

## International Journal of Innovative Research in Science, Engineering and Technology

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# An Experimental Study on Cement Mortar by Mixing of Ureolytic Bacteria

Majjari. Subbarayudu<sup>1</sup>, A.Brahmini<sup>2</sup>

M.Tech (Structural Engineering) Student, Dept. of Civil Engineering, KMM Institute of Technology and Science,  
Ramireddipalli, Tirupati, Andhra Pradesh, India<sup>1</sup>

Assistant Professor, Dept. of Civil Engineering, KMM Institute of Technology and Science, Ramireddipalli, Tirupati,  
Andhra Pradesh, India<sup>2</sup>

**ABSTRACT:** Cement mortar is a leading component of construction material used in infrastructure and most building works. By the precipitation of calcium carbonate (CaCO<sub>3</sub>) in the existence of urea and a calcium source the strength of the cement mortar can be increased by bacteria. *BACILLUS SPHAERICUS* are useful to enhance the compressive strength of cement mortar. Various tests like consistency and initial setting time are done to find the effect of bacterial solution on cement. Compression strength test are favorable to find out the properties of cement mortar. Compressive strength (at 7-day and at 28-day) of mortar cube found to be increasing with the increase of bacteria concentration up to 107 cells/ml. The optimum doses of bacteria found to improve the average compressive strength by 58% (at 7-day) and 23% (at 28-day) over the control specimen. The more increase in strength after 7 day curing may be due to the presence of vitamin medium and it getting depleted as it reaches 28 days and causing end of bacteria. The minimum cumulative water absorption is achieved for a cell concentration of 109 cells/ml.

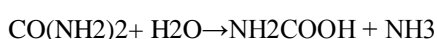
### I. INTRODUCTION

Concrete/cement mortar is an absolutely essential component of construction materials used in infrastructure and most buildings. A lot of research has been carried out around the globe to improve properties of concrete. It can be found that the microbial mineral precipitation arise from metabolic activities of supportable microorganisms in concrete can enhance the overall behavior of concrete/cement mortar. Bio concrete has many benefits over the ordinary concrete; it needs a much shorter time; it is suitable for in-situ process; raw material of bio cement are produced at a lower temperature and can be used as eco-construction material as it consumes less energy and less CO<sub>2</sub>. Alternate cementing materials are generally used in concrete/mortar mixes to decrease cement percentage, improve workability, increase strength and enhance durability through hydraulic activity. This porosity of the building material along with penetration of moisture and other harmful chemicals such as acids and chlorides adversely affect the concrete and reduce the structures strength and life.

This mineral deposition technique can answer for the natural method for the sealing of pores and cracks of concrete and mortar.

### MECHANISM OF CALCIUM CARBONATE PRODUCTION

Bio mineralization is nominated as a biologically produce precipitation in which an organism creates a local micro-environment with conditions that allow optimal extracellular chemical precipitation of mineral phases. The microbial precipitation of calcium carbonate CaCO<sub>3</sub> is resolved by several factors along with: the concentration of dissolved inorganic carbon, the pH, and 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. U.B can induce urease that hydrolysis of urea (CO (NH<sub>2</sub>)<sub>2</sub>) into ammonium (NH<sub>4</sub><sup>+</sup>) and carbonate (CO<sub>3</sub><sup>2-</sup>). Carbonate spontaneously hydrolyses to form additionally 1 mol of ammonia and carbonic acid (Eq. 2).



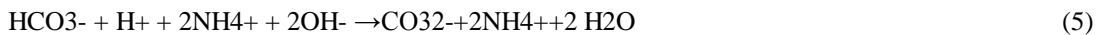
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# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017



Since the cell wall of the bacteria is negatively charged, the bacteria draw cations from the environment, including  $\text{Ca}^{2+}$ , to deposit on their cell surface. The  $\text{Ca}^{2+}$  ions plays  $\text{CO}_3^{2-}$  ions, command to the precipitation of Calcium carbonate  $\text{CaCO}_3$  at the cell surface that plays as a nucleation site (Eq. 6 and 7).



The schematic diagram showing mechanism of calcite precipitation is given in Fig 1.

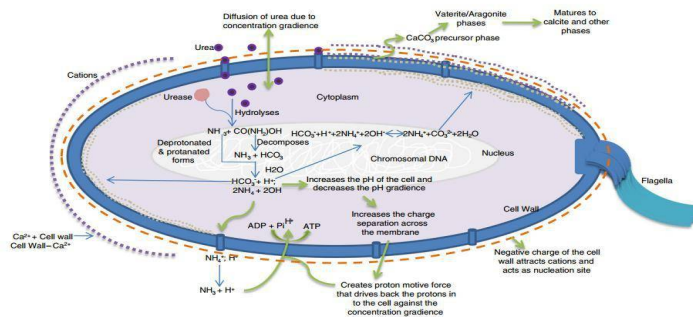


Fig Mechanism of calcite precipitation by bacteria.

## II. EXPERIMENTAL WORK AND RESULTS

An experimental program was outlined to study the effect of bacteria on the different engineering properties of cement mortar. Testing of survival of *Bacillus cereus* in concrete-like environment The following steps are involved in testing the tolerance of this pathogen in concrete-like environment.

200 ml of nutrient medium was prepared for the culture of bacteria.

It was then transferred to 12 fresh clean test tubes and its volume was made to 10ml by adding Noah to increase the pH in the test tubes. It was then incubated for 24 hours at 37°C and 50°C. After incubation of 24 hours at different temperature and different pH level the growth of the bacteria was tested for each of 12 test tubes through turbidity of the solution. The results of this test are listed as follows: Growth of bacteria was not observed in the any of the six cultures incubated at 50°C At 37°C, growth was noticed only in cultures with pH 8 and 9. It can be seen from the table that the bacteria could survived only in the cultures with pH 8.1 and 9.5.

