inevitable and are one of the inherent weaknesses of concrete. Water and other salts seep through these cracks, corrosion initiates, and thus reduces the life of concrete. So, there was a need to develop an inherent biomaterial, a self-repairing material which can remediate the cracks and fissures in concrete. Bacterial concrete is a material which can successfully remediate cracks in concrete.*

This technique is highly desirable because the mineral precipitation induced as a result of microbial activities is pollution free and natural. As the cell wall of bacteria is anionic, metal accumulation (calcite) on the surface of the wall is substantial, thus the entire cell becomes crystalline and they eventually plug the pores and cracks in the concrete. This study discusses the healing of concrete using the bacteria Pseudomonas Putida for different cell concentration of bacteria.

It was found that the use of bacteria improves Compressive strength, split tensile strength, flexural strength and shear strength of concrete. Scanning electron microscope (SEM) is used to document the role of bacteria in microbiologically induced mineral precipitation. Rod like impressions were found on the face of calcite crystals indicating the presence of bacteria in those places. Energy dispersive X-ray analysis (EDAX) spectra of the microbial precipitation on the surface of the crack indicated the abundance of calcium and the precipitation was inferred to be calcite (CaCO₃).

Keywords: *Bacterial concrete, Pseudomonas putida, GGBS*

I. INTRODUCTION

For structures like retaining wall, bridges and under-water structures, durability plays a main role because structure is exposed to the environment over entire lifetime.

If cracks are not sealed the water and other chemicals like chloride and sulphate will penetrate leads to deterioration of reinforcement and leads to failure of structures. For these structures strength is not only important but durability also plays an important role. For durable structures cracks are to be minimized and they should be repaired and maintained. For repair work of crack epoxies are used but they are not compatible, costly.

The alternative method is the self-healing mechanism in concrete induced by metabolic action of bacteria, in which the calcium carbonate precipitation will fill the crack and gives water tight structures. This is an automatic, economic and environment friendly method than other repair methods.

This gives rise to design durable structures. The novel technique is used to repair or make concrete cracks free by microbiologically induced calcium carbonate (CaCO₃) precipitation. The calcite precipitation will occur as result microbial activities. This technique improves the compressive strength and stiffness of concrete and leads to crack free structures. Bacterial concrete is produced by embedding the spore forming bacteria in concrete which can precipitate the calcite continuously. Pseudomonas putida is used in concrete to produce calcite.

The basic idea is the microbial urase get hydrolyses to produce carbon dioxide and ammonia, this ammonia increases the pH in concrete and helps to precipitate the calcite. If concrete is mixed with bacteria, the bacteria go to dominate state. Bacteria need aerobic condition to activate their functions. When crack formation begins bacteria near the cracks start precipitating calcite crystals when a concrete structure comes in contact with water, water seeps through the cracks the spores of bacteria initiate while contact with water and nutrients. They get activated start feeding calcium lactate nutrient. When bacteria utilize the oxygen present in concrete it helps in improving durability of concrete structure.

