

Temperature Effect on Various Bacteria Used in Microbial Concrete

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ABSTRACT: Bio-concrete or bacterial concrete is being extensively studied by many researchers to understand and enhance its mechanical properties by different bacteria in different environment and nutrients. Even though much research has been done, sufficient understanding in the bacteria used in the bio-concrete has not took place. Concrete, even though an excellent construction material has one distinct flaw, it is weak in tension. The heavy loads which are acting on the concrete makes concrete susceptible to creep, strain and causes micro cracks which becomes macro cracks and adversely affect the structural integrity of the structure if not repaired in time. The resins and epoxy's used in repairing concrete are harmful to the human beings and surrounding environment and is quite expensive. However, bio-concrete plays a very important role in creating a sustainable environment by undergoing a kind self –healing called microbiologically induced calcite precipitation (MICP), with addition of a certain species of bacteria which is capable of producing insoluble CaCO_3 bio-concrete is made. The bacteria consumes the calcium chloride which is provided in the concrete as a nutrient in the presence of water which enters the crack due to various natural phenomenon like rain.

This paper deals with the study of the different kinds of bacteria which are used in the bio-concrete and their effect on the strength parameters of the concrete like compressive strength and water absorption under the influence of the factors like bacterial cell concentration at different temperatures is explicitly studied.

KEYWORDS: Compressive strength, Water absorption, Bacterial concrete, Microorganisms, Microbial concrete.

I. INTRODUCTION

Concrete as a construction material has been used in various forms of construction works, and is identified as the most important material and extensively used material in this field [1].improvements have been made throughout the 20th and 21st century to the concrete and various types of concrete have been developed depending on the circumstantial requirement of the engineering sites, but significant interest was not paid on the improving the performance of the concrete by sealing the cracks in the concrete. The cracks in the concrete is caused by several external and internal factors, While internal factors often gives rise to the micro cracking in the structure, the macro cracks are often produced due to the external forces and cycles of repetitive stresses caused due to the loadings which causes the cracks to become enlarged over the time. If these enlarged cracks or macro cracks are not sealed, overtime water carrying the impurities like chlorine, sulphur and like aggressive elements ingress in to the concrete and causes phenomenon like sulphate and chloride attack which results in the failure of the concrete, The oxygen carried by the water reacts with the reinforcement in the concrete and forms rust which is a form of corrosion of concrete and is an electro chemical process [6], destroying the tension bearing capacity of the steel.

Bio-concrete is a type of concrete where a bacterial microbes are used in the concrete for the purposes like 1) sealing cracks 2) increasing compressive strength 3) increase in durability and sometimes used as a water purifier .The mixture of bacteria and it's nutrient medium is called Bio-caulk and nutrient medium of the bacteria must be chosen that it must not affect the setting time or strength of the concrete. The type of the bacteria chosen must be able to survive in high alkaline medium as the PH of the concrete is around 11 to 12 and the by-products generated must not be harmful to the surrounding environment. In the environment there are several microbial microorganisms which precipitates chemical calcium carbonate which are responsible for the bio deposition of calcium carbonate mineral phases in natural or laboratory conditions [2, 3].Moreover, this calcite layer can also improve the impermeability of the specimen, thus

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(An ISO 3297: 2007 Certified Organization)

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increasing its resistance to alkaline, sulphate and freeze-thaw attack[4]. fly ash, silica fume and ground granulated blast furnace slag (GGBS) are commonly used to replace a portion of cement inside concrete along with bacteria to improve various mechanical parameters [5]. An endospore forming bacillus group *S. pasteurii* soil bacterium precipitates calcite in environment by producing a urease enzyme which catalyses urea to produce carbon dioxide and ammonia which results in the increase of pH of the surroundings. It is beneficial for the steel in the concrete as highly alkaline environment abets the corrosion resistance property of steel, where oxidation of steel decreases significantly in such environment. The use of biological approach in concrete is also considered as a green technology as its production does not involve greenhouse gas emission. Therefore bacterial induced Calcium Carbonate (Calcite) precipitation has been proposed as an alternative and environment friendly way for improvement of strength of building materials [19].