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017

Table 3.1: Temperature and pH tolerance of Bacillus Cereus

pH	Presence of Bacteria	
	cultures incubated at 37°C	cultures incubated at 50°C
8	+	-
9	+	-
10	-	-
11	-	-
12	-	-
12.5	-	-

+ Presence of bacteria; - Absence of bacteria

Table: Temperature and pH tolerance of Bacillus sphaericus

pH	Presence of Bacteria	
	cultures incubated at 37°C	cultures incubated at 50°C
8	+	+
9	+	+
10	+	+
11	+	+
12	+	+
12.5	+	+

+Presence of bacillus bacteria

- presence of bacillus bacteria

# International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 6, Issue 5, May 2017

Testing of survival (durability) of B.Sphaericus at in concrete

Growth of bacteria was observed in the cultures incubated at both 37°C and 50 °C for all the pH value from 8 to 12.5. Table 3.2 presents the temperature and pH tolerance of Bacillus sphaericus. It can be seen from the table that the bacteria could survive the pH range of 8 to 12. Therefore it can be concluded that this Bacillus species can be suitable for fresh cement mortar which has pH about 11 to 13. The above result shows that, this species can survive the temperature in concrete (or cement mortar) arising out of the heat of hydration.

Test for CaCO<sub>3</sub> precipitation in agar plate state

In order to confirm that selected bacterial species is capable of producing calcium carbonate following standard test has been undertaken using Calcite Precipitation Agar (CPA).

190ml of distilled water was weighted and taken in a 200ml conical flask.

Total media components are were autoclaved.

Later autoclaving process then urea is added to the medium.

20µl of chowder culture was injecting in the center of a plate, and then hatch at 32°C for 7 days.

Fig. 3.0 presents the precipitation of some material on the plate at points A, B and C and D. The precipitation is detected in a field emission scanning electron microscope. Fig. 3.1 presents the FESEM images of the precipitation. This proves the evidence of the formation of calcite precipitated by bacteria.



Fig. : The CaCO<sub>3</sub> precipitation

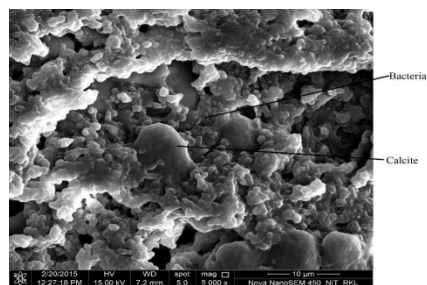


Fig 3.1 FESEM images of the calcite precipitated

## TESTING OF SPECIMEN FOR MECHANICAL PROPERTIES

Specimens were tested after 7-days and 28-days of curing for Compressive Strength and capillary water absorption and the effect of bacteria (Bacillus sphaericus) on these two mechanical properties are studied.

### Compressive Strength Test on Cement Mortar

All the mortar cubes are tested in a load controlled universal testing machine to obtain the unidirectional compressive strength obtained at 7 days and 28 days as shown in Table 3.3.

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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Vol. 6, Issue 5, May 2017

Table 3.3 Effect of bacteria on compressive strength

CELL CONCENTRATION	MEAN	PERCENTAGE	MEAN	PERCENTAGE
	days (MP a)	(%)	DAYS (MP a)	(%)
0 (Regulate)	3.48	-	5.85	-
105	4.48	30.65	6.92	18.48
106	5.32	57.24	7.15	18.99
107	5.45	58.23	7.35	23.86
108	4.96	43.73	6.15	4.67
109	4.74	37.90	6.05	3.42

To regulate same samples of mix proportion were cured in two different curing solution: (i) normal tap water and (ii) mix of tap water, urea and calcium chloride. The purpose of this test was to rule out any possible effect of curing solution on the properties of specimen without bacteria. The test results suggest no significant differences in compressive strength and capillary water absorption among the two control specimens.

