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Ureolytic Bacteria Effect Analysis for Mechanical properties Enhancement of Cement Mortar

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Abstract

In the present scenario where the constructions are increasing, the need to find a supplementary cementing material for the improvement of strength and which has less environmental effects is of great significance. Ureolytic bacteria are the ones which can improve the strength of cement mortar by the precipitation of calcium carbonate in the presence of urea and a calcium source. In the present study Bacillus sphaericus is used to check its applicability in this regard.

INTRODUCTION

History and Source

Cement is a binder, a substance used in construction that sets and hardens and easily can bind other materials with each other. The most important types of cement are used as a component in the production of mortar in masonry, and of concrete- which is a mixture of cement and aggregate to form a strong building material. Improvement in concrete technology can be achieved through its strength improvement and its enhancement in durability using pollution- free and natural methods. As the construction industry is progressing, the usage of cement is also increased exponentially as we are in search of stronger and durable structures.

Brownmillerite ($4CaO{\cdot}Al_2O_3{\cdot}Fe_2O_3$).

This increases the cement productivity globally and in turn increases the carbon dioxide emission to the atmosphere. We need to find a technique which can increase the strength and durability of structures without increasing the use of cement for a better future. Supplementary cementing materials (SCMs) are used in concrete mixes which reduces cement contents, improve workability, increase strength and enhance durability with the help of hydraulic or pozzolanic activity. Silica fume and fly ash are commonly incorporated in concrete as partial cement replacement. All building materials are porous.

This porosity of the building material along with penetration of moisture and some other harmful chemicals such as, chlorides acids and sulphates adversely affect the concrete and reduce the structures strength and life. An additive that seals the pores and cracks and thus reduces the permeability of the structure. Conventionally, a variety of sealing agents such as latex emulsions suffer from serious limitations of incompatible interfaces, unstable molecular structure and high cost, susceptibility to ultraviolet radiations.

One of the encouraging biomimetic processes in nature is done by soil-thriving bacteria. It converts sand to sandstone. Later, it was found out that, a calcite precipitating bacteria,

Bacillus pasteurii, was responsible for the binding agent production for this conversion. This mineral deposition technique can answer for the natural method for the sealing of pores and cracks of concrete and mortar. Biomineralization is defined as a biologically induced precipitation in which a local micro-environment is created by an organism with conditions that allow optimal extracellular chemical precipitation of mineral phases. This can be observed in many biological species living in various natural environments such as soil, geological formations, fresh water biofilms, hot springs, saline lakes and oceans. The exact mechanism behind the microbial calcium carbonate precipitation is not found till date.

Non-hydraulic cement, such as slaked lime (mixture of calcium hydroxide and water), hardens because of carbonation in the presence of CO_2 which is naturally present in the atmosphere. First of all calcium oxide is produced from calcium carbonate by the process of calcinations at temperatures above 825 °C (1,517 °F) for 10 hours at atmospheric pressure.

$$CaCO_3 \rightarrow CaO + CO_2$$

The calcium oxide is then spent (slaked) mixed it with water to make slaked lime (calcium hydroxide)

$$CaO + H_2O \rightarrow Ca(OH)_2$$

When there is no excess water because of completely evaporated, the process of carbonation starts:

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$$

This reaction takes a much amount of time because of the low partial pressure of carbon dioxide in the air. The carbonation reaction always requires the dry cement to be exposed to air, and for this reason the slaked lime is non-hydraulic cement, which cannot be used under water. This whole process is called the lime cycle.

Conversely, when water is added hydraulic cement hardens by hydration. Hydraulic cements (such as Portland cement) are a mixture of silicates and oxides, these are the four main components being:

Belite ($2CaO{\cdot}SiO_2$); Alite ($3CaO{\cdot}SiO_2$);

Tricalcium aluminate ($3CaO{\cdot}Al_2O_3$) (historically, and still occasionally, called 'celite').