II. LITERATURE REVIEW

- A. M. Monishaa, S. Nishanthi., (2017) In this paper an overview of new development obtained in experimental study on self-healing concrete, strength and durability of concrete is mainly affected due to the formation of cracks micro-cracks are the main cause for structural failure. While larger cracks affect structural integrity micro cracks result in durability problems Ingress of water and chemicals can cause premature matrix degradation and corrosion of embedded steel reinforcement Also concrete fails due to insufficient tensile strength In order to overcome this an attempt is made in bacterial concrete with non-pathogenic spore forming calcite mineral precipitating bacterium *Bacillus subtilis* M-20 grade concrete is prepared with different bacterial cell concentration of 10^4 , 10^5 and 10^6 cells per milliliter of water and polyethylene fibre kept at constant 0.4%. The overall development of strength and durability of self-healing concrete using *Bacillus subtilis* and polyethylene fibre has investigated and compared with control concrete The optimum strength is obtained at 10^5 cell concentration. The optimum strength is obtained at 105 cells concentration which increases the compressive strength by 13.2% split tensile strength by 21.4% and flexural strength by 16.04%. The percentage of increment in strength clearly shows that the self-healing concrete is advantageous.
- B. Pawar Bhagyashri, Magdum Archana, Bhosale Megh, Pol Sayali, (2017) When the loads is applied on any structures cracks in concrete are formed which allow water and other chemicals to enter thus making it vulnerable which leads to unwanted corrosion of the steel reinforcement and deterioration of concrete structure For this study they used M-25 grade of concrete because of its superior properties. As mentioned above in cases like this where there is a formation of cracks there is an acute need of Self-healing concrete to achieve this we added *Bacillus subtilis* which is a gram positive bacterium to the mixture Along with this bacteria concrete mixture we use chemical compound calcium lactate ($C_6H_{10}CaO_6$) which is used to activate the healing procedure.
- C. Meera C. M, Dr. Subha V. (2016) In recent years there is increasing interest in the phenomenon of mechanical property recovery in concrete construction using self-healing concrete. The study was motivated by the need to find a solution for the problem of cracking approaching the concept of self-healing concrete. The study was carried out on a bacteria based self-healing concrete using *Bacillus Subtilis* bacteria. The present paper describes the effect of these bacteria on the strength of concrete. An investigation on the strength assessment of the bacteria-based self-healing concrete by finding out the optimum amount of bacterial content to be added to obtain maximum strength is depicted in this.
- D. S. Dinesh, R. Shanmugapriyan & S. T. Namitha Sheen V. Ramakrishnan, Ramesh K (2017) Concrete cracking is a common phenomenon developed due to relatively low tensile strength High tensile stresses may be developed in concrete due to external loads imposed deformations plastic shrinkage plastic settlement and expansive reactions Proper and immediate treatment should be done in order to prevent expansion of cracks which may eventually be of higher cost For crack repair a variety of traditional repair systems are available which possess a number of disadvantageous aspects such as different thermal expansion coefficients environmental and hazards of health. Bacterially induced calcium carbonate precipitation has been proposed as an alternative and environmental friendly crack repair technique. It is found that microbial mineral precipitation as a result from metabolic activities of favorable bacteria in concrete improved the overall behavior of concrete. It is expected that further development of this techniques will result in a more durable sustainable and crack free concrete that can be used effectively for constructions in wet atmospheres where corrosion of reinforcement affects the durability permeability and strength of concrete.
- E. Dr. K. B. Parikh, G.T. Suthar (2016) In this research paper, the behavior of microorganism *Bacillus Subtilis* for enhancement of strength in cracked concrete specimen is discussed. Concrete crack is very important phenomenon due to having low tensile strength and stress which cause settlement shrinkage and expansion in concrete. Without any treatment and precaution crack is expand further more and require expensive repair In this paper the *Bacillus subtilis* a gram positive bacteria was used to induced the precipitation of calcite. This procedure is incredibly applicable due to various facts like it is pollution free and natural. The comparative result is considered for evaluation of strength and durability with addition of bacteria in cracked specimen. Scanning Electron Microscope (SEM) was used to check the role of microorganism precipitation in improving the strength and durability of concrete.

III. OBJECTIVES AND METHODOLOGY

A. Objectives

The aim of the study is experimental analysis of the effect of *Pseudomonas putida* bacteria on strength properties of concrete with partial replacement of cement with GGBS. The replacement of GGBS is 40% by weight of cement.

Different cell concentrations of bacteria used are 10^3 , 10^5 , 10^7 (cells/ml). The comparison is done with respect to conventional concrete.

The strength tests such as compressive strength, split-tensile strength, flexural strength and shear strength are conducted after 28 days of curing. The grade of concrete used is M-25. Mix design is done as per IS-10262:2009.

The objectives of the present study are-

- 1) To investigate the strength properties of concrete on variation of different bacterial cell concentrations.
- 2) To investigate the effect of partial replacement of cement with GGBS (40%) in bacterial concrete with conventional concrete.