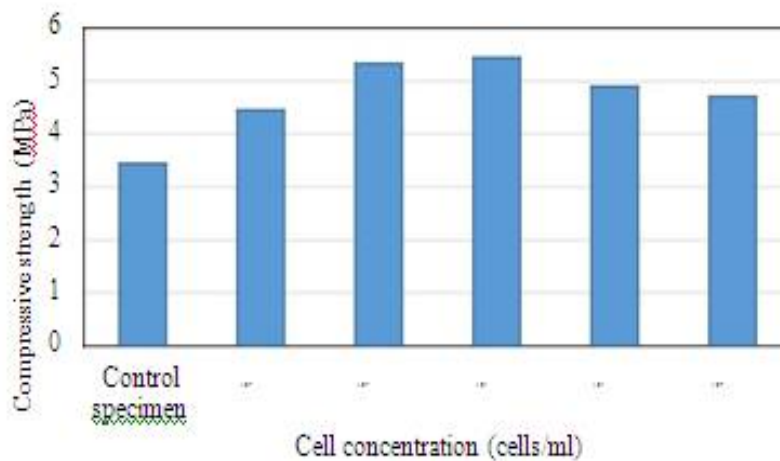


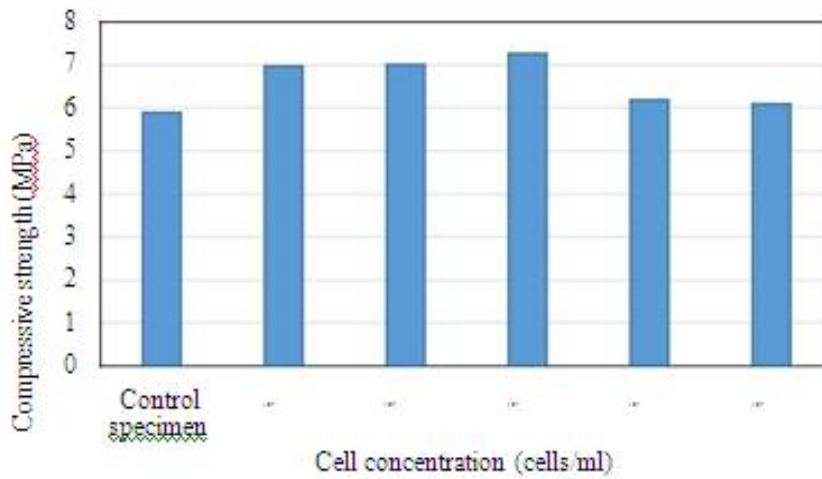
Fig Variation of compressive strength with variation in cell concentration – at 7 day

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017



Cell concentration (cells/ml)  
Fig Compressive strength – cell/ml graph for 28 day cure



Cement Mortar Specimens



Fig compressive strength test of mortar cube

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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Vol. 6, Issue 5, May 2017

## TEST FOR STANDARD CONSISTENCY OF CEMENT

Standard consistency test of the cement as per IS: 4031 Part 4 [24]

- i. Take 300 g of cement and place it in the tray.
- ii. Join the 27% water by weight of dry cement completely to get a cement paste. Total time period of water cement paste mix i.e. "Gauging time" not more than 3 to 5 minutes.
- iii. Level the Vicat's mould, resting upon a glass plate, with this cement paste.
- iv. Next fill the mould fully, smoothen the top surface of the paste, making it level with top of the mould.
- v. Place the whole assembly under the rod bearing plunger.
- vi. Lower the plunger gently so as to touch the surface of the test block and quickly release the plunger allowing it to sink into the paste.
- vii. Calculate the depth of penetration and report it.
- viii. Prepare trial pastes with different varying percentages of water content and follow the steps (ii to vii) as described above, until the depth of penetration is 5 to 7 mm from the bottom. Calculate percentage of water (P) by weight of dry cement required to prepare cement paste of standard consistency by following formula, and express it to the first place of decimal.

$$P = \frac{W}{C} \times 100$$

Where,

W = Quantity of water added

C = Quantity of cement used

## TEST FOR INITIAL SETTING TIME AND FINAL SETTING TIME

Test for initial setting time and final setting time as per Indian Standard IS 4031 (Part 5):1988 [25] and IS 455: 1989 [26]. Initial setting time is that time period between the water is added to cement and the time at which 1 mm square section needle fails to penetrate the cement paste placed in the Vicat's apparatus mould 5 mm to 7 mm from below the mould.

Final setting time is that time period between the water is added to cement and the time at which 1 mm needle makes an impact on the paste in the Vicat's apparatus mould but 5 mm attachment does not make any impression.

### **Test Block Preparation**

- i. Let's take 400 g of cement and prepare a neat cement paste with 0.85P (where P is the consistency) of bacterial solution by weight of cement.
- ii. Gauge time is kept between 3 to 5 minutes. Now, start the stop watch at the immediately when the water is added to the cement. Record this time (t1).
- iii. Fill the Vicat mould, resting on a glass plate, with the cement paste gauged as above. And fill the mould completely smooth off the surface of the paste making it perfect level with the top of the mould. The cement mortar block thus prepared is called test block.

### **Initial Setting Time**

- i. Place the test block confined in the mould and resting on the non-porous plate, under the rod bearing the needle.

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017

- ii. Below the needle gently until it comes in associate with the surface of test block and quickly release, allowing it to penetrate into the test block.
- iii. In the beginning the needle completely pierces the test block. Repeat this procedure i.e. immediately releasing the needle after every 2 minutes till the needle fails to pierce the block for about 5 mm calculated from the bottom of the mould. Note this time ( $t_2$ ).

### Final Setting Time

- i. For determining the final setting time, replace the needle of the Vicat's apparatus by the needle with an annular attachment.
- ii. The cement is considered finally set when upon applying the final setting needle gently to the surface of the test block; the needle makes an impression thereon, while the attachment fails to do so. Record this time ( $t_3$ ).

### Calculation

Initial setting time =  $t_2 - t_1$

Final setting time =  $t_3 - t_1$

Where,

$t_1$  = Time at which water is first added to cement

$t_2$  = Time when needle fails to penetrate 5 mm to 7 mm from bottom of the mould  $t_3$  = Time when the needle makes an impression but the attachment fails to do so.

### **CALCULATION FOR RAW MATERIALS MORTAR CUBES**

Mortar cubes of 1:6 cement to sand ratio is prepared.

