Exploratory Research using Bacteria (Bacillus Subtilis) as a Self-Healing Concrete: A Basis for Strengthening Infrastructure in the Philippine Setting

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ABSTRACT--- This study demonstrates that the utilization of microorganisms-Bacillus Subtlis is productive for development a tough framework and put forth a concentrated effort mending concrete as strategy for break control to upgrade administration life in solid structure. In this paper, the system Microbiologically Induced Calcite Precipitation (MICP) is embraced. It is the utilization of Bacillus Subtilis alongside its nutrients which is the Sodium Bicarbonate (NaHCo3), Ammonium Carbonate (NH4Cl), Calcium Chloride Dehydrate (CaCl2), and nutrient broth. The mixing proporton used is 1:2 ½: 5:0.45 along with 30 ml liquid form of Bacillus Subtilis with the cell concentration of 105 cells/ml. The strength of concrete mix is evaluated by conducting test on 150mm x 150mm x 150mm cube for compressive strength test, 6in x 12in cylindrical mold for split tensile strength test, 21in x 6in x 6in rectangular beams for flexural strength text and 3in x 6in for water absorption test, 3 specimens each test. All specimen utilized for recuperating is 4in x 2in x 2in which is deliberately broken. The investigation demonstrates that there is a noteworthy increment in the quality of cement added with bacteria or bacterial concrete contrasted with conventional concrete and in this manner calcium carbonate precipitation is obvious following 3-4 weeks in small scale splits.

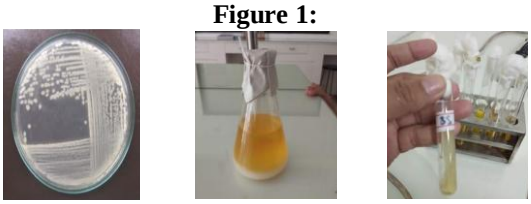
Keywords: Microbiologically Induced Calcite Precipitation, Bacillus Subtilis, Conventional concrete,Bacterial concrete.

I. INTRODUCTION

Cement concrete is the major material used in construction works which is recyclable. It is strong, locally available, durable and versatile. It is a composite material with combined fine aggregates, coarse aggregates, water, cement that hardens over time. And, no matter how the concrete mixture is handled it eventually leads in cracking. We all know that structures are susceptible to cracking which makes the water to enter and degrade the strength of concrete and needs expensive and highly health-risk maintenance in sealing of cracks.

Bacterial Cement is the new imaginative procedure in which the microscopic microorganism are added to blend with cement, fine aggregates, coarse aggregates, water and some selected chemicals like *Bacillus Pasteurii* and *Bacillus Sphaericus*which enhances the strength and acts as an excellent self-healing agent. These bacteria can be used to improve the compressive strength of a concrete. It can lie dormant within the concrete for decades and has the ability to pass the high pH of concrete. When there are cracks on the concrete, water sprayed on it or the moisture in the air triggers the bacteria to germinate and form limestone and seal the cracks. It is observed that the concrete-healing happen in 3 to 4 weeks and the bacteria can seal up to 0.8mm wide. Bacteria based system involves the use of ureolytic bacteria of genus *Bacillus* for the production of calcium carbonate minerals. The digestion of this family includes the enzymatic hydrolysis of urea to smelling salts and carbon dioxide. The response additionally makes an expansion of pH from nonpartisan basic conditions shaping bicarbonate and carbonate particles which hasten with the calcium particles in solid to frame calcium carbonate minerals. The formation of of calcium carbonate mends the pores and splits in the solid. Bacterial concrete is an example of connecting nature to construction

Figure 1:



(a) **(b)** **(c)**

a. Bacillus Subtilis on a Nutrient Agar
b. Bacillus Subtilis in liquid form
c. Bacillus Subtilis

II. METHODOLOGY

This study aims to analyze and evaluate the effectiveness of bacteria *Bacillus Subtilis* in compressive, flexural, split tensile, water absorption, also, how effective it is to seal cracks.

Preparation of Bacteria (Sub-Culturing)

Microbiological culture made by exchanging a few cells from past culture to crisp development medium. Sub-refined is utilized to drag out the life and additionally grow the

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quantity of cells of microorganisms in a culture.The ingredients are urea, Bacillus Subtilis, sodium bicarbonate (NaHCo3), ammonium carbonate (NH4Cl), calcium chloride dehydrate (CaCl2) and nutrient broth. These are the nutrients needed for the bacteria to survive and it also serves as its food or nutrient. The researchers used autoclave machine, test tubes, Erlenmeyer flasks, inoculating wire and biosafety cabinet.