B. Materials Used

- 1) Cement: In this study, cement used is of brand name UltraTech of OPC-43 grade. The cement is checked by preliminary tests, the testing of cement is performed as per Indian standard codal provision of IS: 8112-1989. The specific gravity of cement is obtained as 3.15.
- 2) M-Sand (Fine aggregate): The sand used in the study work is manufactured-sand conforming to zone II of IS 383-1970. The specific gravity of M-sand was obtained as 2.60.
- 3) Coarse Aggregate: Coarse aggregate used is locally available of size 20mm and downsize conforming to IS 383:1970. The specific gravity of coarse aggregate is obtained as 2.70.
- 4) GGBS: The GGBS used in concrete mix is of brand name JK Cement GGBS. Partial replacement of cement by GGBS is done at 40% to reduce the heat of hydration of cement.
- 5) Water: Clean potable water is used for concrete mixes and for curing.
- 6) Bacteria: Bacterium used in the study is *Pseudomonas Putida* which is obtained from Department of Biotechnology, JNMC campus, Belagavi. Culturing process was done at Department of Biotechnology, MO Lab, KLE Dr.MSSCET, Belagavi.

C. Culturing Process of Bacteria

Steps involved in culturing of bacteria are-

- 1) Sterilization: It is done to achieve sterile environment by killing the surrounding microorganisms. It is done in auto-clave at higher temp of 121°C for a time period of 30 minutes.
- 2) Inoculation: This is the process where bacteria are transferred (streaking) with the help of loop on media.
- 3) Incubation: It is the process where the bacteria will grow and maintaining the culture.

D. Procedure for culturing the bacteria

- 1) Initially sterile all the glassware in an autoclave.
- 2) Prepare the media by dissolving 28 gms of nutrient agar to 1000 ml of distilled water.
- 3) The mouth of conical flask is to be closed by cotton plug so that it should not get contaminated.
- 4) Sterile the media once again.
- 5) The media is allowed to cool to room temp then it is poured to petri dish.
- 6) The petri dish is further sterilized by keeping in UV rays to kill bacteria [if any present] for atleast 30 minutes.

After this, the Inoculation is to be done as follows-

- a) First open loop is been taken and it is to sterilize by keeping it near high flame.
- b) The loop is then dipped in bacterial sample; it is scratched on agar media in zig- zag manner.
- c) Prepared Petri dishes are kept in an incubator for 24 hours at temp of 37°C .



Figure 1: Sample of *Pseudomonas Putida* bacteria



Figure 2: Auto-clave



Figure 3: Incubator



Figure 4: Inoculation chamber and Electronic Shaking Incubator



Figure 5: Culturing of bacteria

E. Growth curve study of bacteria

The growth curve study of pseudomonas bacteria was carried out, the growth phase starts at 3 hours and goes on up to 24 hours and maximum beyond that. The spectrophotometer was calibrated and adjusting the wavelength to 600nm for measuring the optical density. As blank solution the plain nutrient broth solution is kept in to sample holder, adjust the needle to read 100% transmission by adjusting the control knob. Blank is removed from the spectrometer. Before placing the bacterial sample, shake the sample well so that there should be uniform distribution of bacteria in the media then take little sample in the quartz sampler place it in sample holder. The optical density is noted down from spectrophotometer. 4th and 5th steps are repeated for every 15 mins of time interval.

Table 1: Growth Curve readings

TIME (mins)	O.D.	TIME (mins)	O.D.
0	0	5:30	1.17
5	0	5:45	1.2
10	0	6:00	1.2
15	0	6:15	1.21
20	0	6:30	1.22
25	0	6:45	1.24
30	0.01	7:00	1.25
35	0.01	7:15	1.26
40	0.01	7:30	1.28
45	0	7:45	1.33
50	0	8:00	1.31
55	0.01	8:15	1.34
1:00	0.02	8:30	1.37
1:15	0.02	8:45	1.36
1:30	0.02	9:00	1.36
1:45	0.02	9:15	1.37
2:00	0.02	9:30	1.39
2:15	0.04	9:45	1.4
2:30	0.11	10:00	1.43
2:45	0.11	10:15	1.41
3:00	0.29	10:30	1.46
3:15	0.4	10:45	1.52
3:30	0.48	11:00	1.53
3:45	0.56	11:15	1.51
4:00	0.68	11:30	1.53
4:15	0.75	11:45	1.54
4:30	0.93	12:00	1.56
4:45	0.98	12:15	1.57
5:00	1.11	12:30	1.59
5:15	1.14	12:45	1.60

