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An Experimental Study on Bacterial Concrete

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Abstract: ***The objective of the present investigation is to obtain the performance of the concrete by the microbiologically induced special growth/filler. One such thought has lead to the development of a very special concrete known as Bacterial Concrete where bacteria is induced in the mortars and concrete to heal up the faults. Researchers with different bacteria have proposed different bacterial concrete's. Here an attempt was made by using the bacteria "Bacillus subtilis". Calcite formation by Bacillus subtilis is a model laboratory bacterium, which can produce calcite precipitates on suitable media supplemented with a calcium source. Cement concrete cubes and Cylinders with four particular cell concentrations were cast and control specimen was also cast. This study showed a significant increase in the compressive strength was observed due to the addition of bacteria for a cell concentration ml of mixing water. By visual analysis, it is noted that pores were partially filled up by material growth with the addition of the bacteria. Reduction in pore due to such material growth will obviously increase the material strength. Concrete cubes with and without addition of bacteria were cast and it is observed that there is an improvement in the compressive strength for the cubes with the addition of bacteria. Concrete Cylinder with and without additions of bacteria were cast and it is observed that there is an improvement in the split tensile strength for the Cylinder with the addition of bacteria.***

Keywords: Bacillus subtilis

I. INTRODUCTION

A. General

Concrete is by far the most widely using building material in the world. Concrete has a large load bearing capacity for compression load, but the material is weak in tension. That is why steel reinforcement bars are embedded in the material to be able to build structures. The steel bars take over the load when the concrete cracks in tension. The concrete on other hand protects the steel bars for attacks from the environment and prevent corrosion to take place. However, the cracks in the concrete form a problem. Here the ingress of water and ions take place and deterioration of the structure starts with the corrosion of the steel. To increase the durability of the structure either the cracks that are formed are repaired later or in the design phase extra reinforcement is placed in the structure to ensure that the crack width stay within a certain limit. This extra reinforcement is then only needed for durability reasons (to keep the crack width small) and not for structural capacity. Especially with current steel prices this extra steel is not desirable. Durability is one reason to prevent cracks or limit crack widths. Other reasons are water tightness of structures, loss of stiffness and aesthetic reasons. If in some way a reliable method could be developed that repairs cracks in concrete automatically, this would increase and ensure durability and functionality developed that repair cracks in concrete enormously. On the other hand it would save a lot of money. Of course repair cracks of cracks that develop in concrete structures would go down. But also the extra steel that is used to limit crack widths could probably be saved to a large extent. Cracks widths in concrete structure should be limited, mainly for durability reasons. If cracks widths are too large the cracks need to be repaired or extra reinforcement is needed already in the design. If a method could be developed to automatically repair cracks in concrete this would save an enormous amount of money, both on the costs of injection fluids fro cracks and also on the extra steel that is put in structures only to limit crack widths. For structural reasons this extra steel has no meaning. A reliable self-healing method for concrete would lead to a new way of designing durable concrete structures, which is beneficial for national and global economy. The "Bacterial Concrete" can be made by embedding bacteria in the concrete that are able to constantly precipitate calcite. This phenomenon is called microbiologically induced calcite precipitation. Calcium carbonate precipitation, a widespread phenomenon among bacteria, has been investigated due to its wide range of scientific and technological implications. Calcite formation by Bacillus subtilisis a model laboratory bacterium, which can produce calcite precipitates on suitable media supplemented with a calcium source. A common soil bacterium, Bacillus subtilis, was used to induce CaCO3 precipitation. The basic principles for this application are that the microbial urease hydrolyzes urea to produce ammonia and carbon dioxide, and the ammonia released in surroundings subsequently increases pH, leading to accumulation of insoluble CaCO3. The favorable conditions do not directly exist in a concrete but have to be created. A main part of the research will focus on this topic. How can the right conditions be created for the bacteria not only to survive in the concrete but also to feel happy and produce as much calcite as needed to repair cracks. Furthermore the bacteria should be

suspended in a certain concentration in a certain medium before they are mixed through the concrete ingredients. Optimization is needed here, which involves experimental testing.