II. RELATED WORK

Jonkers et al (2008) states that the bio-deposition of the calcium carbonate crystals in the between the concrete pores acts as a filler material and helps in reducing capillary pores of the concrete. As a result concrete capillaries are significantly reduced due to the filling of pores and it decreases the permeability of the concrete thus significantly increasing the durability of the concrete. Many research papers have proposed different bacteria that can be used in the bio-concrete but the basic criteria for the bacteria selection depends on the various factors like the bacteria strain used must be able to survive high temperature, High pH conditions, be able to precipitate calcium carbonate in high amounts and must be able to survive in extreme conditions. A.T. Manikandan, A. Padmavathi et al (2015) used Bacillus subtilis strain 121 which confirmed that the bacteria is able to survive at -3c to 70 c high temperature. The paper discussed about the use of bacteria in self-healing of the concrete and improve the Serviceability of the concrete by mineral precipitation. Sathesh Kumar Annamalai et al states that even though the bacillus pasteurii must be able to survive in high pH of around 11, the most optimum growth for the bacteria was at 7.5 which shows that the maximum bio-deposition by the bacteria occurs in optimum conditions of 30 degree Celsius. But the bacteria is often subjected to the extreme environments, therefore the best bacteria selection depends on its survivability factor. S. Soundarya et al (2014) uses four different kinds of bacteria named bacillus pasteurii, escherichiacoli, bacillus sphaericus, bacillus subtilis, bacillus cereus which all belong to bacillus group are urease bacteria and are negatively strained. All the specimens containing these samples were subjected to the extreme heat and its compressive strength parameter are measured. Wang et al (2014) states that the mean size of bacteria is 1-3µm that means the size of the bacteria and the pores of concrete determination is vital for the bacteria mobility in concrete.

In this research paper two bacillus bacterium are selected namely bacillus sphaericus and bacillus cereus. The two bacteria are provided with similar growth medium and nutrient medium. The bacteria containing bacterial concentration of $10^0, 10^4, 10^5, 10^6, 10^7$ is added directly while preparing concrete samples. The samples containing the bacteria are submerged in the nutrient medium for 27 days and then some samples were heated to a temperature of 200°C in the furnace for 3 hr. The tests compressive strength and water absorption were conducted on 28th day and the results are discussed in this paper.

III. EXPERIMENTAL PROGRAM

Bacteria Used: The bacteria samples are bacillus sphaericus LMG 22557 and bacillus cereus were collected from National Collection of Industrial Microorganisms (NCIM) [10].

Growth liquid medium: The growth liquid medium consisted of 5.0 g peptone and 3.0 g meat extract per liter distilled water. A further addition of 1.5% agar in to the medium was introduced along with 0.01g MnSO₄.H₂O. and the pH was adjusted to 7 and autoclaving was done for 20 min at 120 Celsius [7,8]. The mixture is allowed to cool to 37 Celsius as it is ideal for the bacteria growth [9]. The above solution is provided for the bacteria on suggestion of supplier recommendation for the bacteria. The two bacterium species are introduced in the same nutrient but in different containers. The culture were then incubated at 120rpm at 37 celcius for 72 hrs. The bacterial cell concentration is determined by serial dilution technique.

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Nutrient medium: It is the medium where the bacterial concrete specimen are allowed to submerge for 28 days in this experiment. The nutrient medium contains 10g/L yeast extract, 5g/L NaCl, 25mM CaCl₂ and 20g/l urea under aerobic conditions [11].

Concrete specimen preparation: The specimens with size 100×100×100 mm were casted in accordance to the guidelines provided in the IS 10262-2009. The specimens are casted in a number of 6 for each bacterial concentration and note that the two bacterium were used. The bacterial concentration used while casting the specimens is 10⁰, 10⁴, 10⁵, 10⁶, 10⁷. A total number of 30 specimens were casted. The concrete specimens are made of the Fine aggregate, Coarse aggregate, Cement and Water as shown in table 1 [12].

