STUDY ON STRENGTH OF THE BACTERIAL CONCRETE IN BACILLUS MEGATERIUM

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ABSTRACT--Concrete a strong, durable material composed of cement, aggregate and water is the most used building material in the world. Concrete has an ultimate load bearing capacity under compression but the material is weak in tension. The steel reinforced bars take the load when the concrete cracks in tension. To increase the strength and durability of the structure either crack that are formed should be repaired conventionally using epoxy injection or latex treatment or by providing extra reinforcement in the structure during the design phase to ensure that the crack width stays within a permissible limit. A reliable self-healing biological app of water approach is first proposed by V. Ramakrishnan to make use of bacterial concrete to heal cracks in concrete structure.

The experiments are conducted to find the compressive strength, split-tensile strength and flexural strength of the normal concrete mix of 30Mpa with a water–cement ratio of 0.39. In this study mix proportions for M30 grade of concrete with different concentration of bacteria (Bacillus Megaterium) adding 5g in 1lit/m^3 of water.
Keywords: bacillus megaterium, durability, workability, tension, compression, etc.

I. INTRODUCTION

In concrete, cracking is a common phenomenon due to the relatively low tensile strength. High tensile stresses can result from external loads, imposed deformations (due to temperature gradients, confined shrinkage, and differential settlement), plastic shrinkage, plastic settlement, and expansive reactions (e.g. due to reinforcement corrosion, alkali silica reaction, sulphate attack). Without immediate and proper treatment, cracks tend to expand further and eventually require costly repair. Durability of concrete is also impaired by these cracks, since they provide an easy path for the transport of liquids and gasses that potentially contain harmful substances. If micro-cracks grow and reach the reinforcement, not only the concrete itself may be attacked, but also the reinforcement will be corroded when it is exposed to water and oxygen, and possibly carbon dioxide and chlorides. Micro-cracks are therefore precursors to structural failure.

For crack repair, a variety of techniques is available but traditional repair systems have a number of disadvantageous aspects such as different thermal expansion coefficient compared to concrete and environmental and health hazards. Therefore, bacterially induced calcium carbonate precipitation has been proposed as an alternative and environmentally friendly crack repair technique.

Microbial mineral precipitation (biodeposition) involves various microorganisms, pathways and environments. Considerable research on carbonate precipitation by bacteria has been done by using ureolytic bacteria. These bacteria are able to influence the precipitation of calcium carbonate by the production of a urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of the pH and carbonate concentration in the bacterial environment. It is suggested that a new treatment should be applied after 10 years. Bacterial deposition of a layer of calcite on the surface of the specimens resulted in a decrease of capillary water uptake and permeability towards gas.

1. BACTERIAL CONCRETE

Cracks were also repaired by the use of $CaCO_3$ precipitating bacteria. Within the framework of previous research, Bacillus Megaterium and Azotobacter strains had been isolated from soil coming using ureolytic calcification reactor. On the basis of their morphology six uniqu2e strains were distinguished. The purified strains had been deposited in culture medium. For this purpose, the strain was chosen for the treatment of the samples because of its optimal $CaCO_3$ precipitation capabilities. To protect the bacteria from the strong alkaline environment in concrete, the bacteria were, for some of the treatments, immobilized in silica gel. Also the bacteria are cultured to form different cell concentration under peptone condition. The concrete cubes with the standardized cracks show only a crack at one side, so these samples with artificially cracks are made. The organism which has capability of deposition of $CaCO_3$ are,

2.Bacillus pasteurii

3.Bacilla filla

4.Bacillus megaterium

5.Bacillus pseudofirmus

6.Bacillus sabtilis

7.Azotobacter

1.2 NEED FOR THE PRESENT STUDY

- As synthetic polymers, currently used for concrete repair, may be harmful to the environment, the use of a biological repair technique is investigated in this study.
- The bacterial degradation of urea locally increases the pH and promotes the microbial deposition of carbonate as calcium carbonate in a calcium rich environment. These precipitated crystals can thus fill the cracks.
- It was seen that pure bacteria cultures were not able to bridge the cracks in the form glues. However, when bacteria were protected in silica gel, cracks were filled completely.
- As presently about 7% of the total anthropogenic atmospheric CO_2 emission is due to cement production, mechanisms that would contribute to a longer service life of concrete structures would make the material not only more durable but also more sustainable.
- The use of this biological repair technique is highly desirable because the mineral precipitation induced as a result of microbial activities is pollution free and natural.

1.3 OBJECTIVE

To study the growth of bacteria in cracks and also to determine how effective it will decrease the pores in the concrete by using two different concentrations of two different bacteria.

II. LITERATURE REVIEW

1. **Department of Biotechnology, Faculty of Applied Sciences, Julianalaan, The Netherland(2008)** reported on application of bacteria as self-healing agent for the development of sustainable concrete. The

application of concrete is rapidly increasing worldwide and therefore the development of sustainable concrete is urgently needed for environmental reasons. In this study we investigated the potential of bacteria to act as self-healing agent in concrete, i.e. their ability to repair occurring cracks. A specific group of alkali-resistant spore-forming bacteria related to the genus Bacillus was selected for this purpose. Bacterial spores directly added to the cement paste mixture. A continuous decrease in pore size diameter during cement stone setting probably limited life span of spores as pore widths decreased below 1 μm, the typical size of Bacillus spores. Thus potential application of bacterial spores as self-healing agent appears promising.