Standard consistency of cement = 32%

Density of mortar cube = 2162 kg/m<sup>3</sup>

Water cement ratio = 0.55

### Quantity for cubes:

Volume of 6 cubes =  $2.1108 \times 10^{-4} \text{ m}^3$

Weight of 6 cubes = volume x density  
=  $2.1108 \times 10^{-4} \text{ m}^3 \times 2162 = 4.647 \text{ kg}$

Weight of sand required for 6 cubes =  $\frac{6}{7} \times 4.5635 = 4.647 \text{ kg}$

Weight of cement required for 6 cubes =  $\frac{1}{7} \times 4.5635 = 0.7625 \text{ kg}$

Weight of water required for 6 cubes =  $0.6 \times 4.5635 = 0.428 \text{ kg}$



Fig: vicat's apparaters



# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017

## Sorptivity Test on Cement Mortar

Sorptivity test is a material form which represents the impulse of a porous material to absorb and spread water by capillarity. The capillary water absorption is called the sorptivity, which can be considered as a measure of the durability of concrete. This of the can be determined by the measurement of the capillary rise absorption rate on reasonably homogeneous material. The cumulative water absorption (per unit area of the inflow surface) increases proportionally with the square root of elapsed time ( $t$ ) as follows.

$$I \propto S \sqrt{t}$$

$$S \propto \frac{I}{\sqrt{t}}$$

Here the cumulative water absorption per unit area of the inflow surface can be calculated as follows:

Where;  $S$  = sorptivity in mm,  $t$  = elapsed time in min.

$$I \propto \frac{\Delta w}{A \rho d} \tag{2}$$

Where,  $\Delta w$  = change in weight of cube after the elapse time =  $w_2 - w_1$ ;  $w_1$  = oven dry weight

(1a) (1a)

of cylinder in grams;  $w_2$  = weight of cubes after  $t$  time capillary suction of water in grams,

$A$  = surface area of the specimen through which water penetrated; and  $d$  = density of water The steps involved in the test are as follows [22]

- i) The specimen was cured for 7 days and then dried in oven at a temperature of 100°C for a period of 24 hours.
- ii) After drying in oven the flow from the peripheral surface of the cubes is prevented by sealing it properly with non-absorbent coating (knife putty filler)
- iii) Cubes are immersed in the water with water level not more than 5 mm from the bottom of the cube after the filler dries out
- iv) The quantity of water absorbed in time period of 30 minutes, 1, 2, 4, 6, 12, 24, 36, and 48 hours was measured by weighting the specimen using a weighting balance with a precision of 0.1 g. Surface water on the specimen was wiped off with a dampened tissue and each weighting operation was completed within 30 seconds

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017



Fig 3.7 cubes arranged for sorptivity test

Table 3.5 Cumulative water absorption for various concentrations of bacteria

Time of Soaking, t (hours)	Cumulative water absorption (mm)					
	Control specimen	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>8</sup>	10 <sup>9</sup>
0.5	9.57	8.25	8.82	10.98	8.22	6.82
1	9.71	8.68	9.09	11.15	8.48	7.15
2	10.12	9.15	9.85	11.43	8.95	7.59
4	10.44	9.64	9.96	11.83	9.65	7.95
6	10.7	9.89	10.06	12.23	9.87	8.69
12	10.95	10.18	10.45	12.42	10.15	8.62
24	11.4	10.58	11.14	13.10	10.64	9.22
36	11.58	10.75	11.19	13.16	10.49	9.38
48	11.72	10.96	11.3	13.24	10.69	9.96

Fig. 3.7 presents the cubes arranged for sorptivity test. The data analysed from the above test is given in the Table 3.5. The graphical representation of the same is given in Fig. 3.8. It can be observed that as the bacterial cell concentration

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017

increases the cumulative water absorption is also increasing and reaching a maximum value at  $10^7$  cells/ml and then goes on decreasing. The minimum cumulative water absorption is obtained for a cell concentration of  $10^9$  cells/ml

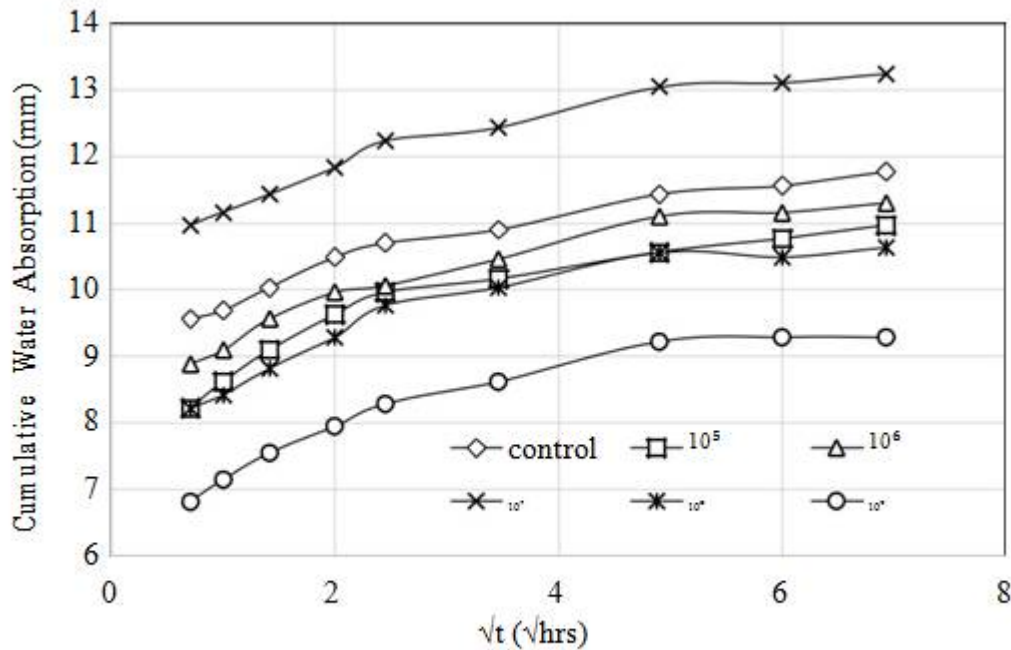


Fig: Cumulative water absorption for various cell concentrations

### III. CHARACTERIZATION STUDIES

Various characterization techniques/ methods are used to analyse the formation of calcite by means of bio-mineralization. Samples were collected from the tested mortar in the form of powders and/or broken pieces to conduct the above studie

#### XRay Diffraction Spectrometry

The crystallinity of the calcium carbonate can be found out using X-Ray diffraction spectrometry. Calcium carbonate can exist in three polymorphic forms. They are calcite, aragonite and vaterite. Calcite is the most stable and the least soluble one among the three [14] Mineralogical composition of the deposited  $\text{CaCO}_3$  crystals were analyzed by X-ray diffraction.