Objectives

The main objective of the present study is identified as to improve the engineering properties of normal strength cement mortar using a single bacterial species. This study was defined to improve the properties of 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. This main objective is divided into following sub objectives:

a) To study the variation of compressive strength of cement mortar with bacteria b) To study the setting time of cement in the presence of bacteria
b) To study the capillary water absorption of cement mortar using bacteria
c) To study the effect of bacterial culture medium on the setting time of cement.

Methodology :

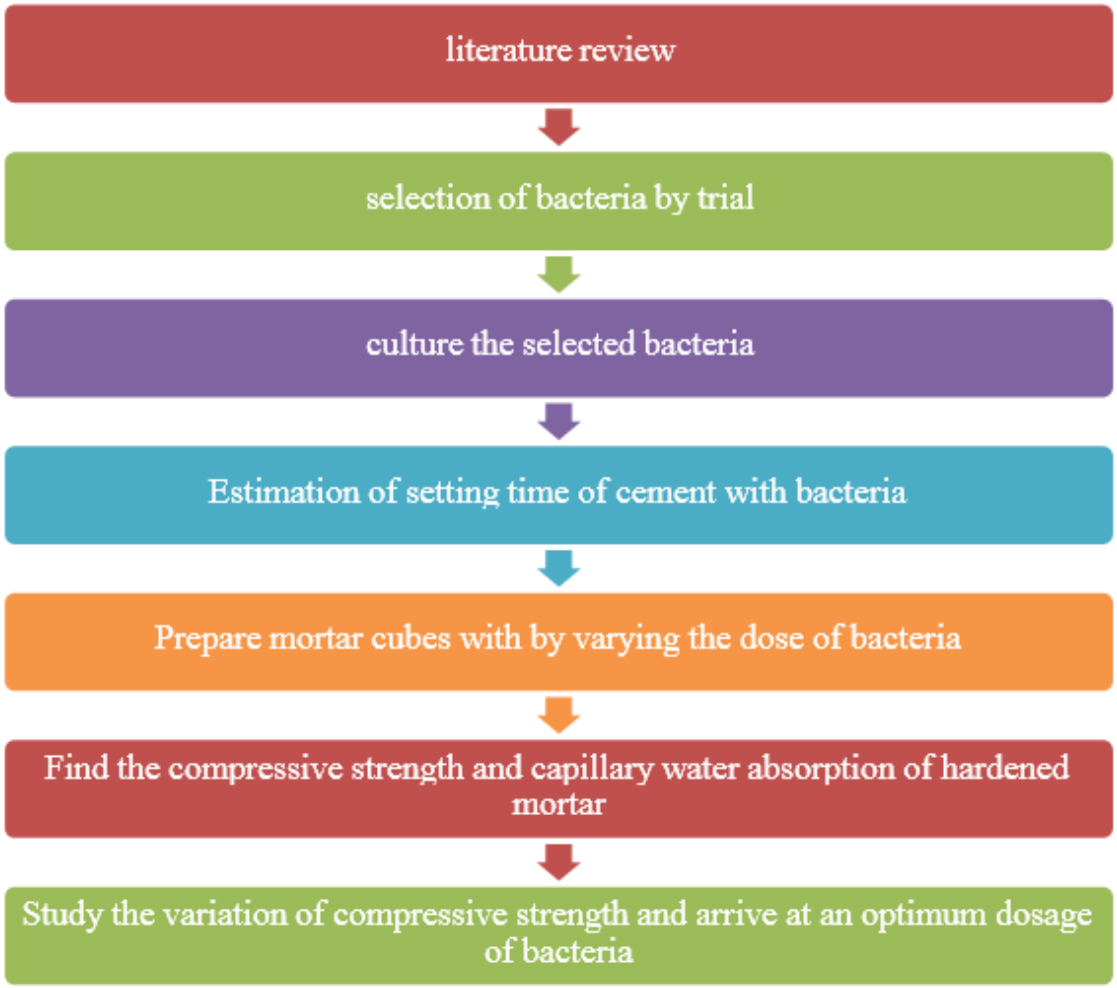

Figure 1. Methodology adopted

EXPERIMENTAL WORK

Selection of Bacterial Species

There are numbers of bacterial species in literature for improve different properties of concrete and cement mortar. Moreover, the present study requires a bacteria which is noncontagious, that survive in the alkaline concrete like environment and that must be capable of producing calcium carbonate through the metabolism. The single celled eukaryotes like bacteria and other microbes can live and reproduce only if they have certain range of environmental conditions. The pH range of a fresh concrete is in between 11.5 to 13 and there will be rise in temperature because of heat of hydration. The ureolytic bacteria used in this study should be alive in these alkaline environments and also have temperature tolerance. Two different noncontagious ureolytic bacteria (bacillus), namely, **Bacillus cereus** and **Bacillus sphaericus** are tested in this study to check its survival in a concrete-like environment.

Testing of survival of Bacillus cereus in concrete-like environment

The following steps are involved in testing the tolerance of this bacteria in concrete-like environment.

i. 230 ml of nutrient medium (Luria Bertani broth) was prepared for the culture of bacteria.
ii. It was then transferred to 12 fresh clean test tubes and its volume was made to 10ml by adding NaOH to increase the pH in the test tubes. (pH of the medium was then found to be 8, 9, 10, 11, 12, and 12.5 in two sets of test tubes respectively)
iii. After the preparation of media, the test tubes were sealed with cotton plug and the sterilized using autoclave.
iv. After autoclaving, *Bacillus cereus* bacteria from the mother culture was scraped and added to the test tubes and mixed well
v. It was then incubated for 24 hours at 37ºC and 50ºC.

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:

i. Growth of bacteria was not observed in the any of the six cultures incubated at 50ºC