1. **Willem De Muynck, Kathelijn Cox Nele De Belie,Willy Verstraete,(2006)** reported on deposition of a layer of calcite on the surface of the specimens resulted in a decrease of capillary suction and a decrease in gas permeability. Bacterial carbonate precipitation as an alternative surface treatment for concrete. B. sphaericus LMG 225 57 (BCCM, Gent) was used for this study. To gain a better insight into the efficiency of the bacterial treatments, results were compared to those obtained from conventional surface treatments. B. sphaericus is unlike to cause human disease. The mortar specimens were coated at the four edges adjacent to the treated side, to ensure unidirectional absorption through the treated side.Morphological differences observed between treatments with pure cultures of B. sphaericus and mixed ureolytic culture . The use of pure cultures resulted in a more pronounced decrease in the uptake of water. The decrease in water absorption resulted from the combined effect of the presence of biomass and carbonate precipitation. The deposition of a layer of calcite resulted in a decrease in gas permeability.

2. **Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, Punjab, India, Department of Civil Engineering, Thapar University, Patiala, Punjab, India(2011)** reported on influence of bacteria on the compressive strength, water absorption and rapid chloride permeability. Influence of Sporoscarcina pasteurii bacteria on the compressive strength and rapid chloride permeability of concrete. Calcite deposition in concrete observed nearly eight times reduction in chloride permeability. Calcium carbonate precipitating bacteria were isolated from Rhizopheric soil (tulsi plant) and alkaline soil. Different concentrations of cells (10^3, 10^5, 10^7 cells/ml) were obtained by growing culture for different time .Concrete cubes were prepared with different concentrations of S. pasteurii. The cell concentration was determined from the bacterial growth curve made by observing optical density at 600 nm. Control concrete cubes were cast without the addition of microbes.

3. **Kim Van Tittelboom, Nele De Belie ,(2003)** reported on use of bacteria to repair cracks in concrete.The use of this biological repair technique is highly desirable because the mineral precipitation induced as a result of microbial activities is pollution free and natural. As synthetic polymers, currently used for concrete repair, may be harmful to the environment, the use of a biological repair technique is investigated in this study. Ureolytic bacteria such as Bacillus sphaericus are able to precipitate $CaCO_3$ in their micro-environment by conversion of urea into ammonium and carbonate. The bacterial degradation of urea locally increases the pH and promotes the microbial deposition of carbonate as calcium carbonate in a calcium rich environment. These precipitated crystals can thus fill the cracks. The crack healing potential of bacteria and traditional repair techniques are compared in this research by means of water permeability tests. Cracked concrete samples were prepared in two different ways. Crack sealing by means of this biological treatment resulted in a decrease in water permeability. However, it was seen that the decrease in water flow was also obtained if autoclaved bacteria were used instead of active bacteria. The use of this biological repair technique is highly desirable because the mineral precipitation induced as a result of microbial activities is pollution free and natural.

4. **Bang et al.(2005)** developed immobilization technique for remediation of cracks in concrete, where microbial cells are encapsulated in polymers has been adapted to enclose calcium carbonate precipitation in the gap to enhance the strength for selective concentration. Microbial calcite precipitation (MCP) occurs as a by-product of common microbial metabolic process, such as urea hydrolysis, photosynthesis, sulfate reduction. These different metabolic processes increase the alkalinity (pH and dissolved inorganic carbon) and thereby favouring the calcium carbonate precipitation.

5. Calcium carbonate precipitation is a general process in the bacterial world under appropriate conditions..

6. **Braissant et al(2002)** studied that Bacillus pasteurii a common soil bacterium can induce the precipitation of calcite. As a microbial sealant, $CaCO_3$ exhibited its positive potential in selectively consolidating simulated fractures and surface fissures in granites and in the consolidation of sand. Besides this, a durability study on concrete beams treated with bacteria, exposed to alkaline, sulfate and freeze–thaw environments were also studied. The durability performance increased with increase in the concentration of bacteria.

7. **Bakoshi et al.(1998)** used coal bottom (10–40%) as replacement for fine aggregate, and observed that the compressive strength and tensile strength of bottom ash concrete generally increases with the increase in replacement ratio of fine aggregate and curing age. The freezing–thawing resistance of concrete using bottom ash is lower than that of ordinary concrete and abrasion resistance of bottom ash concrete is higher than that of ordinary concrete.

8. **GizMag,(2008)** reported on self-destructing bacteria heals cracked concrete. The bacteria is triggered to

start germinating when it senses the specific pH of concrete, reproducing to fill in the crack until they hit the bottom of the fissure and start to clump

9. **Muynck et al.(2002)** Indicated that durability of mortar specimens with different porosity was affected by bacterial carbonate precipitation (biodeposition). The surface deposition of calcium carbonate crystals decreased the water absorption with 85% depending on the porosity of the specimens.

III. MATERIAL PROPERTIES

3.1 CEMENT

The cement used in this study was ordinary Portland cement (OPC) ULTRA TECH, 53 grade. This cement is the most widely used one in the construction industry in India. Cement is the most important ingredient used. One of the important criteria for the selection of cement is its ability to produce improved microstructure. Hence selection of proper grade and quality of cement is important for obtaining rich mix. Some of the important factors, which play a vital role in the selection of the type of the cement or compressive strength at various ages, fineness, heat of hydration, alkali content, tricalcium aluminate (C_3A) content, tricalcium silicate (C_3S) content, dicalcium silicate (C_2S) content and compatibility with admixtures etc., OPC is now available in three grades namely 33,43,53 grades, the number indicating the compressive strength of standard cement sand mortar cubes in MPa at 28 days curing period. Fineness of cement is also one of the parameter, as increasing the fineness will increase the early strength of the concrete, but as the other may lead to rheological problems.