#### *X-Ray diffraction spectrometry on the layer formed over the curing water*

Fig. 3.7 shows the pathogens mortar cube samples in curing solution. It can be observed in this figure that a layer is formed over the surface of water. When this layer was dried it gave a white colour powder. The XRD analysis of this layer was carried out and results are presented in Fig. 3.9. It can be observed that all the peaks obtained from the graph of calcite. Thus we can conclude that the layer was calcite which was produced by bacteria.

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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Vol. 6, Issue 5, May 2017



Fig Pathogens mortar cubes in solution tub

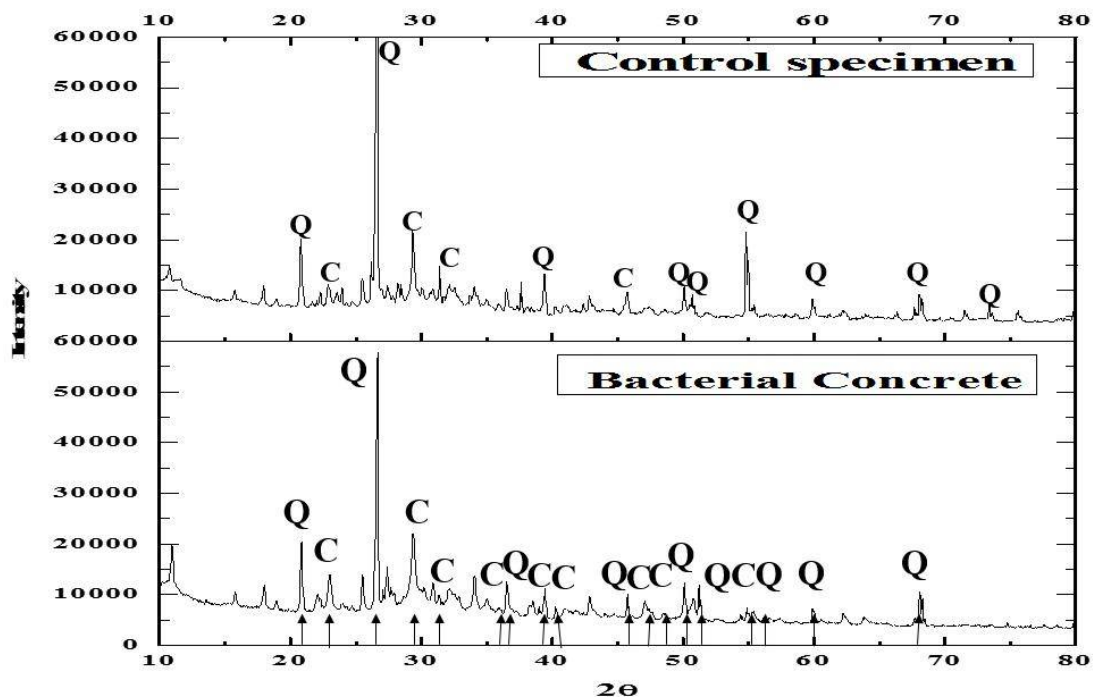


Fig XRD for the mortar cube sample with bacteria and control cubes ('Q' represents quartz or silica and 'C' represents calcite)

## International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017

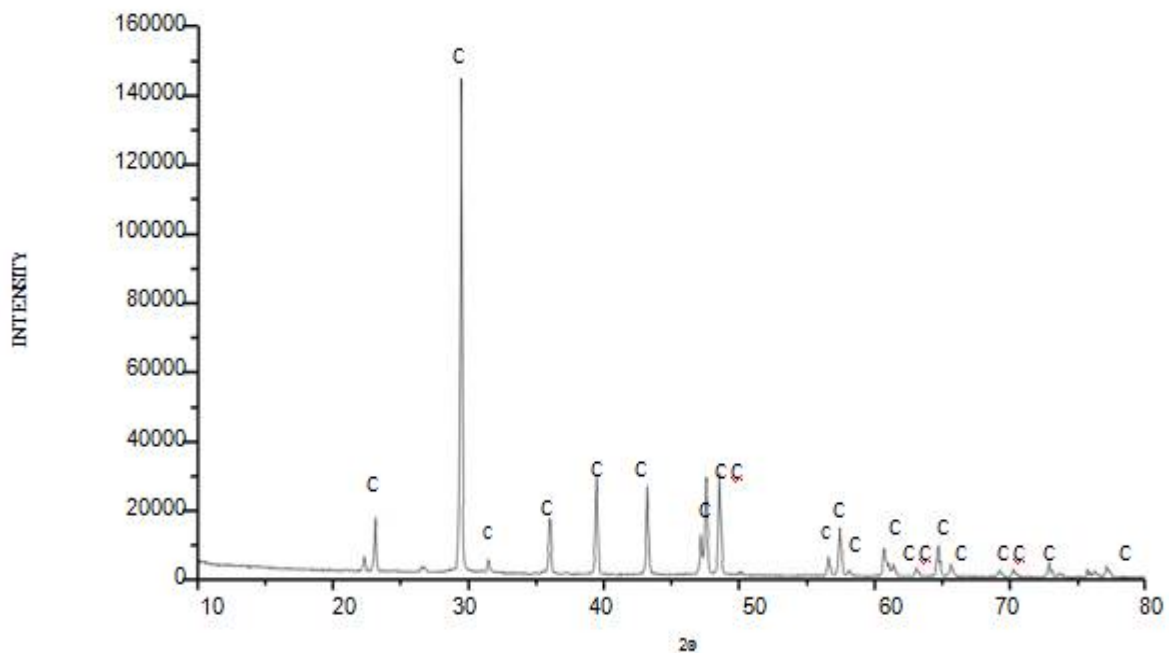


Fig. XR-D for the top surface layer found water for curing (C represents calcite)