ii. At 37ºC, growth was observed only in cultures with pH 8 and 9.

Table 1 presents the result of the growth of *Bacillus cereus* in different cultures. It can be seen from the table that the bacteria could survived only in the cultures with pH 8 and 9. However, the pH of fresh concrete (or cement mortar) is in the range 11.5 to 13, which means that *Bacillus cereus* may not survive in concrete-like environment. Therefore some other species of Bacillus which have a pH tolerance of 11.5 to 13 should be used.

Table 1. Temperature and pH tolerance of *Bacillus Cereus*

pH	Presence of Bacteria	
	cultures	cultures incubated at 50ºC
8	+	-
9	+	-
10	-	-
11	-	-
12	-	-
12.5	-	-
+ presence of bacteria; - Absence of bacteria		

Testing of survival of *Bacillus* sphaericus at in concrete-like environment

As the trial of Bacillus cereus failed a different species of the Bacillus group Bacillus sphaericus was considered next. The same procedure described in the previous section was followed to test *Bacillus sphaericus*. Growth of bacteria was observed in the cultures incubated at both 37ºC and 50 ºC 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 12.5 at both 370C and 500C. Therefore it can be concluded that this Bacillus species can be suitable for fresh concrete (or cement mortar) which has pH about 11.5 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.

Table 2. Temperature and pH tolerance of Bacillus sphaericus

pH	Presence of Bacteria	
	cultures incubated at	cultures incubated
8	+	+
9	+	+
10	+	+
11	+	+
12	+	+
12.5	+	+
+ presence of bacteria; - Absence of bacteria		

Casting and Curing Of Specimens

To check any possible change in the characteristics of cement paste due to addition of bacterial solution, Basic tests of cement such as normal consistency and setting time were carried out before casting of the test specimens

Tests on Cement

The standard tests conducted on cement are given below:

Testing for standard consistency of cement :

Theory:

To measure the quantity of water requires producing a cement paste of standard consistency. To mix with cement a minimum quantity of water is required so as to complete chemical reaction between the water and the cement, water less than this quantity could not complete chemical reaction so that resulting in reduction of the strength and increasing water will increase water cement ratio and so will reduce its strength. So that correct proportion of water in cement is required to be known to gain proper strength when using cement in structure. This could be finding out standard consistency of cement paste.