3.2 FINE AGGREGATE

Fine aggregate used for cement mortar should be properly graded to give minimum void ratio and be free from deleterious materials like clay, silt content and chloride contamination etc., Grading of fine aggregate should be such that it does not cause increase in water demand for the concrete and should give voids so that the fine cementitious particles fill the voids. Hence it is desirable to use coarser variety of fine aggregate having a high fineness modulus for making workable and strong concrete.

The optimum gradation of fine aggregate is determined more by its effect on water requirement than on physical packing. ACI Committee reports that sand with fineness modulus below 2.5 gives a sticky consistency, making it difficult to compact and sand with fineness modulus of about 3 gives the best workability and compressive strength. Properties such as void ratio, gradation, and density have to be accessed to design a dense mix with optimum cement content and reduced mixing water. For the present investigation, locally available river sand was conforming to IS: 383 – 1970 was used.

3.3 COARSE AGGREGATE

Locally available blue metal was used. Crushed granite stones of size passing through 20mm sieve and retained on 4.75 mm sieve as per IS: 383-1970 was used for experimental purpose.

3.4 WATER

Water is an important ingredient of concrete as it chemically participates in the reaction with cement to form the hydration product, C-S-H gel. The strength of cement mortar depends mainly from the biding action of the hydrated cement paste gel. A higher w/c ratio will decrease the strength, durability, water – tightness and other related properties. The quantity of water added should be the minimum for chemical reaction of hydrated cement, as any excess of water would end up only in the formation of undesirable voids (capillary pores) in the hardened cement paste. The strength of cement paste is inversely proportional to the dilution of the paste. Hence, it is essential to use as little paste as possible consistent with the requirements of workability and chemical combination with cement.

Quantity and quality of water is required to be looked very carefully. The water used for making concrete should be free from undesirable salts that may react with cement and admixtures and reduce their efficiency. Silts and suspended particles are undesirable as they interfere with setting, hardening and both bond characteristics. Algae in mixing water may cause a reduction in strength either by combining with cement to reduce the bond or by causing large amount of air entrainment.

3.5 BACILLUS MEGATERIUM

Bacillus Megaterium is a gram positive, endospore forming, rod shaped bacteria. It is considered aerobic. It is found in soil and considered a saprophyte. Bacillus Megaterium has often been used in the laboratory, and is used as an industrial organism that is able to produce a variety of proteins and sources of bioremediation. Bacillus Megaterium is a good source of industrial proteins because it is both a desirable cloning host and produces a large variation of enzymes. The organism does not have alkaline proteases; which allows for recombinant protein synthesis. Using Bacillus Megaterium scientist has developed numerous proteins that are commonly used in the medical and agricultural field. For example, many synthetic penicillin's have been derived using the penicillin amides in the bacteria; harvested glucose dehydrogenise is used in glucose blood tests; ß-Amylases which are often used in the bread industry; and neutral proteases which are used by the leather industry. Several strains have proven to be good hosts for gene expression. One strain, QM B1551, is still used to produce the antigen for HIV Diagnostic Kits. The biotechnological study of the Bacillus Megaterium provides a plethora of different proteins that they are able to employ in important medical, scientific and industrial advances.

IV. Properties

4.1 Bacillus Megaterium is a Gram-positive, mainly aerobic spore forming bacterium found in widely diverse habitats. It is a rod-shaped bacterium. With a cell length of up to 4 µm and a diameter of 1.5 µm, B. Megaterium is amongst the biggest known bacteria. The cells often occur in pairs and chains, where the cells are joined together by polysaccharides on the cell walls. B. Megaterium grows at temperatures from 3 °C to 45 °C, with the optimum around 30 °C. Bacillus Megaterium is the big beast because it is an extremely large bacteria, it is about 100 times as large as E. coli. Due to its immense size, about 60 micrometers cubed, Bacillus Megaterium has been used to study structure, protein localization and membranes of bacteria since the 1950's.

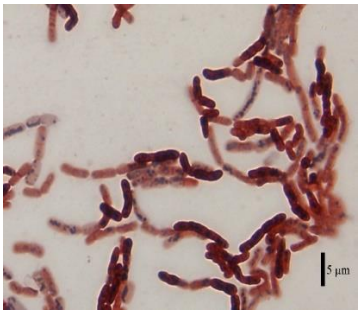


Fig 4.1: Rod shaped

Fig 4.2: Rod shaped Bacillus Megaterium cells

Table 4.1: Class and order

Scientific classification	
Phylum	Firmicute
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	Bacillus
Species	Bacillus Megaterium
Binomial name	
Bacillus Megaterium	