Figure 2:

Ingredients in making Culture Medium (Bacteria's Nutrient Broth)

Process of Sub-culturing

The bacteria are cultured in mass on the culture medium. The culture is prepared using nutrient broth which consists of Urea Broth, Sodium Bicarbonate, Ammonium Chloride and Calcium Chloride Dehydrate. The culture medium is sterilized in autoclave at 121oC at the pressure of 151ps.

1. Dilute the 2.10 grams of nutrient broth, 1.50 grams of sodium bicarbonate, 7 grams of ammonium carbonate, 7 grams of urea broth and 5 grams of calcium chloride dehydrate in 1 liter of water. Stir well.
2. Sterilize the apparatus needed for the inducing of Bacillus Subtilis bacteria to reduce contamination.
3. Sterilize the mixed nutrients on the autoclave machine at a pressure of 151 ps (pressurized steam) for 30 minutes.
4. Inoculating needle will be used to extract a colony of bacteria placed in agar plates/ test tubes. At the biosafety hood, ready the sterilized nutrient broth and the Bacillus Subtilis bacteria. Use the inoculating wire, be sure that the loop is red-hot.
5. Test tube openings are run through the heat to make sure that there are no other contaminants in the tube entrance.
6. Close the test tubes tightly with cotton to reduce contamination.
7. Put the test tubes and the Erlenmeyer flasks with Bacillus Subtilis bacteria at the incubator for 18 to 24 hours at room temperature for it to grow.

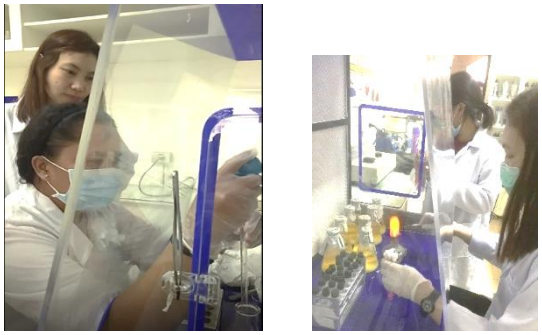
Figure 3
Measuring and mixing of Ingredients for Nutrient Broth

(a) (b)

Figure 4
Autoclaving of Nutrient Broth and Sterilizing of Materials

(a) (b) (c)

Figure 5
Inducing of Bacillus Subtilis on the Sterilized Nutrient Broth



(a) (b)

Bacterial urease chemical corrupts into alkali and carbon dioxide, which lead to increment in pH of the media and carbonate precipitation. Different looks into have affirmed the steadiness of natural CaCO3 (Calcium Carbonate) hasten in condition for an all-inclusive timeframe utilizing Bacillus Subtilis.

Mixing of Conventional Concrete

The researchers opted to use the concrete mixture 1:2 1/2:5:0.45. Which is 1 cement bag, 2 and a half bags of sand, 5 bags of coarse aggregates and 0.45 for the water-cement ratio.

1. Mix the cement, sand and coarse aggregate manually on a platform.
2. Add water and mix until the desired mixture was attained.
3. Place it in the mold, fill it in three layers.
4. Compact each layer with 25 strokes using a tamping rod and smoothen it with a trowel.
5. Level the top surface and smoothen it with a trowel.

III. RESULTS

Preparation of Specimen

Standard grade concrete design mix is made with cylinders (6"x12") and (3"x3"), rectangular molds (21"x 6"x 6") and cubes (6"x6". These specimens are casted with bacteria and without bacteria. Hardened and demolded after 24 hours.

Table 1

	Strength Test		
	Compressive	Flexural	Split Tensile
7 Days			
Conventional	3	3	3
Bacterial	3	3	3
14 Days			
Conventional	3	3	3
Bacterial	3	3	3
28 Days			
Conventional	3	3	3
Bacterial	3	3	3
	Water Absorption Test		
Conventional	3		
Bacterial	3		
TOTAL			60

1. Compressive Strength Test - Compressive loading tests were conducted a compressive testing machine, the Universal Testing Machine (UTM) with capacity of 3000KN, at a loading rate of 2.5kN/s was applied as per ASTM C-39/C-78; AASHTO T22.