Standard consistency:

Standard consistency of cement paste could define as the consistency which permits vicat plunger to penetrate up to a point 5 to 7 mm from the bottom of the vicat mould. It expressed as a percentage (by weight) of dry cement as amount of water.

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

i. First keep the vicat apparatus on a level base (while using vicat apparatus with dash pot, the bearing movable rod should be kept to its highest position and pin it.) Unscrew the top of the dash pot. Fill the dash pot half with any suitable oil of viscosity and screw the top. Work with the plunger a number of times.

ii. For determining standard consistency attach the plunger to the movable rod. Work with the plunger a number of times.

iii. Take 300 gm of cement in a pan and weighed quantity of water in a beaker. Then prepare a paste with the water to cement. At the time of adding water to cement start a stop watch.

iv. Vicat mould kept on a non porous plate and in it fills the cement paste.

v. After completion of filling the mould, shake it to expel the air. Then smooth off the surface of the paste make it level with the top of molder. The cement paste then prepared for the test block.

vi. Place these test block on the non porous plate under movable rod, bearing the needle.

vii. Lower the plunger easily to touch the surface of cement paste and quickly release; (while vicat apparatus with dash pot used, place the mould filled with cement paste and non absorbent plate on the base plate of the vicat apparatus. Then raise the plunger from the dash pot now bring it in contact with the top cap of the bearing rod. Remove the pin and holding the movable bearing rod to the surface of the cement paste after that quickly release by pushing down the plunger to sink in to the cement paste). This operation should be done immediate after filling the mould.

viii. Prepare trial test specimens with various percentages of water until plunger penetrates from a point 5 to 7mm from the bottom of the vicat mould, which is taken on the scale. Expressing the water required as percentage by weight of the dry cement.

Calculation:-

1. Percentage of water = volume of water / quantity of water X 100

   = 70/300 X 100

   = 23.33%

2. Percentage of water = volume of water / quantity of water X 100

   = 85/300 X 100

   = 28.33%

3. Percentage of water = volume of water / quantity of water X 100

   = 100/300 X 100

   = 33.33%

4. Percentage of water = volume of water / quantity of water X 100

   = 106/300 X 100

   = 35.33%1

5. Percentage of water = volume of water / quantity of water X 100

   = 110/300 X 100

   = 36.67%

Observation:-

Table 3. Different composition variation

Sr. No.	Quantity of cement	volume of water	Penetration of plunger from bottom	% of water
1.	300 gm	70 ml	28 mm	23.33
2.	300 gm	85 ml	23 mm	28.33
3.	300 gm	100 ml	13 mm	33.33
4.	300 gm	106ml	11 mm	35.33
5.	300 gm	110 ml	7 mm	36.67

Result: - Standard tests on cement is conducted for the consistency of cement as per Indian standard IS 4031 Part 4 (1988). The consistency of the cement paste was found to be 36.67%

Testing for initial setting time and final setting time

Theory:-

- This test is used to determine the setting time of the hydraulic cement by a VICAT needle apparatus.
- The known setting time of the cement is always helpful to decide the time duration to mix, transport, place and compact the concrete effectively.
- Always prefer a more initial setting time so we can mix, transport and place the concrete easily. According to ASTM specifications, initial setting time should not be less than 45 min but in the field we could prefer an initial setting time not less than 90 min.
- Smaller value of the final setting time will always preferred in order to avoid large expenditures on the formwork. According to mostly specifications, the final setting time should not be greater than 10 hrs and should not be less than (90 + 1.2 x (initial setting time)) min.

  i.e (90 + 1.2 x (initial setting time)) min < **final setting time** < 10hrs

Prepare a paste of cement of standard consistency and put it in the ring of the Vicat apparatus within the allowable time of 4±1/4 min. Clear and level any extra paste by the means of a trowel.

1- Initial Setting Time

At the start determine the penetration of the 1 mm needle. A penetration reading of 4-7mm will obtained then note the time as called the initial setting time otherwise in every 10min keep checking the penetration reading until a penetration reading of 4-7 mm is obtained which would be the initial setting time of the cement. Take each penetration test at least 5 mm difference from any previous penetration and at least 10 mm difference from the inner side of the mold.