4.2 Properties of Material Used In Present Investigation

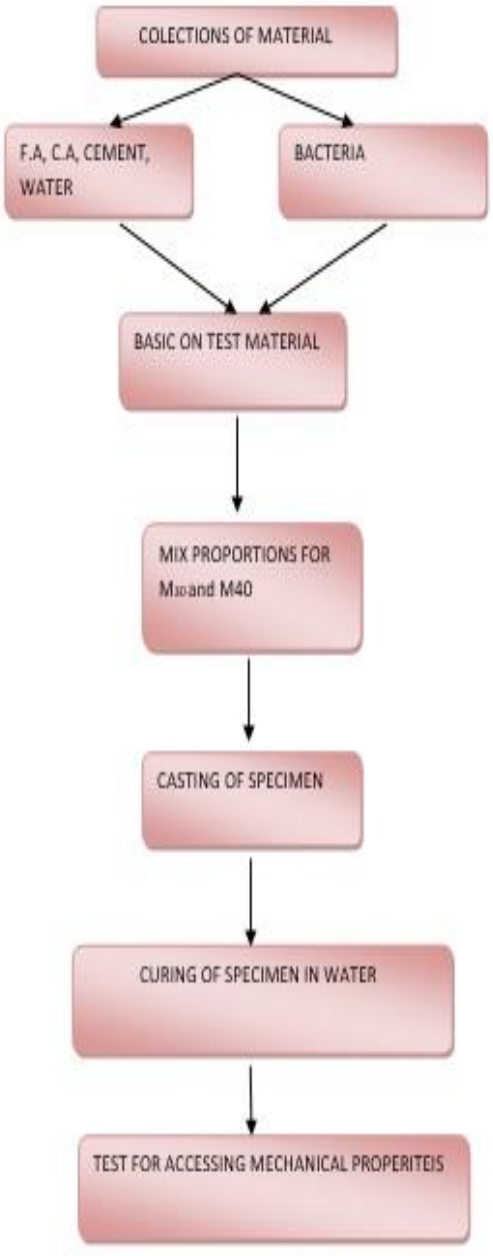
Table 4.2: Sieve Analysis of River Sand

Sieve size	**% passing**
4.75mm	98
2.36mm	96
1.18mm	78
600µm	51
300 µm	26
150 µm	7

Table 4.3 Sieve Analysis of Natural Coarse Aggregate

Sieve Size (mm)	**% Passing**
25	100
20	100
16	100
12.5	100
10	33
6.3	3
4.75	0

V. METHODOLOGY



VI. MIX DESIGN

Mix design is the process of selecting suitable ingredients of concrete and determines their relative proportions with the object of producing concrete of certain minimum strength and durability as economically as possible. The first object is to achieve the stipulated minimum strength. The second object is to make the concrete in the most economical manner. Cost wise all concretes depend primarily on two factors, namely cost of materials and cost of labour. Labour cost, by way of formworks, batching, mixing, transporting and curing is nearly same for good concrete and bad concrete. Therefore, attention is mainly directed to the cost of the materials. Since the cost of cement is many times more than the cost of their ingredients, optimum usage of cement is sought out by designing the mix.

6.1MIX DESIGN M30 GRADE OF CONCRETE (IS 10262-2009)

1) STIPULATION FOR PROPORTIONING

a) Grade of designation = M30
b) Type of cement = OPC 53 grade
c) Maximum size of aggregate = 20mm
d) Type of quality control = Good
e) Type of exposure = Mild
f) Type of aggregate = Angular

2) TEST DATA FOR MATERIAL

a) Cement used = OPC 53 grade
b) Specific gravity of cement = 3.05
c) Specific gravity of aggregate
1) Coarse aggregate = 2.63
2) Fine aggregate = 2.5
e) Water absorption
1) Coarse aggregate = 0.6%
2) Fine aggregate = 1.0%

3) TARGET STRENGTH FOR CONCRETE

F'ck= fck + (1.65 s)
= 30 + (1.65x5)
Therefore, the target strength = 38.25 N/mm^2

4)SELECTIONOF WATER CEMENT RATIO

Max water cement ratio= 0.39

5) SELECTION OF WATER CONTENT

Maximum water content = 186 litres

6) CALCULATION OF CEMENT

Water cement ratio = 0.39
Cement content = $\frac{186}{0.39}$
= 476.92 kg/m^3> 300 kg/m^3

7) VOLUME OF COURSE AND FINE AGGREGATE (TABLE4 IS 10262)

Volume of Coarse aggregate = 0.64
Volume of Fine aggregate = 1-0.64
= 0.36

Mix calculation

Volume of concrete = 1m^3

Volume of cement = $\frac{mass of cement}{specific gravity of cement} \times \frac{1}{1000}$
= $\frac{476.92}{3.05} \times \frac{1}{1000}$
= 0.156 m^3

Volume of water = $\frac{mass of water}{specific gravity water} \times \frac{1}{1000}$
= $\frac{186}{1} \times \frac{1}{1000}$
= 0.186 m^3

Volume of all in aggregate (e)= a- (b+c)
= 1-(0.156+0.186)
= 0.658m^3

8) MASS OF COARSE AGGREGATE

= e× volume of fine aggregate× Specific gravity of CA
= 0.658×0.64×2.63×1000
= 1107.54 kg/m^3

9) MASS OF FINE AGGREGATE

= e× volume of fine aggregate× Specific gravity of FA
= 0.658×0.36×2.5×1000
= 592.2 kg/m^3

10) MIX PROPORTIONS FOR TRIAL (WEIGHT)

Cement = 476.92 kg/m^3
Fine aggregate = 592.2 kg/m^3
Coarse aggregate = 1107.54kg/m^3
Water = 186 kg/m^3
Water cement ratio = 0.39

Table6.1 Concrete mix design proportion for (M30grade)

UNIT	WATER	CEMENT	FA	CA
Kg/m^3	186	476.92	592.2	1107.54
Ratio	0.39	1	1.24	2.32

6.2 MICRO ORGANISMS CULTURE

6.2.1 CULTURE OF BACTERIA

The basic culture of the Bacillus Megaterium and Azotobacter 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.