### *X-Ray diffraction spectrometry on mortar cubes*

From the specimens used for compressive strength testing small amount of sample was collected from the core of the cube and sieved through 100 $\mu$  IS sieve and was tested by XRD. The result obtained was analysed using XPert High Score software and the result was plotted using Origin software.

### **FESEM on Mortar Cubes**

The morphological, qualitative and semi-quantitative analysis of the deposited CaCO<sub>3</sub> crystals were investigated with scanning electron microscopy coupled with Energy Dispersive X-Ray.

. From Fig 3.11 and 3.12 which corresponds to samples collected from the core of mortar cubes used for compressive strength study after 7 day and 28 day curing, the amount of calcite crystals is more in the bacterial mortar cubes than in the control cubes. It is the presence of this crystalline calcite that lead to the improvement of compressive strength.

# International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 6, Issue 5, May 2017

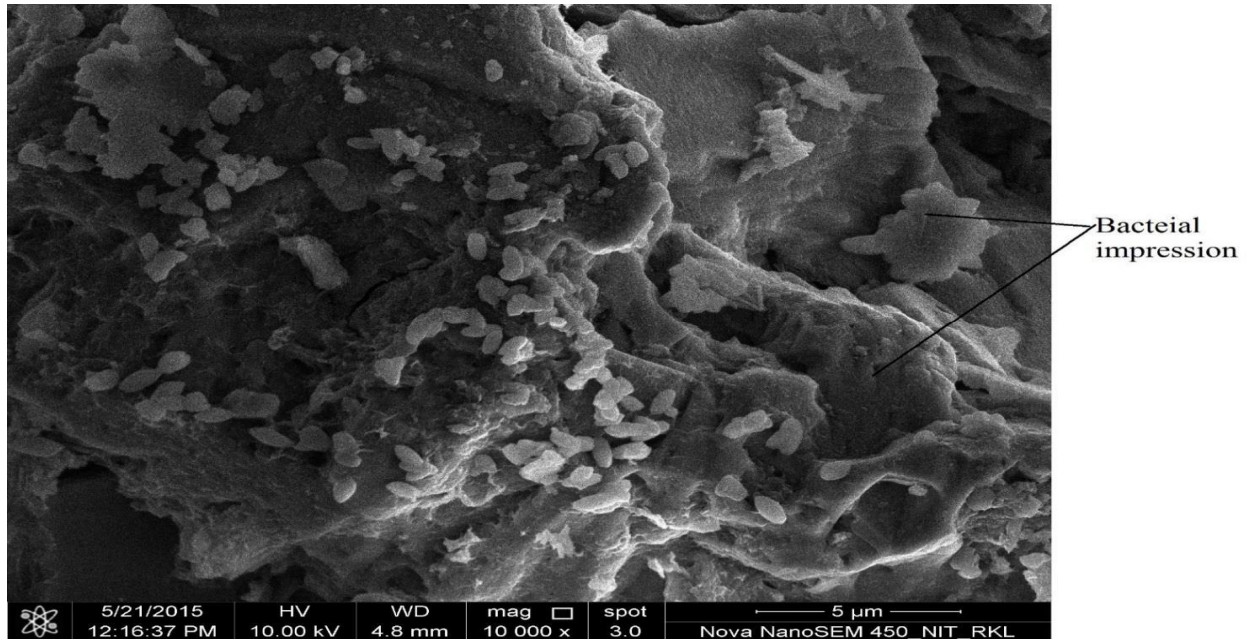


