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Effect of Various Calcite Precipitating Bacteria on Compressive Strength of Concrete: A Comparative Study

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Abstract

The presence of micro pores in concrete makes the concrete weak and less durable, in this study an ecofriendly attempt has been made to minimize these micro pores by calcite precipitating bacteria i.e. Escherichia Coli, bacillus subtilisand pseudomonas aeruginosa which produce calcium carbonate when they blend with calcium lactate in presence of water and air thus filling up the micro pores, thus comparing their effects on compressive strength of concrete. Various proportions of E. coli, B. subtilisand P. aeruginosa bacterial media were impregnated in concrete viz. 10%,20% and 30% replacing the proportion of mixing water, small proportion of fine aggregate was also replaced by calcium lactate and silica gel. It was observed that concrete with 10% replacement of P. aeruginosa culture media with mixing water showed maximum compressive strength with 79.6% increase in the compressive strength of concrete where as concrete with B. subtilisbacterial media showed 61.1% increase in the compressive strength of concrete with E.coli bacterial media showed 23.5% increase in the compressive strength of the concrete, keeping 10-6 cells/ml the cell concentration for all bacterial concretes.

Key Words: bacterial concretes, Escherichia Coli, bacillus subtilis and pseudomonas aeruginosa, calcium carbonate, compressive strength

1 Introduction

Any fault in the design of concrete may lead to failure of structure; the presence of micro pores in the concrete leads to the cracking of concrete which may cause the knocking down of steel reinforcement by corrosion by ingression of moisture and oxygen. These cracks are undesirable and should be eliminated as the crack repairing has proven to be feverish in nature especially when it comes to repairing of inaccessible cracks which also require highly skilled workmanship and other repairing materials which has proven to be extravagant. Thus taking an enterprise before the crack occurs can prove to be advantageous as increasing the strength

can curtail this hiccup in concrete. The introduction of calcite bacteria in concrete helps in enhancing the properties of concrete in both natural and laboratory conditions .With the reference from the previous researches it has been found that MICCP(microbiologically induced calcium carbonate precipitation) technology has been already used for improvement in compressive strength of concrete. Thus more the compressive strength of concrete less will be its chances of cracking. Moreover the elimination of micro poresin concrete increases the compressive strength of concrete. The calcite precipitating bacteria fills up the



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micro pores increasing the compressive strength of concrete.

The genus Bacillus has been mostly used for thebiological development of calcium carbonate-based mineralsas, which considered to be as ureolytic bacteria. Theformation of calcium carbonate using this type of bacteria isbecause of the hydrolysis of urea to carbon dioxide and ammonia (1). Bacillus megateriumwhich produces calcite canimprove the properties of ash brick. A significant reduction in water absorption was noticed in the treated bricksalong with the increasing compressive strength due to thedeposition of calcite on the voids and surface of bricks. Theextracellular deposition of calcite crystals on the surfaceof bricks are due to the microbial activity as seen from thescanning electron micrographs. These findings show that thistechnology has a better potential towards the development ofeco-friendly and durable building blocks [2].

The use of aerobic microorganisms *Pseudomonas aeruginosa*as self-healing agents have shown 18% improvement in the compressive strength of cement mortar [3]. Durability of cementitious materials can be improved along with the deposition of carbonate by *Bacillus sphaericus*as surface treatment [4]. The use of bacteria for enhancing the durability of concrete as to show resistance towards the alkali, freeze-thaw attack, sulfate, drying and shrinkage [5].

The increase in the compressivestrength of cement mortar (25 %) at 28th day was observed with the addition of thermophillic and anaerobic bacteria (E.Coli) in the range of 10^5 cells/ml to the mixing water. The strengthimprovement was due to the

growth of filler material withinthe pores of cement—sand matrix (6). The use suitable bacteria in concrete canincrease its durability and resistance even in the presence of strong acids such as sulphuric acid and hydrochloric acid [7].

Among the various positive effects of using calcite precipitating bacteria in concrete, self healing of concrete is also the major parameter. The potential of crack-healing using a novel selfhealing agent that was embedded in a porous clay particle acted as reservoir which replaced the minor portion of regular concrete aggregate. The self-healing agentconsisting of bacterial spores and calcium lactate werereleased through the crack ingress water, whenever crackformation occurred. The bacterial induced formation of calcium carbonate helped in sealing of the micro cracks up to0.46 mm-wide. Therefore, it was concluded this novel biochemicalselfhealing agent has a true potential towardsincreasing the durability concrete structures existing in the wet environment [8]. In addition to be applied externallyfor crack remediation, microbial CaCO3is being investigated for selfhealing of concrete cracks. Concrete cracks arealmost unavoidable and often result in ahigh expense related successivemaintenance and repair work. Therefore, a self-healing concrete, that could heal itsdamage (specifically cracks) automaticallyby itself, has been gaining moreand more interest. Note that various methodologies for autonomous concreteself-healing exist, and microbial calciumcarbonate precipitation is only one ofthem [9].