6.3 BACTERIAL CULTURING AND SUB - CULTURING

6.3.1 Methods of obtaining pure culture

To make the bacteria of pure culture of Bacillus Megaterium and Azotobacter the following procedure is followed.

6.3.1.1 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.

6.3.1.2 Materials Required

Petri plates, Test tubes, inoculating loop, needle, Bunsen burner, culture.

6.4. Procedure

6.4.1 Culturing:

- The nutrient agar was prepared for 100ml and pH of the medium was checked and then autoclaved.
- The petri plates and test tubes were sterilized in the hot air oven.
- Under sterile condition, the stab, slant and agar plate were inoculated with the given culture.
- The culture was inoculated in the agar plate by streak plate method.
- Then the plates, stabs and slants were inoculated 24-48 hours at 37°C.

6.4.2 Sub – culturing

- The broth solution is prepared for the given composition.

- The broth solution is inoculated in the laminar condition.
- Then it is placed in the shaker at 37◦C and a speed of 125rpm for 12 hours.

6.4.3 Broth solution composition

Peptone:5 g/lt.

NaCl :5 g/lt.

Yeast extract: 3 g/lt.

6.4.4 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 Hemocytometry.

6.4.5 Result

Isolated colonies of bacterial cells were obtained.
The Number of cells present in the broth solution after sub- culturing for 12hrs by Haemocytometr = 5.8 x 10^3 cells/ml

6.5 HAEMOCYTOMETRY

6.5.1 Introduction

To calculate the number of cells, present in the sub- culture solution. Hemocytometry 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.

(Fig 6.2)

6.5.2 Materials required:

Counting chamber (Hemocytometer), cell suspension, pipette, microscope.

6.5.3 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.

6.5.4 Observation:

Total number of cells = 150 cells/0.025mm^2

6.5.5 Result:

The total number of cells in the suspension is 5.8 x 10^3 cells per ml.

6.6 BACTERIAL PRECIPITATION

6.6.1 BACTERIAL PRECIPITATION

Bacteria undergo several reactions to precipitate the compound $CaCO_3$. The microbial precipitation of $CaCO_3$ is determined by several factors including

- The concentration of dissolved inorganic carbon,
- The pH,
- The concentration of calcium ions and
- The presence of nucleation sites.

The first three factors are provided by the metabolism of the bacteria while the cell wall of the bacteria will act as a nucleation site.

6.6.2 REACTIONS

The bacteria used in this research produce urease which catalyzes the hydrolysis of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}). First, 1 mol of urea is hydrolyzed intracellular to 1 mol of carbonate and 1 mol of ammonia (Eq. (6.1)).

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad \text{Eq. (6.1)}$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad \text{Eq. (6.2)}$$

Carbonate spontaneously hydrolyses to form additionally 1 mol of ammonia and carbonic acid (Eq. (6.2)). These products subsequently form 1 mol of bicarbonate and 2 mol of ammonium and hydroxide ions (Eqn. (6.3) and (6.4)).

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad \text{Eq. (6.3)}$$

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad \text{Eq. (6.4)}$$

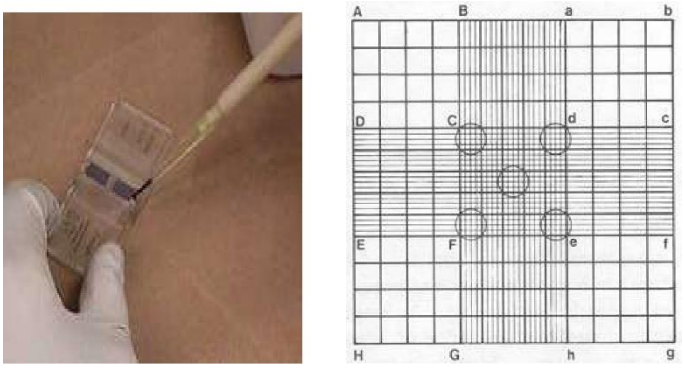
The last 2 reactions give rise to a pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of carbonate ions (Eq. (6.5)).

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O \quad \text{Eq. (6.5)}$$

Since the cell wall of the bacteria is negatively charged, the bacteria draw cations from the environment, including Ca^{2+}, to deposit on their cell surface. The Ca^{2+}-ions subsequently react with the CO_3^{2-}-ions, leading to the precipitation of $CaCO_3$ at the cell surface that serves as a nucleation site (Eqn. (6.6) and (6.7)).

$$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+} \quad \text{Eq. (6.6)}$$

$$Cell\text{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3\downarrow \quad \text{Eq. (6.7)}$$



(Fig 6.3)

Several bacteria have the ability to precipitate calcium carbonate. These bacteria can be found in soil, sand, natural minerals. Calcium carbonate precipitation has been used for consolidation of sand columns, healing of cracks in granite or for surface treatment of limestone. For these applications the use of bacteria, precipitating $CaCO_3$, proved its efficiency.