2- Final Setting Time

After that fix the final setting time plunger in which 1mm diameter of the smaller needle and the 5mm diameter of the outer needle. Drop the rod of the Vicat apparatus and note the time while the smaller 0.5 mm diameter needle has completely penetrated into the paste and the outer needle will leave no impression on the cement surface.

Observations & Results:-

Time of Starting the experiment = 9:20 am

Time at taking initial setting reading = 10:45 am Time at taking final setting reading = 1:40 pm Initial Setting Time = …**1 hour 25 minutes**…. Final Setting Time = …**4 hours 20 minutes**….

The values of setting time obtained are presented in the Table 3.3. It can be seen from the table that the presence of bacteria does not have considerable effect on the setting time.

Table 4. Setting time of the cement paste

Specimen	Initial Setting time	Final Setting time
Cement paste with tap water	1 hour 25 minutes	4 hours 20 minutes
Cement paste with tap water and	1 hour 10 minutes	4 hours 50 minutes

Details of Mortar Cube Test Specimens :

As one of the objectives is to study the variation of compressive strength of mortar cubes with various concentrations of bacteria, cement to sand ratio of 1:6 and water cement ratio of 0.55 are considered to prepare the mortar cubes. Accordingly, the amount of cement, sand and water are calculated as shown in the Table 3.4. The table presents identifications for different mortar cubes such as control, B1, B2, B3, B4 and B5 with number specimens casted and its constituents. The specimens B1-B5 is prepared by replacing appropriate amount of water with bacterial solution to get the desired level of concentration of bacteria in water. Section 2.4.2.1 presents the procedure to prepare the cell culture whereas Section 2.4.2.2 presents the detailed procedure to prepare the mortar cubes.

Table 5. Details of mortar test specimen for mechanical properties

Mortar cube ID	Bacteria concentration (cells per *ml*)	Number of specimen for			Mix proportion			Curing Soln.
		7 day comp. strength	28 day comp. strength	Capillary water absorption	Cement (kg)	Sand (kg)	Water (ml)	
Control	0	3	3	3	0.13	0.77	72ml	†
Control	0	0	3	0	0.13	0.77	72ml	Ø
B1	10^5	3	3	3	0.13	0.77	72ml*	Ø
B2	10^6	3	3	3	0.13	0.77	72ml*	Ø
B3	10^7	3	3	3	0.13	0.77	72ml*	Ø
B4	10^8	3	3	3	0.13	0.77	72ml*	Ø
B5	10^9	3	3	3	0.13	0.77	72ml*	Ø

* indicates that the volume of water includes bacteria and culture medium
† indicates tap water as curing solution
Ø indicates a mix of tap water, urea and calcium chloride as curing solution

The mortar cubes are de-moulded after 48 hours as indicated in Section 3.3.2.2 and then placed in curing solution of tap water, 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. Calcium chloride of 25 mM/lit was added to supply a source of calcium to the system in order bacteria can produce the desired CaCO3. One set of control specimen were also cured in tap water without any admixture (urea and calcium chloride) for reference.

Making of cell culture for bacterial mortar cubes :

i. 500ml of Luria Bertani broth was prepared in two fresh clean conical flask of 1l
ii. After autoclaving the nutrient medium bacteria is inoculated into it for 24hours

The water is replaced by bacteria culture in bacterial mortar cubes

Making of mortar cubes of 1:6 cement to sand ratio:

i. Take required amount of cement and sand and mix them dry thoroughly.
ii. Add the calculated volume of water to the dry mix of cement and sand and mix thoroughly fro not more than 4 minutes.(potable water was used for the preparation of control specimen and bacterial medium was used for the preparation of bacterial concrete cubes),
iii. Place the mortar in the mould and mount it in the holder of the vibrating machine and clamp it in position
iv. Fill the mould with required amount of mortar during vibration and the vibration should be done as per specified speed to attain the required compaction.
v. After attaining required compaction, remove the mould from the holder and keep it in a place 48 hours for setting.