Fig 3.11 FESEM image showing bacterial impression on calcite crystals

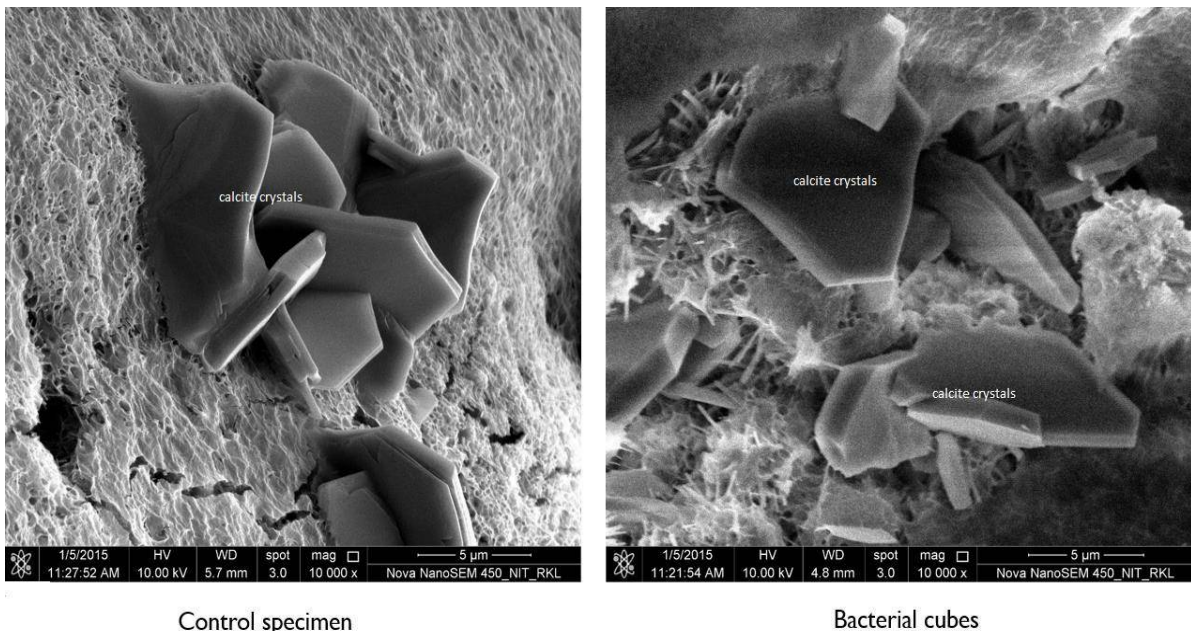


Fig 3.11.1: FESEM image of cubes after 7 day curing

# International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: [www.ijirset.com](http://www.ijirset.com)

Vol. 6, Issue 5, May 2017

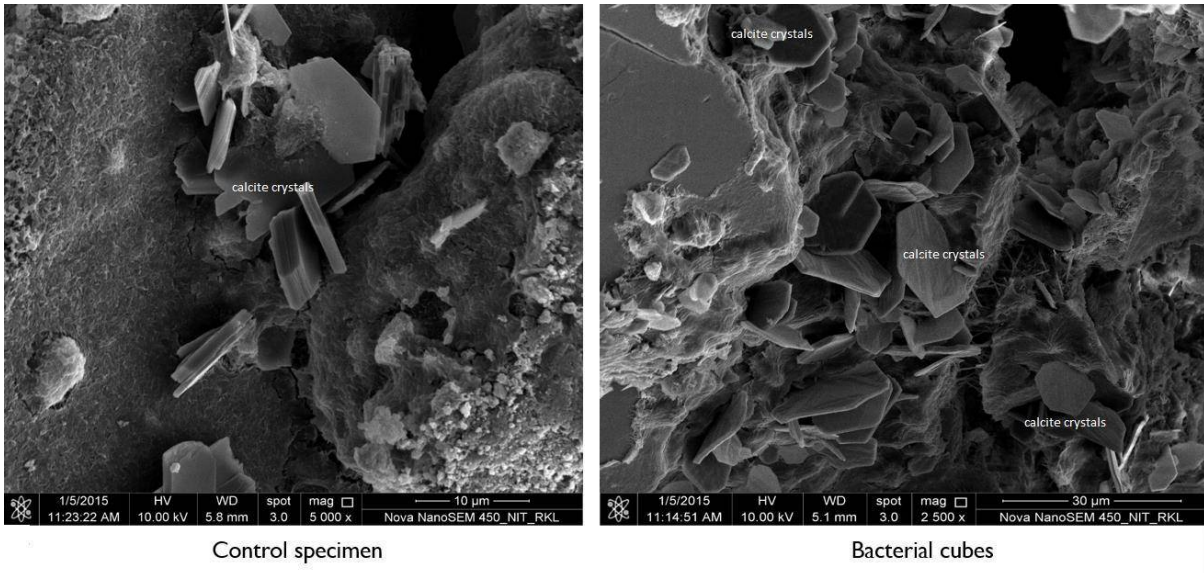


Fig 3.12: FESEM images of cubes after 28 day curing

To examine the continuous different variation in the microstructure of the cement mortar due to the presence of bacterial calcite precipitation, bacterial mortar cubes were casted and analysed by FESEM for 7, 14 and 28 days and it was observed that needle shaped structures are seen more in concentration and growing as the number of days of curing increases and are less when it reaches a curing period of 28 days. We can see that the lamellar rhombohedra structures concentration increases as the curing period increases. Fig's.14 and 16 shows the FESEM pictures of cement mortar cubes after 7, 14 and 28 days curing properly.