6.7 EXPERIMENTAL INVESTIGATIONS ON STRENGTH CHARACTERISTICS OF CONCRETE

6.7.1 INTRODUCTION

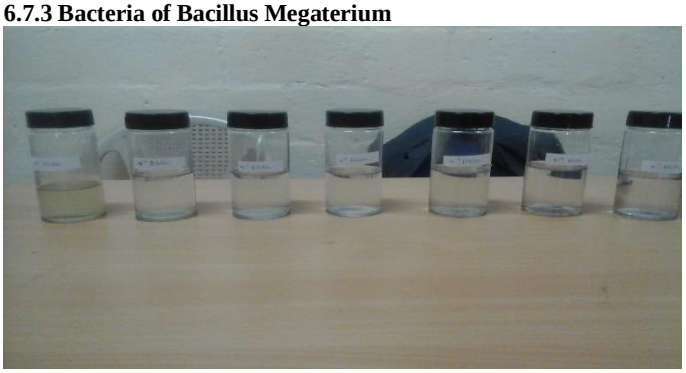
This chapter presents the details of experimental investigations carried out on the test specimens to study the strength-related properties of concrete using bacteria. Here, an attempt was made to study the strength development at different concentration levels at different levels with bacteria and the results were compared.

The strength-related property compressive strength is studied. Minimum three specimens were tested for each mix for each test. The entire tests were conducted as per specifications required.

6.7.2 CASTING AND CURING SYSTEM

Cracked concrete samples were prepared in two different ways. The first method resulted in samples with standardized cracks while the second method gave rise to more realistic cracked samples

6.7.3 Bacteria of Bacillus Megaterium



(Fig 6.4) Collecting of bacteria bacillus megaterium

6.7.4 Mixing of Concrete

Concrete a strong, durable material composed of cement, aggregate and water is the most used building material in the world. Concrete has an ultimate load bearing capacity under compression but the material is weak in tension. The steel reinforced bars take the load when the concrete cracks in tension. To increase the strength and durability of the structure either crack that are formed should be repaired conventionally using epoxy injection or latex treatment or by providing extra reinforcement in the structure during the design phase to ensure that the crack width stays within a permissible limit. A reliable self-healing biological app of water approach is first proposed by V. Ramakrishnan to make use of bacterial concrete to heal cracks in concrete structure. The mix proportions for M30 grade of concrete with adding bacteria (Bacillus Megaterium) of 5g in 1lit/m3 of water.

(Fig 6.5)

(Fig 6.6) mixing of concrete

6.7.5 Natural cracks

More realistic cracks were obtained by performing compressive strength on concrete cube. From that concrete cube, cylinders are made with cracks.

(Fig 6.7)

(Fig6.8) after the growth of bacteria

VII. CUBE COMPRESSIVE STRENGTH

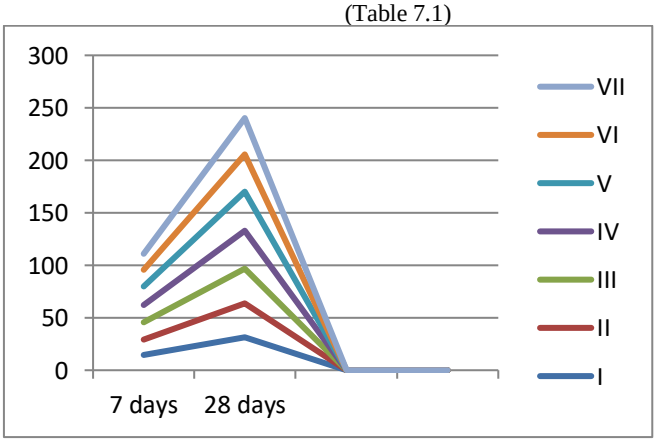
The cube compressive strength results at various ages such as 7, 28 days for different concentration levels such as 10^4, 10^6 of Bacillus Megaterium and Azotobacter bacteria.

From the test results it was observed that the maximum compressive strength is obtained for mix with 10^6 cell concentration Bacillus Megaterium bacteria, in the case of Azotobacter it is found that compressive strength is same as that of the nominal mix. Main factor for bacillus Megaterium shows more result compared to Azotobacter is that it can survive in very hot condition. Formation of glue like material will fill the cracks.

(Table7.1) Characteristic compressive strength of cubes in MPa(M30grade)

Days of curing	Type of bacterial concrete	Different concentration of bacillus megaterium concrete						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	**10^{-5}**	10^{-6}	10^{-7}
	Type I	14.76	14.3	16.5	16.6	**17.05**	16.45	15.08
	Type II	14.5	14.9	16.8	16.9	**17.7**	16.2	15.4
	Type III	14.2	14.7	16.4	16.4	**17.3**	15.9	15.1
Average		14.4	14.6	16.5	16.9	**17.3**	16.1	15.1
	Type I	31.5	32.3	33.5	36.02	**36.58**	35.03	34.2
	Type II	31.1	32.8	34.08	36.5	**37.57**	35.9	34.04
	Type III	31.8	31.9	33.5	35.9	**36.3**	35.6	35.7
Average		31.4	32.3	33.6	36.1	**37.2**	35.5	34.6

(Table 7.1)