At the end of 48 hrs remove the cube from the mould and immediately submerge in the water for obtaining the required curing.

Testing of Specimen for Mechanical Properties

Testing of specimen for these two mechanical properties, Compressive Strength and capillary water absorption were carried out and find out the effect of bacteria (*Bacillus sphaericus*) on these two mechanical properties. These specimens were tested after 7-days and 28-days of curing. The following section presents the results of these two tests.

Compressive Strength Test on Specimen

All the mortar cubes are tested in a compression testing machine to obtain the unidirectional compressive strength obtained at 7 days and 28 days as shown in Table 3.5. The same results are also plotted in Figs. 3.8 and 3.9 for 7-day and 28-day compressive strength respectively. It can be observed from the table and the figures that as the cell concentration increase the compressive strengths at both 7 days and 28 days increases initially and then decreases. The maximum strength occurs at a cell concentration of about 107 cells/ml and hence this cell concentration can be treated as Optimum dosage.

Two control specimen of same mix proportion ware cured in two different curing solutions:

(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 indicate no significant differences in compressive strength and capillary water absorption among the two control specimens.

Table 6. Effect of bacteria on compressive strength

Cell concentration (cells/ml)	Mean compressive strength at 7	Percentage increase (%)	Mean compressive strength at 28	Percentage increase (%)
0 (Control)	3.10	-	4.80	-
10^5	3.91	26.12	5.92	23.33
10^6	4.25	37.09	6.15	28.13
10^7	5.16	66.45	6.92	44.17
10^8	4.89	57.74	5.60	16.67
10^9	4.44	43.22	5.55	15.25

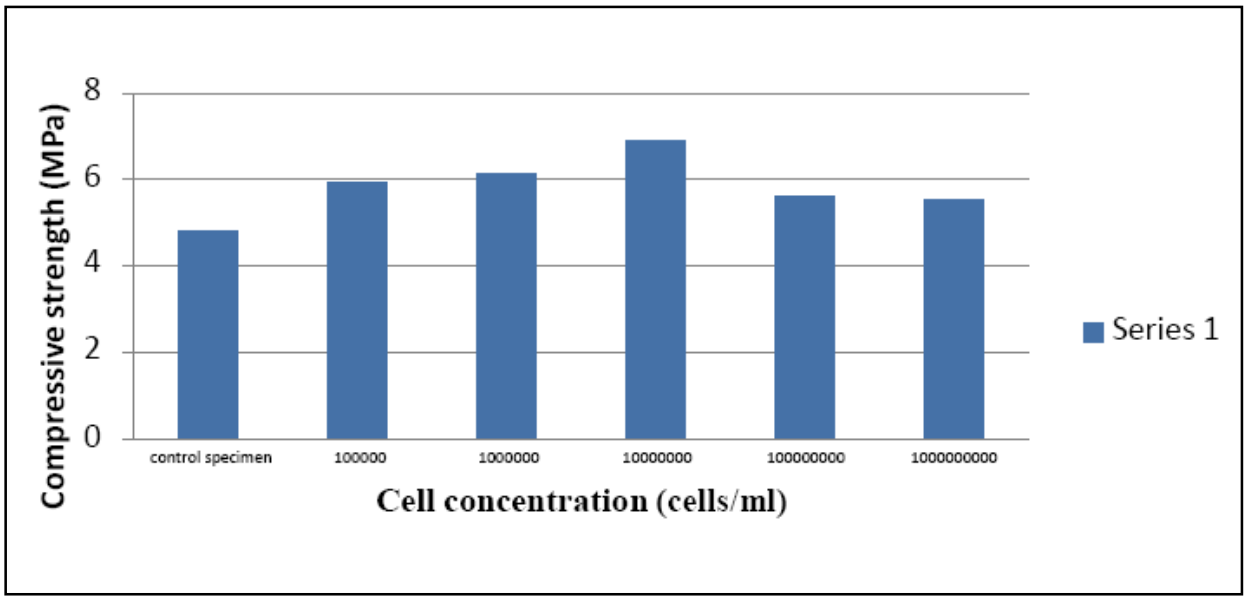

Figure 2. Variation of compressive strength with variation in cell concentration – at 7 day