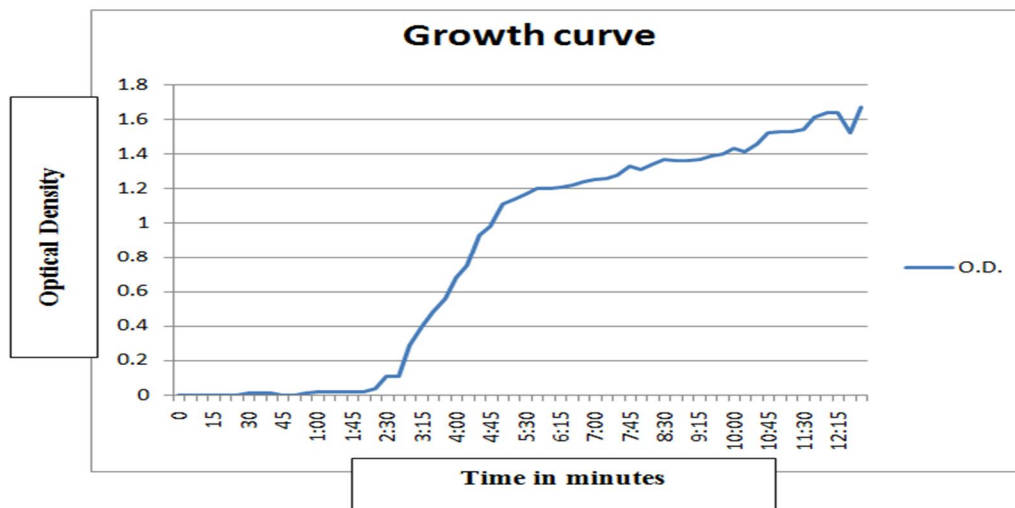


Figure 6: Growth Curve of bacteria

F. Observations from Growth Curve study

Growth sequence of bacteria was studied under three stages. In the first stage, when bacteria were introduced in high nutrient content media cells need to adapt to new media environment, in this stage the cell biosynthesis is very slow, hence this stage is called as lag phase. In second phase, the biosynthesis rate is very fast, the required protein for growth of bacteria are releases hence in this stage the cell are more in number, hence this stage is called as logarithmic phase or exponential phase. During log phase nutrients released rapidly until one of nutrient becomes depleted. This depleted will start the third stage that is stationary phase where the bacteria growth is very least. The final stage is death phase where the cell reduces their activities.

G. Mix Proportion of concrete

Table 2: Proportion of Concrete mix

W/C RATIO	CEMENT	FINE AGGREGATE	COARSE AGGREGATE
0.50	1.00	1.88	3.20

H. Specimen Casting

The ingredients cement, sand, aggregates and water are weighed and mixed as per the mix-ratio for M-25 grade concrete (1:1.88:3.20:0.50). Then, Cement is replaced by GGBS (40% by weight of cement). First dry mixing is done in mixer and then the water which was replaced by 25% of bacterial water is added to the dry mix and mixing is done to get a uniformly mixed concrete. Then fresh concrete is poured into moulds in three layers with each layer tamped. Then moulds are kept on vibrator to remove the entrapped air.





Figure 7: Specimen casting

IV. RESULTS AND DISCUSSIONS

A. Compressive Strength test results

Table 3: Test results of Compressive strength test

Description of Concrete	Bacteria Concentration (cells/ml)	Average Compressive strength (Mpa)	% increase in compressive strength w.r.t conventional concrete
Concrete with 40% GGBS as replacement of cement	Conventional concrete (0)	31.55	-----
	Bacterial concrete (10^3)	33.03	4.69%
	Bacterial concrete (10^5)	34.81	10.33%
	Bacterial concrete (10^7)	31.99	1.39%