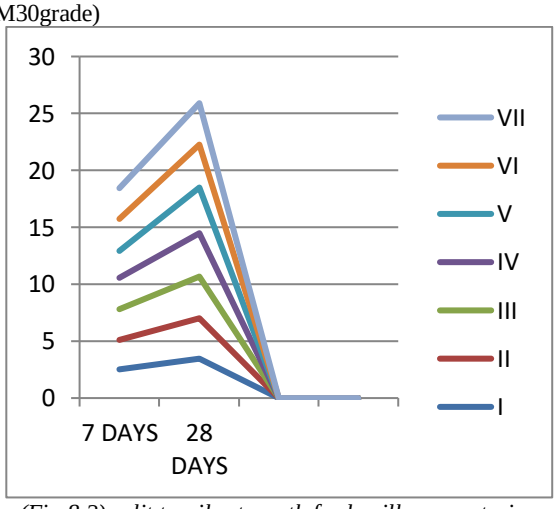
(Fig 7.1) compressive strength for bacillus megaterium

VIII. TENSILE SPLITTING TEST

The split tensile strength of a concrete is carried on cylindrical specimen of diameter 150mm and length 300mm.Two wooden-bearing strips are placed. The specimen was loaded until it fails. The test is done at the age of 7, and 28 days. The machine used was the same UTM that used for compression test.

Days of curing	Type of bacterial concrete	Different concentration of bacillus megaterium concrete						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	**10^{-5}**	10^{-6}	10^{-7}
	Type I	2.53	2.64	2.78	2.85	**2.95**	2.89	2.63
	Type II	2.61	2.58	2.67	2.72	**3.01**	2.82	2.62
	Type III	2.49	2.57	2.61	2.73	**2.99**	2.81	2.74
Average		2.54	2.59	2.68	2.73	**2.98**	2.84	2.66
	Type I	3.52	3.66	3.628	3.85	**3.96**	3.79	3.60
	Type II	3.49	3.53	3.75	3.87	**4.08**	3.78	3.69
	Type III	3.39	3.46	3.63	3.73	**3.99**	3.76	3.65
Average		3.46	3.55	3.66	3.81	**4.01**	3.77	3.64

Table 8.1 Characteristic Split Tensile strength in MPa(M30grade)



(Fig 8.2) split tensile strength for bacillus megaterium

IX. FLEXURAL STRENGTH TEST

The flexural strength of concrete was determined using prism and beam of size 15x15x70cm and 20x23x150cm respectively at an age of 28 days. The bearing surfaces of the supporting and loading rollers shall be wiped clean and any loose sand or other materials. The axis of the specimen shall be aligned carefully with the axis of the loading device. The load shall be applied without shock and increased continuously at a rate such that the extreme fibre stress increases at 7kg/sqcm/min at a load rate of 400kg/min. The load shall be increased until the specimen fails and the maximum load applied to the specimen during the test shall be recorded. The flexural

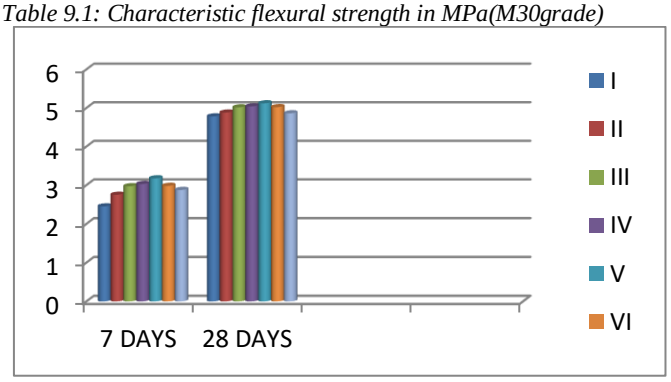
strength of the specimen shall be expressed as the modulus of Rupture and is determined by the following formula

$$f_b = \frac{P \times l}{b \times d^2}$$

Days of curing	Type of bacterial concrete	Different concentration of bacillus megaterium concrete						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	**10^{-5}**	10^{-6}	10^{-7}
	Type I	2.5	2.8	2.95	3.1	**3.2**	3.02	2.8

	Type II	2.4	2.7	2.99	3.0	**3.15**	2.95	2.83
Average		2.45	2.75	2.97	3.05	**3.17**	2.98	2.87
	Type I	4.8	4.95	5.05	5.1	**5.15**	5.08	4.85
	Type II	4.76	4.82	4.98	4.99	**5.09**	5.00	4.87
Average		4.78	4.885	5.015	5.04	**5.12**	5.04	4.86

Table 9.1: Characteristic flexural strength in MPa(M30grade)