II. LITERATURE REVIEW

A. *Newcastle University*

1) *As part of the research during the year 2010:*A bacteria that can knit together cracks in concrete structures by producing a special 'glue' has been developed by a team of students at " Newcastle University ".They named the concrete as a Bacterial Concrete.The "Bacterial Concrete" can be made by embedding bacteria in the concrete that are able to constantly precipitate calcite. This phenomenon is called Microbiologically Induced Calcite Precipitation (MICP) .Under favorable conditions for instance BacillaFilla, a common soil bacterium, can continuously precipitate a new highly impermeable calcite layer over the surface of an already existing concrete layer.The genetically-modified microbe has been programmed to swim down fine cracks in the concrete. Once at the bottom, Bacilla Filla produces a mixture of calcium carbonate and a bacterial glue which combine with the filamentous bacterial cells to 'knit' the building back together. The nine students, whose backgrounds range from computer science, civil engineering and bioinformatics to microbiology and biochemistry, took part in the International Genetically Engineered Machines contest (iGEM), is run out of the Massachusetts Institute of Technology (MIT) in Cambridge, Boston. Over 130 teams took part in this year's event and it is now the third time Newcastle University has won Gold. Around five percent of all man-made carbon dioxide emissions are from the production of concrete, making it a significant contributor to global warming. Finding a way of prolonging the lifespan of existing structures means we could reduce this environmental impact and work towards a more sustainable solution. This could be particularly useful in "Earthquake zones" where hundreds of buildings have to be flattened because there is currently no easy way of repairing the cracks and making them structurally sound. The Bacilla Filla spores only start germinating when they make contact with concrete and they have an in-built self-destruct gene which means they would be unable to survive in the environment. Once the cells have germinated, they swarm down the fine cracks in the concrete and are able to sense when they reach the bottom because of the clumping of the bacteria. This clumping activates concrete repair, with the cells differentiating into three types: cells which produce calcium carbonate crystals, cells which become filamentous acting as reinforcing fibers and cells which produce a Levans glue which acts as a binding agent and fills the gap. Ultimately hardening to the same strength as the surrounding concrete, the Bacilla Filla has been developed to prolong the life of structures which are environmentally costly to build.

B. *A research by Delft University*

The long-term novel goal of the research is to remediate cracks in granite, concrete, and structures utilizing calcite that is induced by common soil bacteria such as Bacillus pasteurii.

Cracks in concrete allow water and chemicals to enter, a process that may lead eventually to the unwanted corrosion of the steel reinforcement and the deterioration of the concrete structure.

Within the framework of the "Self Healing Materials Research Project of the Delft Center" for Materials, the possible application of bacteria to extend the lifetime of concrete is studied. The goal of this project is to incorporate dormant but viable bacteria in the concrete matrix which will contribute to the concretes self-healing potential.

Water entering freshly formed cracks will activate the dormant bacteria which in turn will seal these cracks through the process of metabolically mediated calcium carbonate precipitation. Concrete, however, is due to its high internal pH (>12), relative dryness and lack of nutrients needed for growth, a rather hostile environment for common bacteria.

Yet, certain extremophilic bacteria may be able to endure this artificial environment for their growth of bacteria. In this study they tested the applicability of alkaliphilic spore-forming bacteria of the genus Bacillus as self-healing agent in concrete.They found that incorporation of high numbers of bacteria (10^{9} cm^{-3}) as well as some suitable organic growth substrates in concrete did not negatively affect compressive- and flexural tensile strength. ESEM analysis revealed furthermore the self-healing potential of immobilized cells, as bacterial- but not control cement stone samples were found to deposit a new layer of calcium carbonate minerals on its surface.

This research was awarded by the Florida Department of Environmental Protection (FDEP), Florida.So we are eagerly undergone, similar research by using Bacillus subtilis producing cheapest rehabitation glue for arresting fine cracks. In order to modify conventional concrete properties like Texture, Compression Strength & Spilt Tensile strength.

III. MATERIALS AND METHODOLOGY

A. *Materials used*

The following are the details of the materials used for concrete making:

1) *Cement:* Ordinary Portland cement of 53 grade available in local market is used in the investigation. The cement used has been tested for various properties as per IS: 4031-1988 and found to be confirming to various specifications of IS: 12269-1987 having specific gravity of 3.15.
2) *Fine Aggregate:* The sand available in the market is used in the investigation. The sand has been tested as per IS: 2386-1963 and found to be to conforming various specifications of IS: 383-1970 having specific gravity of 2.72 and falls under zone III.
3) *Coarse Aggregate:* Crushed broken stone angular aggregate of size 20 mm nominal size from local source was used as coarse aggregate having specific gravity of 2.85.
4) *Water:* Locally available portable water confirming to IS 456 is used.
5) *Micro Organisms:* Bacillus subtilis, a commonly available soil bacterium is used
6) Biochemical characteristics of the pure culture bacillus subtilis
7) Characteristics Bacillus subtilis