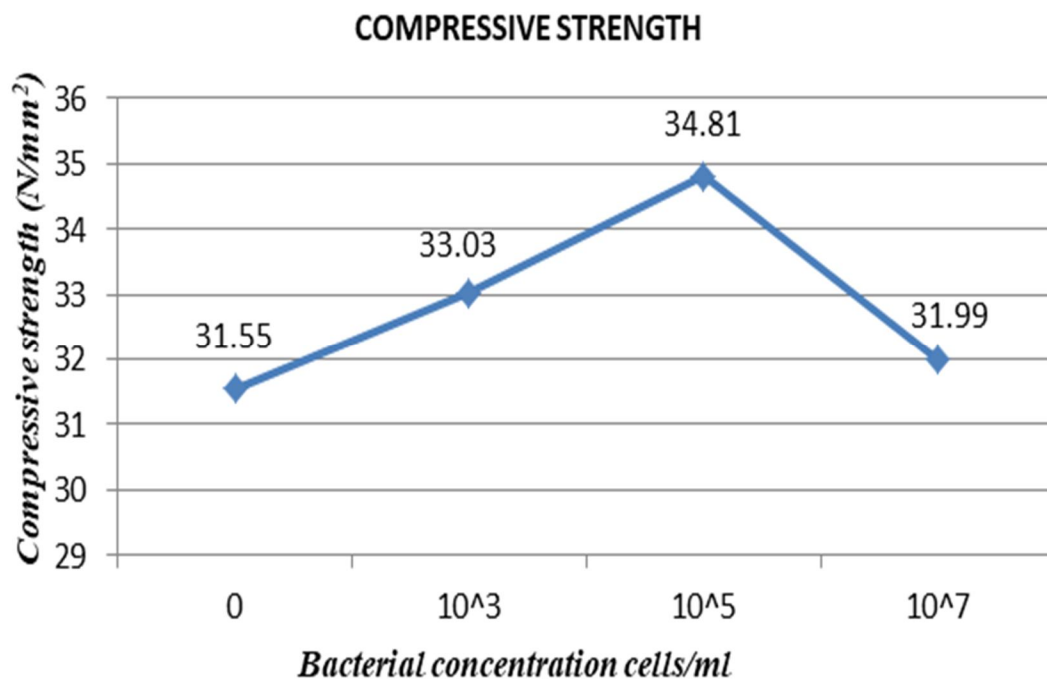


Figure 7: Variation of Compressive strength

B. Split-Tensile Strength test Results

Table 4: Test results of Split-Tensile strength test

Description of Concrete	Bacteria Concentration (cells/ml)	Average Split-Tensile strength (Mpa)	% increase in split-tensile strength w.r.t conventional concrete
Concrete with 40% GGBS as replacement of cement	Conventional concrete (0)	3.20	-----
	Bacterial concrete (10^3)	3.48	8.75%
	Bacterial concrete (10^5)	3.72	16.25%
	Bacterial concrete (10^7)	3.29	2.81%

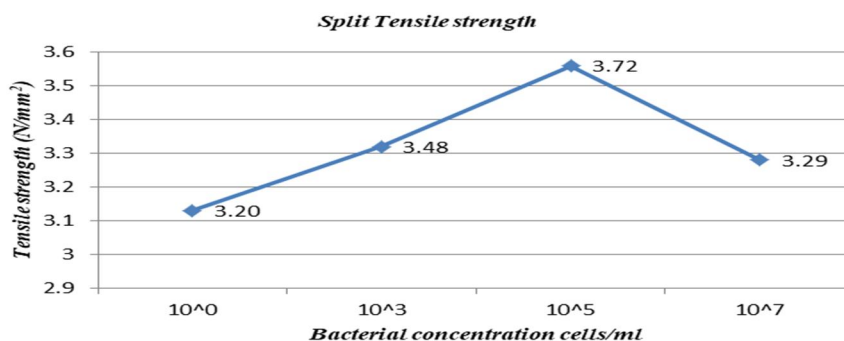


Figure 8: Variation of Split-Tensile strength

C. Flexural Strength test Results

Table 5: Test results of Flexural strength test

Description of Concrete	Bacteria Concentration (cells/ml)	Average Flexural strength (Mpa)	% increase in flexural strength w.r.t conventional concrete
Concrete with 40% GGBS as replacement of cement	Conventional concrete (0)	3.13	-----
	Bacterial concrete (10^3)	3.32	6.07%
	Bacterial concrete (10^5)	3.56	13.73%
	Bacterial concrete (10^7)	3.28	4.79%