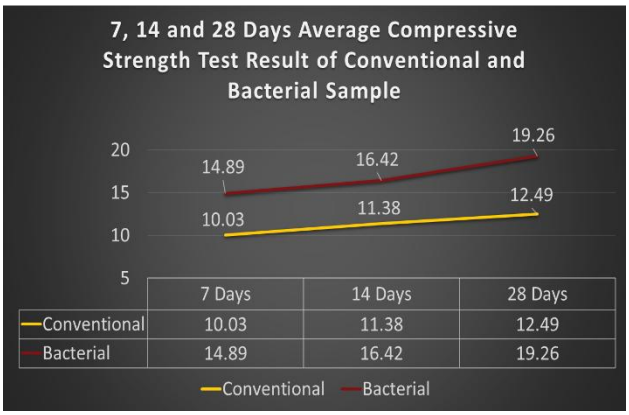


Table 2

2. Split Tensile Strength Test - This property for concrete relates to its tension strength. This is obtained by performing split tensile test on concrete specimen. The concrete specimen in this test is taken as cylindrical in shape. Tensile strength for concrete specimen is defined as the tensile stresses developed due to application of the compressive load at which the concrete specimen may crack.

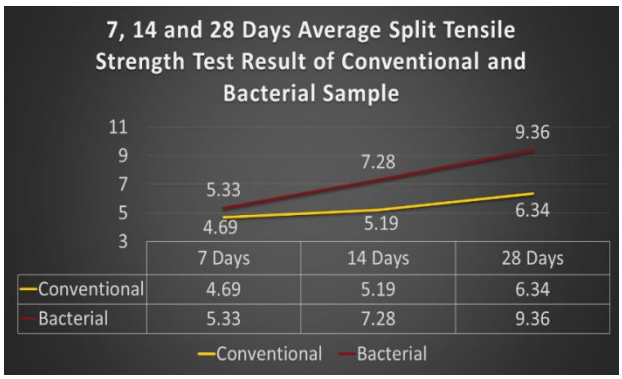


Table 3

3. Flexural Strength Test - Turn the specimen on its side with the regard to its position when formed. What's more, focus it on the supporting bearing squares. The heap-applying squares will be acquired contact with backings.

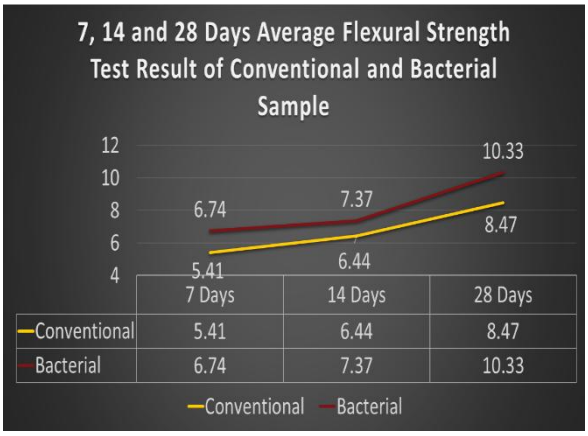


Table 4

4. Water Absorption Test – This test is likewise emphatically influenced by the dampness state of the solid at the season of testing including the length of introduction and temperature. The data sheds light on the performance of the materials in water or humid environment.

Conventional Concrete				
Sample	Hardened	Immersed	Oven Dry	Water Absorption
1	981	996	947.6	4.86
2	982	997	949.8	4.73
3	980	995	958.6	3.66
Average	981	996	952	**4.42**
Bacterial Concrete				
Sample	Hardened	Immersed	Oven Dry	Water Absorption
1	920	938	918	2.13
2	932	950	920	3.16
3	929	947	925	2.32
Average	927	945	921	**2.54**

Table 5

Sealing of Cracks due to Calcium Carbonate Precipitation

Bacillus Subtilis has been used to precipitate calcium carbonate in order to seal cracks. Microbiologically Induced Calcite Precipitation or MICP is a phenomenon concerning the urease enzyme and can be effectively used wherein the bacteria is mixed with nutrient broth to create conditions where they can seal cracks and pores. Calcite deposits where observed to be effective at sealing cracks and consolidating the surface finish of the concrete. This process can be considered as an organic remediation in healing concrete and that can be used in other industrial applications. Calcite precipitation is able to reduce moisture ingress. The conditions for the crack to heal is when the concrete or the crack is in contact with moisture that is the time the bacteria will activate or will self-heal.