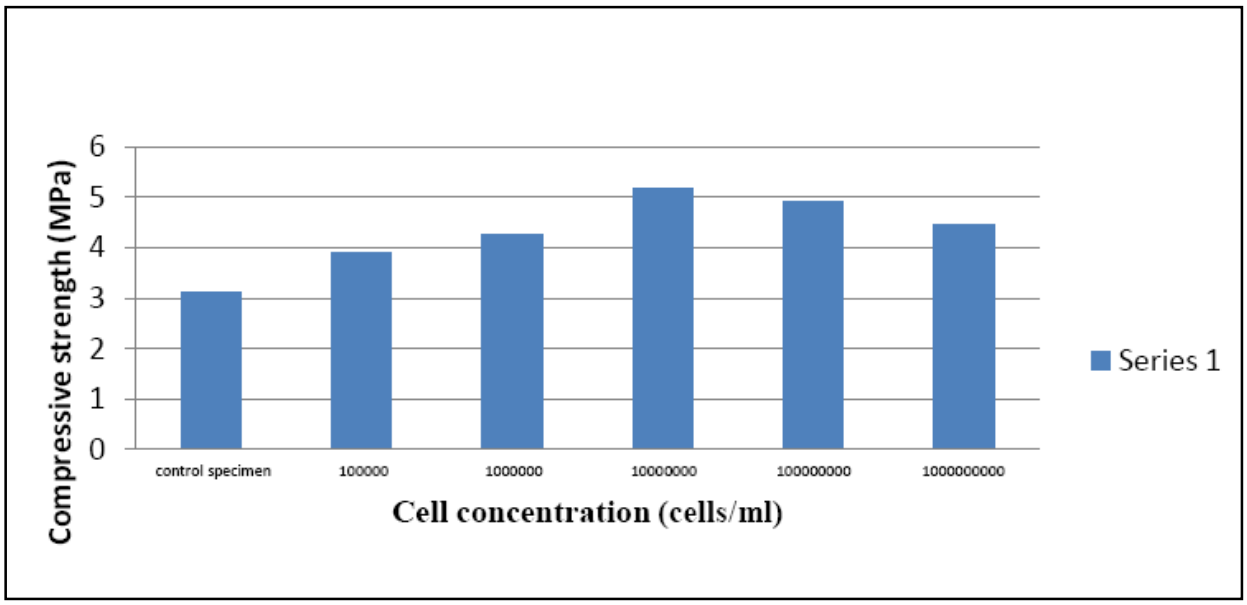

Figure 3. Compressive strength – cell/ml graph for 28 day curing

Sorptivity Test on Specimen

Sorptivity is defined as a measure of the medium to absorb or desorb liquid by capillarity of the capacity. Sorptivity explain the tendency of a material to transmit and absorb water and other liquids by capillarity. Sorptivity is mostly used in characterizing soils and the porous construction materials such as stone, concrete and brick. Require numerical iterative procedures for calculating the true sorptivity dependent on soil water content and diffusivity. Sorptivity could be determined by the measurement of the capillary rise absorption rate on homogeneous material.

The cylinders after casting have immersed in water for 90 days curing. The specimen size 100mm diameter x 50 mm height after drying in the oven at temperature of 100 + 10°C were drowned with water level should not be more than 5 mm above the base of the specimen and the flow from the peripheral surface prevented by sealing it properly with non-absorbent coating. The water quantity absorbed in time period of 30 minutes measured by weighting the specimen on a top pan balance weighting up to 0.1 mg. and surface water on the specimen was wiped off with a wet tissue and weighting operation was completed within 30 seconds.