Shape, size, gram stain width and 2.0 to 3.0 µm in Length, gram positive (Figure: 3.2)	Long rods, 0.6-0.8 µm in
Colony morphology (on Nutrient agar plate) opaque colonies (Figure: 3.3)	Irregular, dry, white,

Fermentation	
Lactose	No acid, no gas
Dextrose	No acid, no gas
Sucrose	Acid and gas
H2S production	-
Nitrate reduction	-
Indole production	-
Methyl Red test	-
Vogesproskauer test	-
Citrate utilization	-
Catalase activity	+
Gelatin liquefaction	+
Starch hydrolysis	+
Lipid hydrolysis	+

Note: “+”:- Present “-“:- Absent

Figure: 3.1 Microscope

Figure: 3.2 Colony morphology on nutrient agar plate (irregular, dry, white, opaquecolonies)

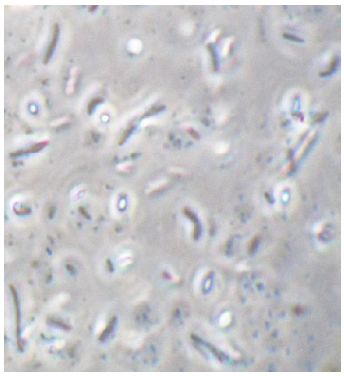


Figure: 3.3Phase contrast microphotograph

8) *Culture of Bacteria:* The basic culture of the Bacillus subtilis was obtained from micro biological laboratory. Whenever required a single colony of the culture is inoculated into nutrient broth of 25 ml in 100 ml conical flask and the growth condition are maintained at 37° C temperature and placed in 125 rpm orbital shaker.

B. *Bacterial Culturing And Sub - Culturing*

1) *Methods of obtaining pure culture:* To make the bacteria of pure culture of Bacillus subtilis the following procedure is followed,.
2) *The streak plate method:* Bacteria are picked on a sterile loop and streaked on the agar plate. When streaking is properly performed the bacterial cells will be sufficiently far apart in some areas of plate to ensure that the colony developing from one cell will not merge with that growing another.
3) *Methods of making Bacterial culture The pour plate method:* It is useful in enumeration of the bacterial cells. The disadvantages of pour plate method is that some of the organisms grow beneath the surface of the medium when it gets, therefore produces both surface and subsurface colonies. This is unsuitable for psychrophilic bacterial culture.
4) *The spread plate method:* In this method a drop of liquid contain a suspension of microorganisms is placed on the centre of an agar plate and spread over the surface of the agar using an L shaped glass rod. This is useful in isolating and enumerating the

bacterial cells present in suspension. The disadvantage of spread plate technique is its lengthy inoculation time. The cultures will be easily contaminated. We use streak plate method for making Bacillus subtilis culture

5) *Media:* A solution containing the nutrients necessary for the survival and growth of micro organisms is known as cultural media. Agar is an extract of weed; a complex carbohydrate composed mainly of galactose and is without nutritional value. It liquefies at hundred degrees Celsius and solidifies at 40 degree Celsius, so pathogens can be cultivated at 37.5° C or slightly higher temperature without the fear of the medium liquefying. When the agar is taken in test tube and hardened in a slant position, agar slants are produced. These are useful for pure maintaining pure cultures for longer periods of time. Similarly agar deep tubes are used to study the gaseous requirements of microorganisms and also to absorb the air motility.

6) *Materials Required:* Petri plates, Test tubes, inoculating loop, needle, Bunsen burner, culture.

7) *Procedure*

a) *Culturing*

i) The nutrient agar was prepared for 100ml and pH of the medium was checked and then autoclaved.

ii) The petri plates and test tubes were sterilized in the hot air oven.

iii) Under sterile condition, the stab, slant and agar plate were inoculated with the given culture.

iv) The culture was inoculated in the agar plate by streak plate method.

v) Then the plates, stabs and slants were inoculated 24-48 hours at 37◦C.

b) *Sub – culturing*

i) The broth solution is prepared for the given composition.

ii) The broth solution is inoculated in the laminar condition.

iii) Then it is placed in the shaker at 37◦C and a speed of 125rpm for 12 hours.

c) *Broth solution composition*

Peptone: 5 g/lt.