Table 1
Combination used for casting

MIX DESIGNATION	CEMENT(kg/m ³)	FINE AGGREGATE (kg/m ³)	COARSE AGGREGATE (kg/m ³)	WATER (kg/m ³)
CONTROL CONCRETE	435	800.4	1010	197.6

Methodology: The specimens which are casted are submerged in to the nutrient medium for a period of 28 days and left for the bacteria to grow and deposit calcium carbonate inside the concrete pores. All the specimens are separated as triplicates in to two classes based on temperature to which they have to be heated and each triplicate series has a bacterial concentration of 10⁰, 10⁴, 10⁵, 10⁶, 10⁷ respectively. The first triplicate series were maintained at room temperature. Second triplicate series were heated to a temperature 200 Celsius for 3 hrs. In a furnace on 27th day and again immersed in nutrient media for 24 hour, before testing them for water absorption and compressive strength. The average compressive or water absorption values obtained from triplicate series are chosen as the final values. Note that the two bacterium series have total 60 specimens to test. The cubes are immersed in the medium as shown in fig-1 and second class of cubes are heated at a temperature of 200°C as shown in fig-2.



Figure-1

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Fig-1 shows the immersion of cubes and before immersion of cubes, the cubes must be completely cleaned on the surface by a wet cloth to clear any dirt or grease present on the cube which might be present due to the grease applied on the mold while casting, grease may obstruct the water from entering the cube. Sufficient care must be taken while placing the cubes in the nutrient medium.



Figure-2

Fig-2 shows the cube placed in oven to be heated. The furnace must be cleaned thoroughly and cubes must be placed with sufficient gaps between them such that no two cubes must be in contact with each other and must be placed at a gap to let hot air pass between them. The test cubes must be heated to a temperature of 200°C for 3 hrs and must be left in the furnace for 30 min to let them cool before testing them.

Compressive Strength Test: The 28 day compressive strength of the control specimen and two bacterial specimens of concentration 10^0 , 10^4 , 10^5 , 10^6 , 10^7 is tested according to the guidelines prescribed in the IS: 516-1959 [13] the results are shown in table 2.

Water Absorption Test: It is performed to observe the increase in resistance of the cubes to water penetration. A sorptivity test is performed based on the guidelines provided by the RILEM 25 PEM (II-6) [14,18]. All the four sides adjacent to the testing side are coated with the polysiloxane and one layer of silicon layer. The cubes were then dried in the oven at 45 Celsius for 30 minutes. The second class of the specimens were heated in furnace at 200 Celsius for 3 hours before coating them. The results are shown in table 4 and 5.

IV. RESULTS

The compressive strength of the specimen with bacillus sphaericus LMG 22557 and bacillus cereus after 28 days at a temperature of 30 Celsius and after heating the specimens in an oven at 200 Celsius for 3 hrs at 27th day and immersed in nutrient medium for 24 hr is taken.

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Table 2
Shows the compressive strength results

SL.NO	BACTERIAL CONCENTRATION (cells/ml)	At 28 days and 30°C		At 28 days and 200°C	
		BACILLUS SPHAERICUS (N/mm ²)	BACILLUS CEREUS (N/mm ²)	BACILLUS SPHAERICUS (N/mm ²)	BACILLUS CEREUS (N/mm ²)
1)	10 ⁰	29.8	29.8	28.2	28.2
2)	10 ⁴	32.5	31.4	30.5	29.7
3)	10 ⁵	38.7	37.2	35.7	32.2
4)	10 ⁶	37.8	36.5	37.8	39.5
5)	10 ⁷	35.8	35	34.8	32.7

Table -2 shows the compressive strength results tested at 28th day and the results are shown in the table. The first class of cubes are at room temperature but second class of cubes are heated to a temperature of 200°C before testing them.