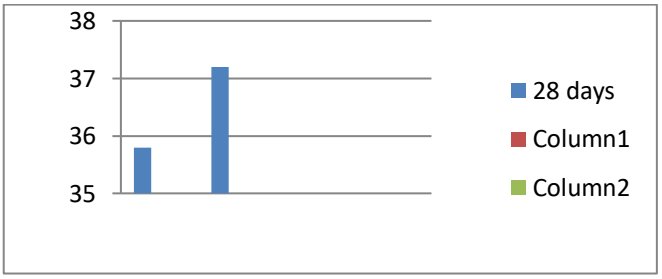


(Fig 9.1) flexural strength for bacillus megaterium

X. COMPARISON OF TEST RESULT

10.1 **Compressive test result for Bacillus Megaterium**

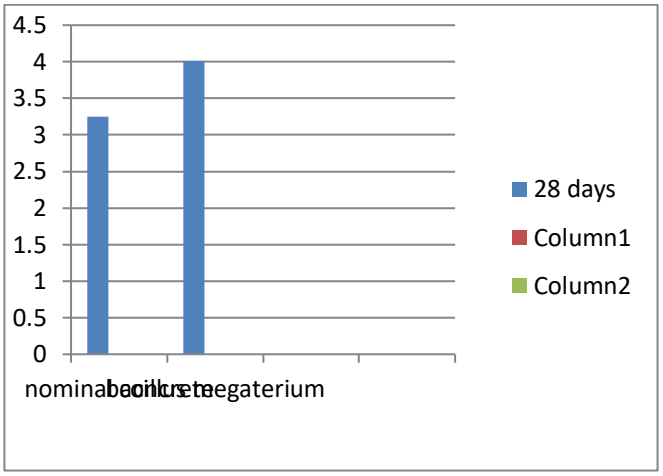
On comparing the compressive strength of different concentration of Bacillus Megaterium with nominal mix , the test result show at 28th day the strength get increased slightly compared to that of nominal mix and 10^5 cell concentration of Bacillus Megaterium .



(Fig 10.1) Comparison of compressive strength of concrete(MPa)-M30 grade at 28 days

10.2 **Split tensile test result for Bacillus Megaterium**

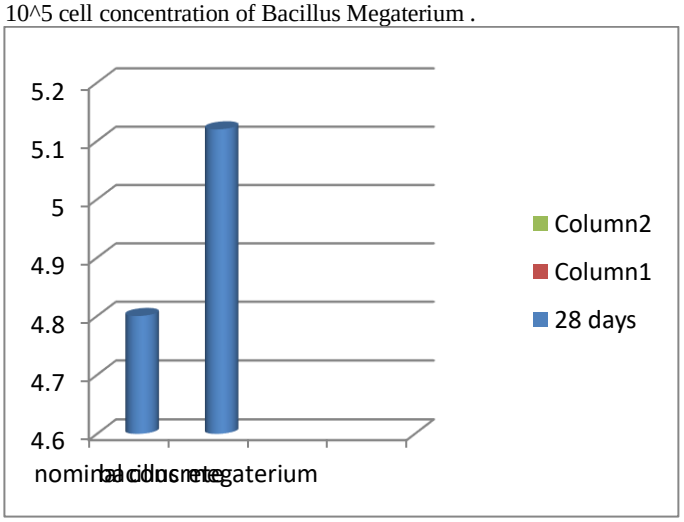
On comparing the split tensile strength of different concentration of Bacillus Megaterium with nominal mix, the test result show at 28th day the strength get increased slightly compared to that of nominal mix and 10^5 cell concentration of Bacillus Megaterium.



(Fig 10.2) Comparison of split tensile strength of concrete(MPa)-M30 grade at 28 days

10.3 **Flexural test result for Bacillus Megaterium**

On comparing the flexural strength of different concentration *(Table 10.1): Flexural Strength of bacterial beam* of Bacillus Megaterium with nominal mix , the test result show at 28th day the strength get increased slightly compared to that of nominal mix and 10^5 cell concentration of Bacillus Megaterium .



(Fig 10.3) Comparison of flexural strength of concrete(MPa)-M30 grade at 28 days

(Fig 10.4) Setup of the Test Specimen before Loading

(Fig 10.5) *The specimen after the load*

XI. RESULTS AND DISCUSSION

11.1 GROWTH OF BACTERIA

Initially before addition of bacteria to cracks in the concrete, the cracks are sprayed with water. Then the bacteria which are kept in lab condition are injected into it using syringe. After addition of bacteria the nutrition are added to it after 5-10 minutes. The amount of bacteria added to concrete is increased by .5ml in each trial starting from .5ml. Addition of bacteria are done until the growth of growth is found. The nutrition is added to it until it triggers the growth of bacteria. It is found that 2ml shows the growth of bacteria inside the cracks of concrete.

SI. NO	Load (KN)	Deflection(mm)	Observation	SI. NO	Load (KN)	Deflection(mm)	Observation
1	20	0.09		9	180	2.97	
2	40	0.29		10	200	3.27	
3	60	0.65		11	220	3.52	
4	80	1.12		12	240	3.84	
5	100	1.69		13	260	4.08	
6	**120**	**2.03**	**First crack**	14	280	4.22	
7	140	2.48		15	300	4.51	
8	160	2.78		**16**	**320**	**4.82**	**Ultimate load**

XI. RESULTS AND DISCUSSION

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11.2 SUGGESTION FOR WORKS

- The present study deals only with compressive strength, split tensile strength, flexural strength and repairing of cracks.
- The arresting of cracks by using bacteria.
- Also to determine how effective it can be used in buildings.
- Use of different culture for different precipitation of substance.

XII. CONCLUSION

12 .1 CONCLUSIONS

From the obtained compressive strength and porosity results the incorporation of more numbers of bacteria in the cracks of the concrete cube, result in a significant gain of strength due to self healing property of bacteria. As the repairing of cracks in concrete is increased with the increase in the concentration of bacteria number. Due to the inclusion of bacteria in concrete, we achieved slight increase in compressive strength and also 12% increase in porosity. From the results it can be concluded that easily cultured Bacillus Megaterium can be safely used in improving the performance and characteristics of concrete. Hence we can effectively use the bacteria to repair cracks.

- Bacteria can be produced from lab which is proved to be a safe.
- The Bacillus Megaterium is found effective due to the fact that it can survive in hot condition.
- The 5ml of bacteria will be the optimum proportion.
- Also addition of nutrition to bacteria will trigger the growth of bacteria.

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