Sorptivity (S) is a property of material which characterizes the tendency of a porous material to transmit and absorb water by capillarity.

John R. Philip (1969) showed that sorptivity could be determined from horizontal infiltration from where water flow is mostly controlled by capillary absorption:

$I = S \sqrt{t}$

$S = I / \sqrt{t}$

Where; *S* = sorptivity in mm, *t* = elapsed time in min. Here the cumulative water absorption per unit area of the inflow surface can be calculated as follows:

$I = \Delta w / (A \times d)$

Where, Δw = change in weight of cube after the elapse time = $w_2 - w_1$; w_1 is oven dry weight of cylinder in grams and w_2 is weight of cubes after t time capillary suction of water in grams, *d* = density of water and *A* = surface area of the specimen through which water penetrated.

The steps involved in the test are as follows

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 wet tissue and every weighting operation was completed within 30 seconds.

Table 6. Cumulative water absorption for various concentration

Time of Soaking, t	Cumulative water absorption (mm)					
	Control	10^5	10^6	10^7	10^8	10^9
0.5	8.96	8.13	9.01	11.1	8.12	7.34
1	9.00	8.34	9.05	11.15	8.35	7.91
2	10.10	8.59	9.61	11.48	8.64	8.09
4	10.35	9.77	9.78	11.79	8.97	8.13
6	9.58	10.03	9.99	11.97	9.25	8.59
12	10.12	10.34	10.07	12.45	9.84	8.81
24	11.37	10.78	10.57	12.89	10.23	9.18
36	12.39	11.03	10.84	13.10	10.58	9.30
48	13.25	11.15	11.01	13.15	11.01	9.87

The data analyzed from the above test is given in the Table 3.6. The graphical representation of the same is given in Fig. 3.1. It can be observed that as the bacterial cell concentration increases the cumulative water absorption is also increasing and reaching a maximum value at 107 cells/ml and then goes on decreasing. The minimum cumulative water absorption is obtained for a cell concentration of 109 cells/ml

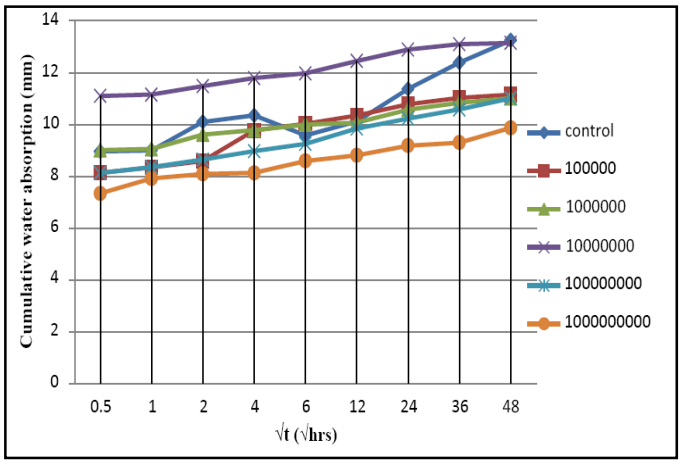

Figure 4. Cumulative water absorption for various cell concentrations

CONCLUSION

a. Now, as previous study we have found a bacteria which can use to improve the properties of cement mortar or concrete the appropriate bacteria. For example, *Bacillus cereus* could not survive in the given environment whereas another Bacillus species Bacillus sphaericus survived.

b. Compressive strength (at 7^{th} day and at 28^{th} day) of mortar cube found to be increasing 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.

b) Ureolytic bacteria require urea and a source of calcium to produce CaCO3 however addition of bacteria alone cannot improve the properties of concrete/cement mortar

c) The optimum doses of bacteria found to increase the average compressive strength by 58% (at 7^{th} day) and 23% (at 28^{th} day) over the control specimen. After 7 day curing more increase in strength may be due to the presence of nutrient medium and it getting depleted as it reaches 28 days and causing death of bacteria.

d) Bacillus sphaericus found to be not altering the normal consistency and setting time of the cement paste.

e) In order to see if the increase 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.

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