Table-3
Table 3 shows Water Absorption Test results

SL.NO	BACTERIAL CONCENTRATION (cells/ml)	At 28 days and 30°C		At 28 days and 200°C	
		BACILLUS SPHAERICUS (Kg/m ²)	BACILLUS CEREUS (Kg/m ²)	BACILLUS SPHAERICUS (Kg/m ²)	BACILLUS CEREUS (Kg/m ²)
1)	10 ⁰	5.2	5.2	7	7
2)	10 ⁴	3.9	4.8	6.2	6.4
3)	10 ⁵	1.2	2	5	5
4)	10 ⁶	2.4	2.8	4.2	3.8
5)	10 ⁷	3.1	3.2	4.4	4

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Table-3 shows water absorbed by the cubes at 28th day, the water used for the experiment must be pure and specimens are tested in the sequence of its bacterial concentration the cubes must be dry before testing them for water absorption.

V. DISSCUSSIONS

Compressive strength: Fig-3 shows that the optimum bacterial cell concentration for maximum compressive strength is 10^5 cells/ml. The strength attained by bacillus sphaericus is greater than that of the bacillus cereus. This might be because of the small size of the bacillus sphaericus when compared to the size of bacillus cereus which enables it to penetrate better in to the pores of the concrete. The mean size of bacteria is about 1-3 μm [15] and it bio-deposits the CaCO_3 which results in better strength (Further research required). The strength decreases after 10^5 cells/ml is because the bacteria produces more than necessary amount of the CaCO_3 this affects the structural integrity of the concrete cube, the crystals arrangement in the concrete gets distorted due to excess deposition and results in decrease of compressive strength in the concrete after certain bacterial concentration. Fig-3 shows that the optimum bacterial concentration is 10^6 cells/ml, while the overall compressive strength is less while compared to the cubes at 30°C but strength increase in bacillus cereus is greater than that of the bacillus sphaericus at this concentration. This might because of the dead cell deposition in the pores of concrete when the bacteria dies due to high temperature. The strength correspondingly increases with the surface area of the bacteria. Having a greater surface area [17], bacillus cereus gives additional strength at 10^6 cells/ml concentration. The maximum strength obtained at the 10^6 cells/ml is due to extreme temperature which causes loss of bacteria due to its death and the dead bacteria acts as a better filler material (which is covered with CaCO_3) which helped in increase of strength in case of bacillus cereus.

Water absorption test: Fig-4 shows the amount of water absorbed by the cubes heated to 200°C is more than that of the amount of the cubes at 30°C. It is because when the cubes are heated beyond 100°C the hydrates bonding with the Silica gel gets vaporized due to the heat, this causes reduction in strength because the autogenously healing capacity of the concrete decreases significantly because of missing hydrates. Therefore when placed in water this cubes absorb more water than the conventional ones as there is no moisture present in specimen. At 200°C the water absorption of the bacillus cereus at 10^6 cells/ml is less than that of bacillus sphaericus this might be because of pores of the concrete getting occupied by the large size bacillus cereus which gives reduces the water absorption into the concrete which occurs due to capillary effect (more research needed).