NaCl : 5 g/lt.

Yeast extract: 3 g/lt.

d) *Observation:* After inoculation the agar plates, stabs and slants were observed. The bacterial cells were found to be grown on the plates along the lines streaked on the medium. Also bacterial cells were absorbed in the slants and stabs. Also the number of cells present in the broth solution after shaking is observed by Haemocytometry.

Figure: 3.4 Sub – culture Bacteria

e) *Result:* Isolated colonies of bacterial cells were obtained.The Number of cells present in the broth solution after sub- culturing for 12hrs by Haemocytometry = 5.8×10^3 cells/ml

C. *Haemocytometry*

1) *Aim:* To calculate the number of cells present in the sub- culture solution.

2) *Principle:* Haemocytometry is a special microscopic slide with a counting chamber 0.1 cubic mm originally deviced for counting blood cells. It is used for counting the bacteria in the liquid suspension. The counting chamber has a total of 9 squares, each of 1mmX1mm graved over it but only one square per field is visible under 100 X microscope magnification. A 1mm

square is divided into 25 medium sized squares, 0.2 mm X 0.2 mm each, each of which is further subdivided into 16 small squares (0.05 mm X 0.05 mm each), thus a total of 400 squares of 1 mm. each medium sized square is separated by triple lines, the middle one acts as the boundary. Each large square has a volume of 1 mm X 1mm X 0.1 mm. The cell suspension is introduced and total cell number is determined mathematically by counting the number of cells in the chamber.

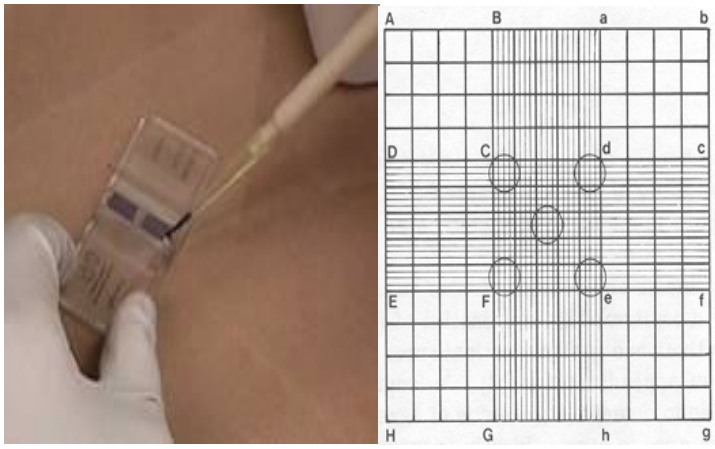


Figure: 3.5 Heamocytometer

3) *Materials required:* Counting chamber (Haemocytometer), cell suspension, pipette, microscope.
4) *Procedure:* The cover glass was placed over the grid and a drop of bacterial suspension was introduced in between cover slip and grid.The position of the microscope was adjusted till the cells were made clearly visible and the bacterial cells were counted in the chamber.
5) *Observation:* Total number of cells = 150 cells/0.025mm^2
6) *Result:*The total number of cells in the suspension is 5.8 x 10^{3}cells per ml.

D. Concept Of Bacterial Concrete

1) *Role of microorganisms in $CaCO_3$ precipitation :*The overall equilibrium reaction of calcite precipitation can be described below.

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3\downarrow$$

The solubility of $CaCO_3$ is a function of pH and affected by ionic strength in the aqueous medium (Stumm and Morgan, 1981). In urea-$CaCl_2$ medium that supports microbial growth, NH_4^+ and Cl^- also react with OH^- and H^+, respectively, at different pH, further interfering with chemically-induced $CaCO_3$ precipitation. Microbiologically-induced $CaCO_3$ precipitation occurs via far more complicated processes than chemically-induced precipitation. The bacterial cell surface with a variety of ions could nonspecifically induce mineral deposition by providing a nucleation site. Especially, Ca^{2+} is not likely utilized by microbial metabolic processes, rather accumulates outside the cell. In medium, it is possible that individual microorganisms produce ammonia as a result of enzymatic urea hydrolysis to create an alkaline micro-environment around the cell. The high pH of these localized areas, without a significant increase in pH in the entire medium at the beginning, apparently commences the growth of $CaCO_3$ crystals around the cell.