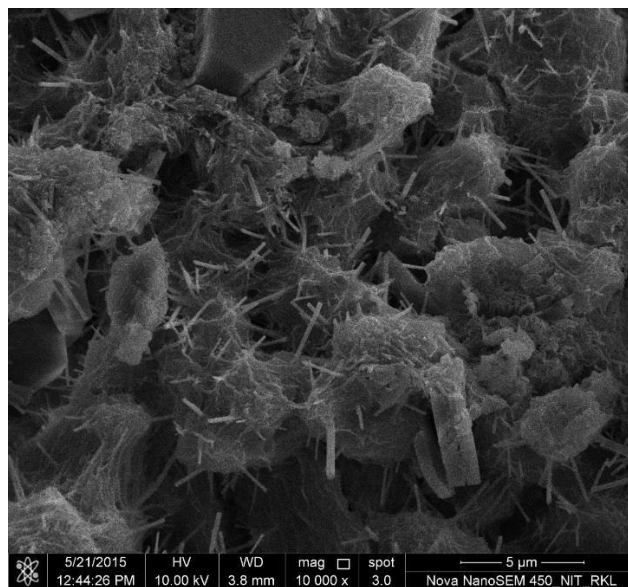


Fig 13 FESEM images of cubes after 7 day curing

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Vol. 6, Issue 5, May 2017

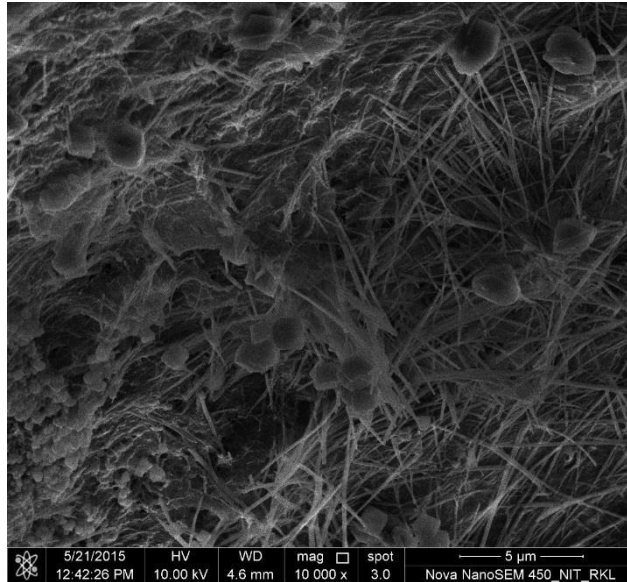


Fig 14: FESEM image of mortar cube after 14 days of curing

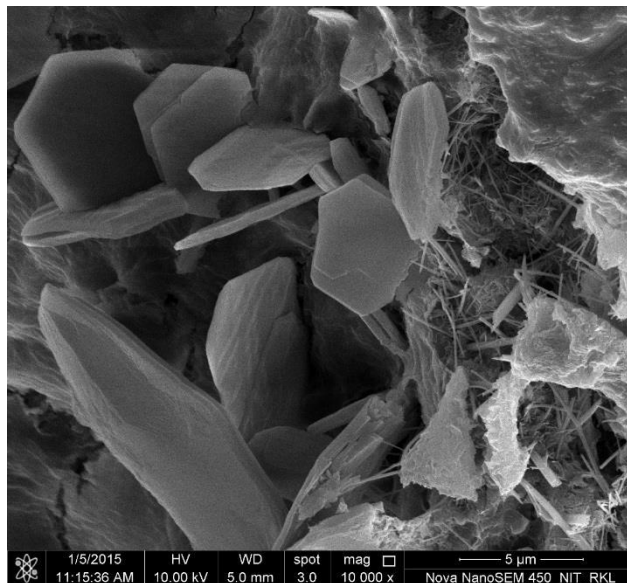


Fig 15: FESEM image of mortar cube after 28 days of curing



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Vol. 6, Issue 5, May 2017

## SUMMARY

The objective of this study was defined to enhance the engineering properties of normal strength cement mortar using a single bacterial species. There are many bacteria reported in literature which can improve the strength, durability and other mechanical properties of concrete and cement mortar. To analyze the variation of compressive strength and capillary water absorption of cement mortar cubes with various combinations of pathogens, cement to sand ratio of 1:6 and water cement ratio of 0.57 are dealt with to prepare the mortar cubes. Accordingly, different mortar cubes with bacterial concentration of 0, 105, 106, 107, 108, and 109 cells/ml are prepared. The mortar cubes then cured in water solution of urea and calcium chloride. 2% of urea (in terms of volume of total water) was added in to solution to activate the urease enzyme used for the metabolism of bacteria. Various characterization techniques are used to analyse the formation of calcite due to bio-mineralization. These techniques involve all modes of microbial analysis like imaging, diffraction and spectroscopy, including X-rays. Samples were collected from the tested mortar in the form of powders and/or broken pieces to conduct the above studies.

## IV. CONCLUSIONS

The important conclusions drawn from the above study are listed as follows:

To improve the properties of cement mortar or concrete the appropriate bacteria should be selected judiciously *Bacillus sphaericus* found to be not altering the normal consistency and setting time of the cement paste. Compressive strength (at 7-day and at 28-day) of mortar cube found to be raise with the increase of bacteria concentration up to 107 cells/ml. However, further increase of bacteria concentration found to reduce the compressive strength of cement mortar. The optimum doses of bacteria found to enhance the average compressive strength by 58% (at 7-day) and 23% (at 28-day) over the control specimen. The more increase in strength after 7 day curing may be due to the presence of nutrient medium and it getting depleted as it reaches 28 days and causing death of bacteria [20] In order to notify if the improve in strength is due to the addition of urea and calcium chloride in curing water one set of control cubes were cured in the same solution and it was found that there was negligible (4%) variation in the strength. The minimum cumulative water absorption is achieve for a cell concentration of 109 cells/ml. Optimum dose of bacterial cell concentration found to increase the cumulative water absorption over the control specimen. The morphology of the bacterial calcite was found out by FESEM. It displays the straight accomplice of bacteria in calcite production. This shows the system allows for the continuous formation of calcite [20]. It can be clearly seen that the rod shaped ones are more in 7 day cured cubes and hexagonal ones are less and as days increases the hexagonal crystal concentration increases. From the literatures [20] it can be noted that the rod shaped structures are aragonite crystals of which are the precursors of formation of calcite crystals The XRD analysis was conducted for bacterial and control mortar cubes. The XRD result after 28 day curing shows the presence of more calcite peaks in bacterial mortar sample than the control specimen. Presence of more calcite peaks signifies the presence of more calcite in the sample [5]. A layer was consider to be formed over curing water of bacterial specimen after a few days. The XRD analysis of this layer proved that this layer is of calcite. This layer was not notified on the curing solution with control specimen. It can be concluded from this information that calcite was produced by bacteria which is important for the increase compressive strength of mortar cube.

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