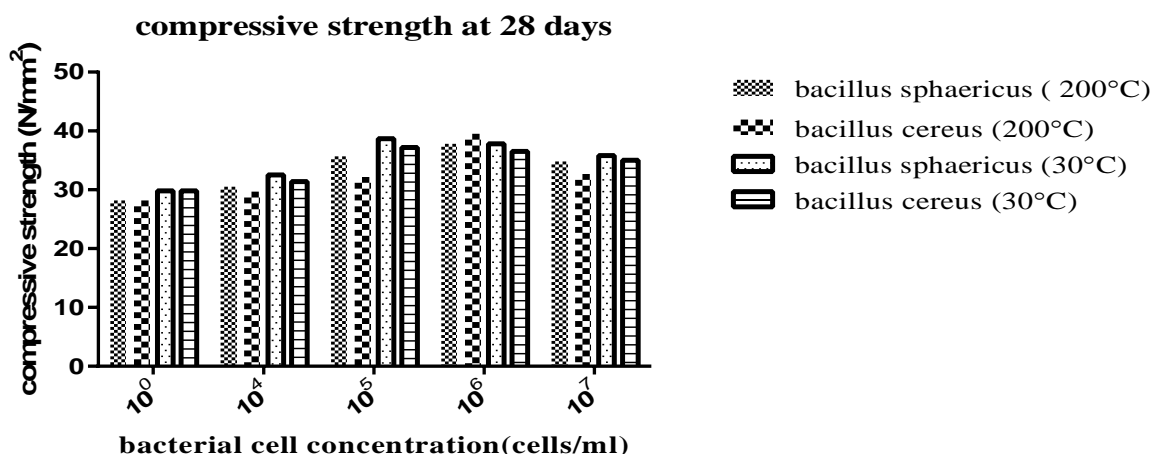


Figure-3

Fig-3 shows the graph of compressive strength of the test specimen at 28 days as we can see from the graph bacillus sphaericus at 30°C attains maximum strength at 10^5 cells/ml.

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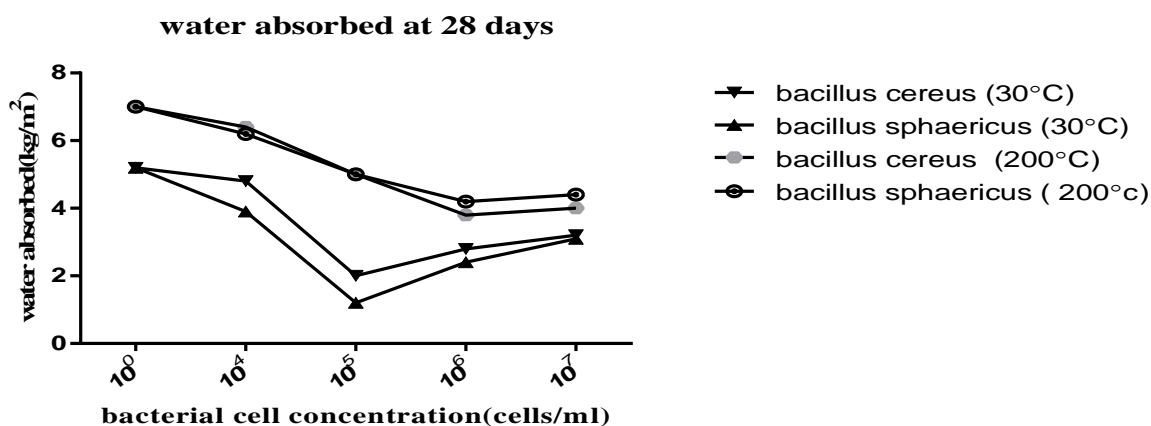


Figure-4

Fig-4 shows the water absorbed by the test specimens at 28 days the lowest amount of water is absorbed by the bacillus sphaericus at 10⁵ cell/ml concentration at 30°C.

VI. CONCLUSIONS

The most optimum pH for the bacteria growth is 7.4. The maximum compressive strength attained by the cube containing bacillus sphaericus is 38.7 kN/mm² at 10⁵ cells/ml at 30°C and bacillus cereus achieved 39.5 kN/mm² at 10⁶ cells/ml at 200°C. The most resistance offered to the water absorption is bacillus sphaericus as its water absorption is only 1.2 Kg/m² at 30°C (10⁵ cells/ml) and water absorption by bacillus sphaericus is least at 3.8 (10⁶ cells/ml). Therefore it can be concluded that bacillus sphaericus can be used as in bacterial concrete at lower temperature and bacillus cereus can be used in bacterial concrete subjected to high temperature. The optimum cell concentration of bacillus species at 10⁵ cell/ml is in accordance with seshagirirao et al (2013) and atayers et al(2015).

ACKNOWLEDGEMENTS

The author acknowledges the cooperation extended by various lab in-charge during the project and grateful thanks to the Head of Department of Civil Engineering and personally thanks Dr.Jagannathan for his guidance, providing the bacterial culture and his kind support throughout the project.

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