Bacterial Concrete Sample

An intentionally cracked specimen

WEEK 1

Figure 6

(a) Week 4 (b). Week 8

Figure 7

IV. DISCUSSION

1. The researchers conclude that the concrete added with 30 ml of liquid form of Bacillus Subtilis can withstand the compressive, flexural and split tensile strength test.
2. The water absorption of bacterial concrete has lower rate compared on conventional concrete, this is because of the microorganisms actuated development of calcium carbonate in the voids in concrete, leading to a lesser void and hence a lesser permeability.
3. Bacillus Subtilis can be produced in the laboratory and it is proven to be safe because its biosafety level is only 1 and it is a bacterium that can be found in soil.
4. The compressive strength test, the researchers found out that there is increase in strength compared to conventional concrete and its highest percentage increase is 35.15%.
5. In split tensile strength test, the bacterial concrete with cell concentration of 105 gives the optimum result which is proven by 12.01% in 7 days, 28.71% in 14 days and 32.26% in 28 days of curing.
6. In flexural strength test, the researchers observed that among the three (3) curing days, the 7 days have an increase of 19.73%, the 28 days have an increase of 18.01%, while the 14 days have an increase of 13.98%. Its average increase is 17.24%.
7. 7, 14- and 28-days test results shows that absorption rate in concrete with bacteria lead to the reduction in water absorption compared with conventional concrete.

V. CONCLUSION

The trial consider demonstrates the expansion of microscopic organisms that the addition of bacterial solution-*Bacillus Subtilis* in concrete shows improvements in different properties of concrete as far as compressive quality test, split tensile strength test, water absorption test, and flexural quality test. As the microbes can delivers in the research center, it could be ended up being exceptionally protected and doesn't give treat to human health. The examination achieved utilization of microscopic organisms in cement enhances its strength and durability hence using this type of bacteria for self-healing mechanism in concrete can produce cost effective strong or durable structures.

VI. RECOMMENDATION

With the data and computations presented in this study, the researchers analyzed and studied the performance of bacteria Bacillus Subtilis in concrete and recommend the following for better production of concrete mixtures

- The use of other method in applying bacteria in concrete like microencapsulation system.
- The use of different ureolytic endospore-forming bacteria such as E-Colii, SporosarcinaPasteurii, Pseudoformis, and Shewanella Species.
- Considering other brands of cement as it may varies in some properties such as strength and durability
- For future work, the researchers would also recommend to test other parameters such as porosity, acid resistance and chloride resistance
- The researchers would also recommend to check if paint can affect the healing process of bacterial concrete
- The use of SEM (Scanning Electron Microscopy) is also recommended to check/see the healed percentage of cracks in concrete
- More trials with different volume/cell concentration of bacteria are recommended to get different outcomes and possible higher strengths.
- To likewise do strength tests on specimen/s that was proposed distinctly for recuperating and check whether there was an increase in strength and also have specimen/s that is 3 months or more progressively seasoned and have it go through a concrete strength test.

REFERENCES

1. SalmabanuLuhar, SutharGourav 1. Research Scholar, Malaviya National Institute of Technology, Jaipur 2. U.G. Student, Civil Engineering Department, Jaipur J. U., '*A Review Paper on Self-Healing Concrete'*
2. GauravAgarwal, Rahul Kadam Civil, Mumbai University, India-'*Bacterial Concrete – A Solution to Crack Formation'*

3. P. Arulsivanantham, R. Gokulan, Divyalakshmi.M.V, Karthikeyan.S, ShilpaAchuthan. Lecturer, Department of Civil Engineering, Ambo University, Ambo– 19, Ethiopia, '*Bacterial Self-Healing Concrete – An Innovative Repair Technique*'
4. ASTM C192 – Standard Practice for Making and Curing Concrete Test Specimens in the Laboratory
5. ASTM C143 – Standard Test Method for Slump of Hydraulic-Cement Concrete
6. L.Soundari Associate Professor Department of civil Engineering, IFET college of Engineering, Villupuram, C.S.Maneesh Kumar Senior Assistant professor Department of civil Engineering, IFET college of Engineering, Villupuram, S.Anthoniraj UG graduate Department of civil Engineering, IFET college of Engineering, Villupuram, E.Karthikeyan UG graduate Department of civil Engineering, IFET college of Engineering, villupuram,- "AN EXPERIMENTAL STUDY ON STRENGTHENING OF CONCRETE BY USING BACTERIAL MINERAL PRECIPITATION, International Journal of Core Engineering & Management (IJCEM) Volume 2, Issue 9, December 2015