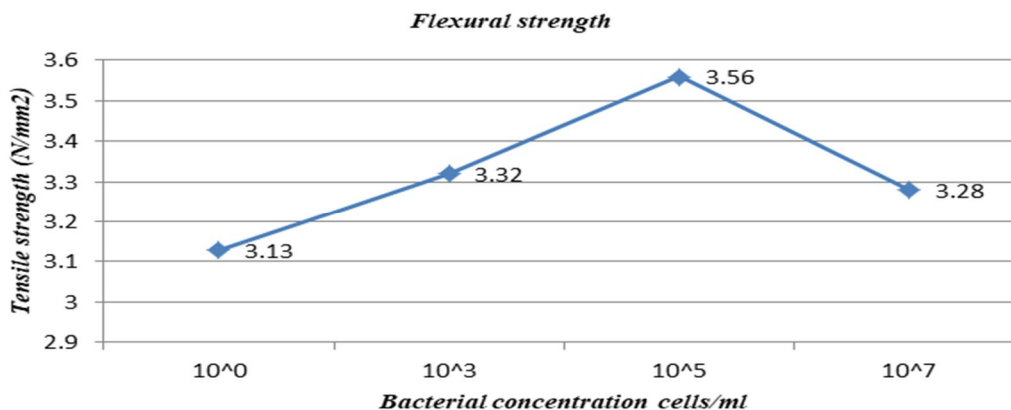


Figure 9: Variation of Flexural strength

D. Shear Strength test results

Table 6: Test results of Shear strength test

Description of Concrete	Bacteria Concentration (cells/ml)	Average Shear strength (Mpa)	% increase in shear strength w.r.t conventional concrete
Concrete with 40% GGBS as replacement of cement	Conventional concrete (0)	3.44	-----
	Bacterial concrete (10^3)	3.70	7.55%
	Bacterial concrete (10^5)	4.05	17.73%
	Bacterial concrete (10^7)	3.53	2.61%