Possible biochemical reactions in urea-$CaCl_2$ medium to precipitate $CaCO_3$ at the cell surface can be summarized as follows.

$$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+}$$

$$Cl^- + HCO_3^- + NH_3 \rightarrow NH_4Cl + CO_3^{2-}$$

$$Cell\text{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3\downarrow$$

In addition, the results of kinetic studies render an explanation that the rate of $CaCO_3$ precipitation correlates with the cell growth and urease exhibits higher enzymatic activities and stronger affinity to urea at higher pH levels (pH 8-9) where calcite precipitation is favorable.

7) *Role of the microbial urease in calcite precipitation:* The role of the microbial urease was defined from the data that the calcite precipitation by B. pasteurii and E. coli expressing B. pasteurii urease was inhibited in the presence of a urease inhibitor and a significant amount of calcite precipitation was induced by E. coli, whereas little was induced by E. coli lacking urease genes. These observations support our assumption that the urease enzyme is a primary factor that initiates microbiologically-induced calcite precipitation. In detail, reaction 1 produces calcium carbonate and protons in aqueous medium where CO_3^{2-} primarily stays as HCO_3^-.

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$$

In biological systems, many calcareous organisms couple calcification to their metabolic assimilation processes to scavenge protons. In urease-based reactions, NH_3 released by the enzymatic hydrolysis of urea uses the protons generated from the calcite precipitation to produce NH_4^+.

$$NH_3 + H^+ \rightarrow NH_4^+$$

The subsequent increase of pH in surrounding medium due to the presence of ammonia ions and the additional release of CO_2 from the enzymatic urea hydrolysis further accelerate the rate of the urease-induced calcite precipitation. Thus, an active participation of urease is of essence in biochemical calcite precipitation**.**

8) Mix Proportion

Water	Cement	Fine aggregate	Coarse aggregate
191.6 litres	383 Kg	525.98Kg	1349.30 Kg
0.5	1.0	1.5	3.0

9) Note: We were made six concrete cubes of size 15 x 15cm, nine cubes with bacteria and three cubes without bacteria and six cylinders, diameter of 15cm & length 300cm, in this three cylinders with bacteria and three cylinders without bacteria.

IV. RESULTS & DISCUSSIONS

A. Compressive Strength Test

Figure: 4.1 Compressive Strength Test of Concrete

Table no: 4.1 Compressive Strength of Conventional Concrete

S.no	Size (mm)	Age of loading in days	Maximumload in Tones	Compressive Strength (N/mm^2)	Avg. (N/mm^2)
1.	150 x 150	7	28	12.20	12.20
2.	150 x 150	7	26	11.33	
3.	150 x 150	7	30	13.08	
4.	150 x 150	14	43	18.75	19.77
5.	150 x 150	14	48	20.93	
6.	150 x 150	14	47	19.62	
7.	150 x 150	28	53	23.11	24.83
8.	150 x 150	28	58	25.23	
9.	150 x 150	28	60	26.16	

Table no 4.2 Compressive Strength of Concrete with Bacteria

S.no	Size (mm)	Age of loading in days	Maximumload Tones	Compressive Strength (N/mm^2)	Avg. (N/mm^2)
1.	150 x 150	7	32	13.95	13.95
2.	150 x 150	7	34	14.83	
3.	150 x 150	7	30	13.08	
4.	150 x 150	14	53	23.11	23.84
5.	150 x 150	14	56	24.42	
6.	150 x 150	14	55	23.98	
7.	150 x 150	28	64	27.91	27.76
8.	150 x 150	28	61	26.60	
9.	150 x 150	28	66	28.78	