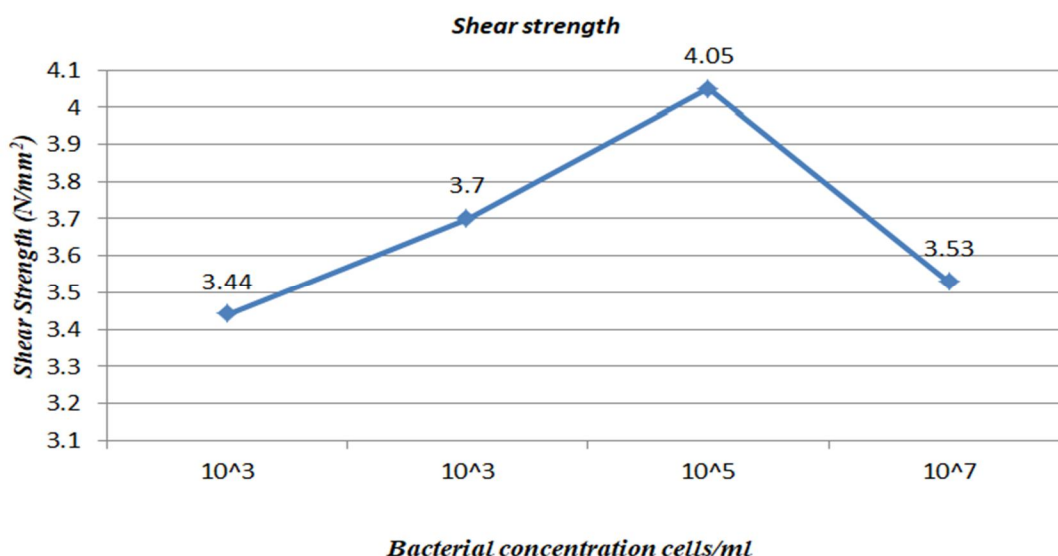


Figure 10: Variation of Shear strength

E. Discussions

- 1) **Compressive strength Test:** The effect of Pseudomonas putida bacteria on the 28-day compressive strength of GGBS bacterial concrete and conventional concrete is given in Table 3 and is graphically illustrated in Figure 7. It is noted that the compressive strength of conventional and bacterial concrete is increased by increasing the bacterial cell concentration up to 10^5 cells/ml. Then there is a decrease in strength at 10^7 cells/ml. The maximum compressive strength was reached at 10^5 cells/ml for GGBS bacterial concrete. There was an improvement of 10.33% in the compressive strength for the bacterial concrete respectively; with the addition of 10^5 cells/ml. Improvement in the compressive strength of bacterial concrete is probably due to the deposition of $CaCO_3$ on the surfaces of the micro-organisms and the pores that clog the pores in the matrix of the binder.
- 2) **Split-Tensile strength Test:** The effect of Pseudomonas Putida on the 28-day split-tensile strength of conventional and bacterial concrete and is given in Table 4 and graphically represented in Figure 8. It was observed that the tensile strength of GGBS bacterial concrete increased with an increase in the concentration of bacterial cells of up to 10^5 cells/ml, then a reduction of the tensile strength at 10^7 cells/ml. The maximum tensile strength was reached at 10^5 cells/ml for GGBS bacterial concrete. In bacterial concrete, there was a 16.25% improvement in the tensile strength of concrete with the inclusion of 10^5 cells/ml of bacterial cells. The results of the study showed that due to the inclusion of Pseudomonas Putida bacteria in bacterial concrete, the deposition of $CaCO_3$ on the surfaces of the microorganism cells and in the pores of the binding matrix.
- 3) **Flexural Strength Test:** The effect of Pseudomonas putida bacteria on the 28-day flexural strength of conventional and GGBS bacterial concrete is given in Table 5 and shown graphically in Figure 9. It was observed that the flexural strength of GGBS bacterial concrete increased with increasing bacterial cell concentration up to 10^5 cells/ml, and then there was a reduction in strength at 10^7 cells/ml. The maximum flexural strength was reached at 10^5 cells/ml for GGBS bacterial concretes. In GGBS bacterial concrete, there was a 13.73% improvement in the flexural strength of concrete with the inclusion of 10^5 cells/ml of

bacterial cells. The improvement in the flexural strength is probably due to the deposition of CaCO_3 on the cell surfaces of the micro-organisms and in the pores of which clog the pores in the binder matrix.

- 4) *Shear Strength Test*: Effect of *Pseudomonas Putida* bacteria on the 28-day shear strength of conventional and GGBS bacterial concrete is given in Table 6 and graphically represented in Figure 10. It is observed that shear strength of GGBS bacterial concrete increased with increase in bacteria cell concentration up to 10^5 cells/ml, and then there was reduction in the strength at 10^7 cells/ml. Maximum shear strength was achieved at 10^5 cells/ml for GGBS bacterial concrete. In GGBS bacterial concretes, there was 17.73% improvement in shear strength of concrete with the inclusion of 10^5 cells/ml bacterial cells. The improvement in shear strength of bacterial concrete is probably due to deposition of CaCO_3 on the microorganism cell surfaces and within the pores of, which plug the pores within the binder matrix.

V. CONCLUSIONS

The following conclusions are given based on the experimental investigations conducted-

- A. *Pseudomonas putida* bacteria plays a significant role in increasing the compressive strength of GGBS bacterial concrete up to 10.33% as compared to conventional concrete at a particular cell concentration i.e. at 10^5 cells/ml.
- B. *Pseudomonas putida* plays a significant role in increasing the split tensile strength of GGBS bacterial concrete up to 16.25%, as compared to conventional concrete at a particular cell concentration i.e. at 10^5 cells/ml.
- C. Bacteria *Pseudomonas Putida* plays a significant role in increasing the flexural strength of GGBS bacterial concrete up to 13.73%, as compared to conventional concrete at a particular cell concentration i.e. at 10^5 cells/ml.
- D. Bacteria *Pseudomonas Putida* plays a significant role in increasing the shear strength of GGBS bacterial concrete up to 17.73%, as compared to conventional concrete at a particular cell concentration i.e. at 10^5 cells/ml.
- E. The increase in Compressive strength, Split-Tensile strength, Flexural strength and Shear strength is mainly due to the consolidation of pores inside the conventional and bacterial concrete with bacteria induced calcium carbonate precipitation.
- F. The cell concentration of 10^5 cells/ml can be considered as optimum for the given study.

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