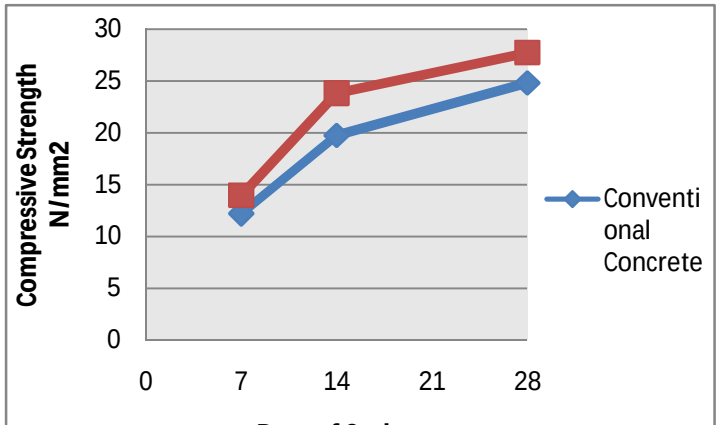


Figure: 4.2 Comparison of Compressive Strength

B. Result

Average Compressive Strength of the Concrete for

7 days = 12.20 N/ mm^2 14 days = 19.77 N/ mm^2 28 days = 24.83N/ mm^2

Average Compressive Strength of the Concrete for

7 days = 13.95 N/ mm^2

14 days = 23.84 N/ mm^2 28 days = 27.76N/ mm^2

C. Comparison

The Compressive Strength of Bacterial Concrete is more than 11.80% of the Conventional Concrete for 28 days Curing.

D. Split Tensile Strength Test

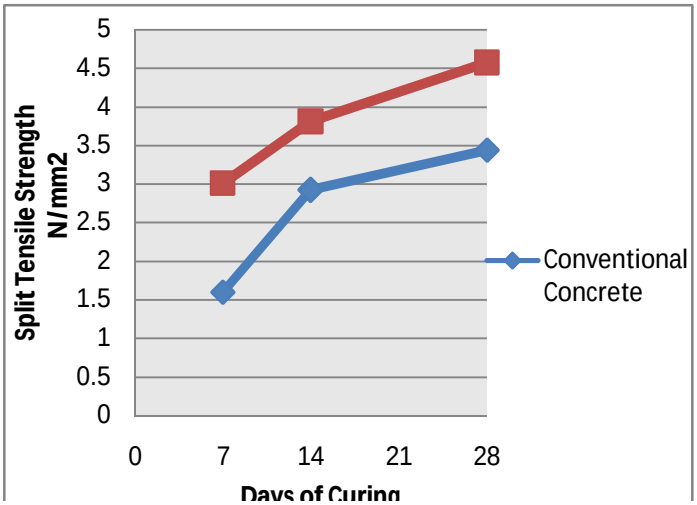
Figure: 4.3 Split Tensile Strength Test of Concrete

Table no: 4.3 Split Tensile Strength of Conventional Concrete

S.no	Dimension		Age of loading in days	Maximum loadTones	Split Tensile Strength =2P/πDL (N/mm^2)	Avg. (N/mm^2)
	Diameter (mm)	Length (mm)				
1.	150	300	7	14	1.98	1.60
2.	150	300	7	11	1.55	
3.	150	300	7	9	1.27	
4.	150	300	14	18	2.54	2.92
5.	150	300	14	21	2.97	
6.	150	300	14	23	3.25	
7.	150	300	28	24	3.39	3.44
8.	150	300	28	26	3.68	
9.	150	300	28	23	3.25	

Table no: 4.4 Split Tensile Strength of Bacterial Concrete

S.no	Dimension		Age of loading in days	Maxload Tones	Split Tensile Strength =2P/π DL (N/mm^2)	Avg. (N/mm^2)
	Diameter (mm)	Length (mm)				
1.	150	300	7	21	2.97	3.01
2.	150	300	7	20	2.82	
3.	150	300	7	23	3.25	
4.	150	300	14	25	3.53	3.81
5.	150	300	14	27	3.82	
6.	150	300	14	29	4.10	
7.	150	300	28	31	4.39	4.57
8.	150	300	28	34	4.81	
9.	150	300	28	32	4.52	



4.4 Comparison of Split Tensile Strength

E. Result

Average Split Tensile Strength of the Concrete for

7 days = 1.60 N/ mm^2

14 days = 2.92 N/ mm^2

28 days = 3.49 N/ mm^2

Average Split Tensile Strength of the Concrete for

7 days = 3.02 N/ mm^2

14 days = 3.81 N/ mm^2

28 days = 4.57 N/ mm^2

F. Comparison

The Split Tensile Strength of Bacterial Concrete is more than 30.94% of the Conventional Concrete for 28n days curing.

V. CONCLUSION

From the obtained split tensile strength and compressive strength results the incorporation of high numbers of bacteria in the concrete mix, result in a significant gain of strength due to self healing property of bacteria's. As the durability of bacterial concrete is increased with the increase in the concentration of bacteria more number of bacteria's may be added. Due to the inclusion of bacteria in concrete, we achieved approximately 10% of increase in compressive strength and also 30% increase in flexural strength. From the results it can be concluded that easily cultured Bacillus Subtiliscan be safely used in improving the performance and characteristics of concrete. Hence we can effectively use the Bacterial concrete in the structures, to get more strength and durability.

VI. SCOPE OF THE PROJECT

A. In future bacterial precipitated glue play vital role in the production of habitation agent to arresting of fine cracks formation the structure.

B. Due to culture of bacteria our surrounding Eco- System and fertility of soil will be preserved.

C. It serves as good remedial measures for Earthquake affected building.

D. The bacterial concrete widely used in earthquake resistance structure because of precipitation of calcite layer in the concrete offers lateral stability.

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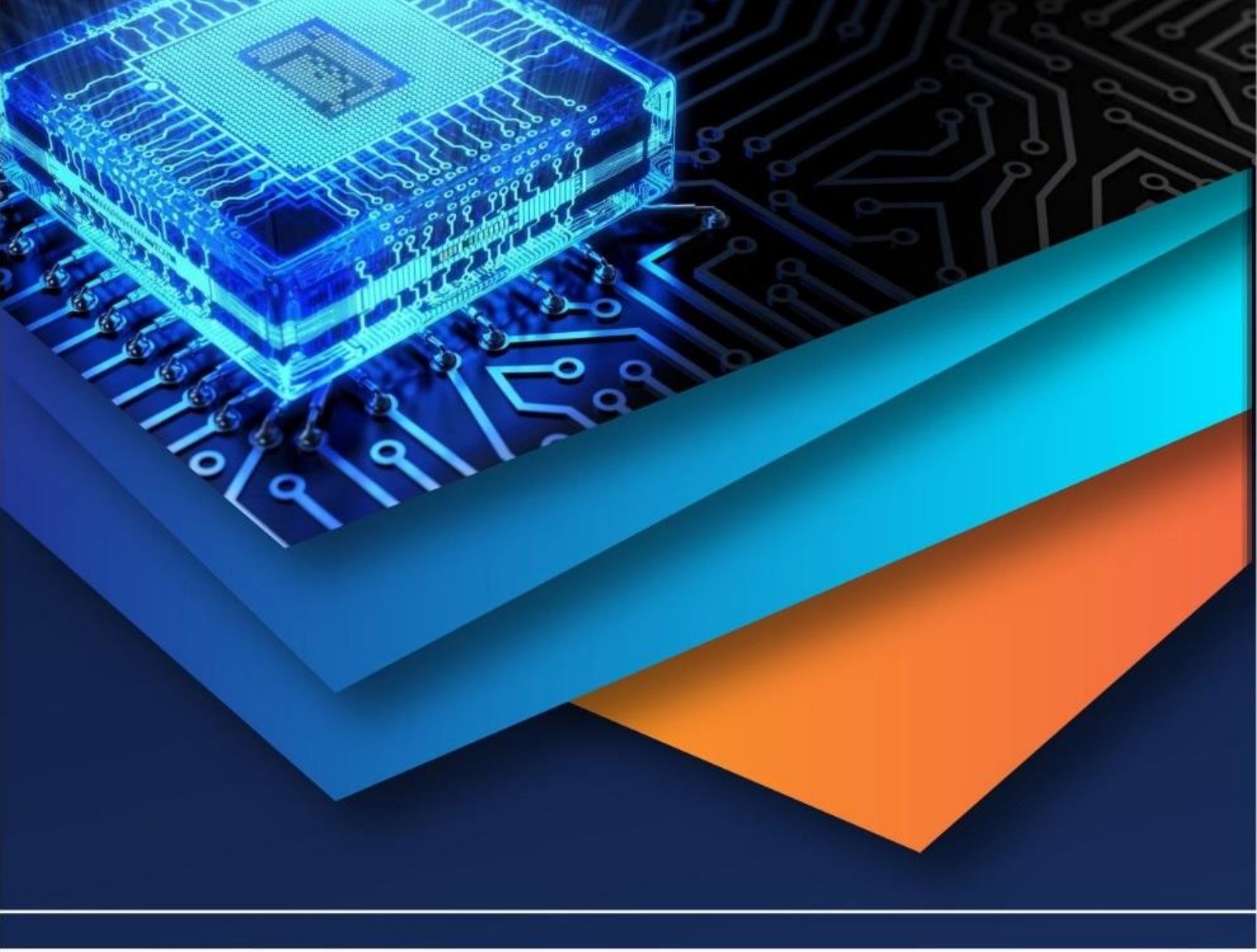

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