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The biggest challenges arethe survival of bacteria in the harsh concreteenvironment (stay alive, but notactive), compatible carriers (both withbacteria and concrete matrix) for immobilization, in situ bacterial activity to producesufficient precipitation and lifespanof the bio-agents. Utilizing silica gel as the filling materialand as protective carrier for bacteria from the high pH environment in concrete has proved to be beneficial in previous study.Bacterial cells were well mixed with silicasol and injected into the cracks manually. Crack closure was shown byultrasonic pulse velocity and water permeabilitytests; however, the greater partof the effect was due to the filling of thecrack by silica gel. precipitationof Nevertheless, crystals inside the gelmatrix may enhance the durability of thisrepair material [10]. porosimetry confirmed Mercury modification in pore size distribution due to the addition of microorganisms; a cell concentration of 10⁵cell/ml generated the greatest reduction in porosity and thus an increased compressive strength. the modification of attributed pore properties to the new formed silicate phase, which was induced by a silica related enzyme excreted frombacteria. But at higher cell concentration, the matrix integrity may be disrupted due to excessive bacterial activity and thus result in a decrease incompressive strengthof mortar. A control experiment was done by adding the isolated protein (from the bacterium) into mortar specimens (around 1 μg protein/ 1 g cement). With such a trace amount, an obvious increase in compressive strength was observed. The protein was characterized and found to dissociate silica from silica rich substances

and form new silica phases, resulting in enhanced coherence between particles and cement matrix at the microscale, and hence an increased strength [11]. The rate of urea hydrolysis is dependent more on bacterial concentration than the initial urea concentration. If the bacteria have a lowaffinity nickel transporter system, which is e.g. the case for E. coli in comparison with Bacillus pasteruii, a Ni2+ supplement will help to increase the calcite precipitation rate [12].

The increase in strength of concrete by method of microbiologically induced Calcium carbonate precipitation (MICCP) has proved to be eco-friendly as there is no involvement of various products used for increasing strength of concrete. The process can trivialize the use of super plasticizers, high grade of cement in conventional concrete, GBBS, fly ash and many more techniques of improving strength of concrete and this is where bacterial concrete can help in not only preventing bars from corrosion but also in increase and durability of concrete structures. The method was first used for repairing of cracks to prevent leaching of channel. [13]. The method can also be used as remediation of granite, mortar and limestone. There are researches which end up concluding that bacillus subtilis worked best with cell concentration of 10⁵ cells/ml and increased the compressive strength by 15%, such bacteria also does not affect the human health, hence environment friendly and safe [14]. The use of bacteria in concrete can enhance durability, mechanical and permeation aspect of concrete [15]. The life of bacterial



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concrete is more than that of conventional concrete [16].

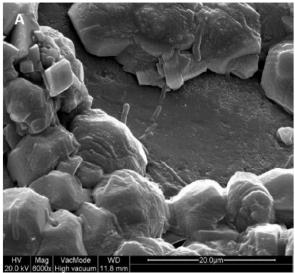


Figure 1: SEM micrograph of bacterial CaCO3 precipitation on concrete samples (Courtesy of Lien Standaert)

According to H.M Jonker (2011) there are two methods of impregnating concrete with bacteria first methods includes direct application of bacteria in concrete and other by replacement of aggregates by light weighted bacterial encapsulated aggregates[17]. Several strategies have been tried for the bacteria (or nutrients): silica gel or polyurethane in glass [9], expanded clay capsules [8]etc protecting the bacterial spores and acting as water reservoir for spores germination and bacterial activity. In the present study direct application of bacteria method was used for which the bacteria was isolated, inoculated, incubated and then induced in the concrete, the proportions of mixing water were replaced by various bacterial media i.e. Escherichia Coli, bacillus subtilis and pseudomonas aeruginosa with different replacement proportions i.e. 10%, 20% and 30% at the time of mixing along with the nutrient (Calcium Lactate) and the defending agent i.e. silica gel. Nominal

mix of M20 grade was prepared with 1: 1.5: 3 ratio, small amount of calcium lactate and silica gel was mixed replacing the equal proportion of fine aggregate. Cell concentration of 10⁶ cells/ml was kept as this concentration resulted in maximum compressive strength as per literatures which were followed by normal concreting process i.e. batching, mixing, compaction and curing.

2. Materials and Methodology

2.1 Bacterial strain.

250 ml of bacterial media was prepared in three flasks of 1000 mleach after autoclave, inoculation and incubation from *E.Coli, P.Aeruginosa and B. Subtilis* media and kept as stock (Fig 1).



Figure 2.1: E. Coli, P. Aeruginosa and B. Subtilisgrowth at 37°C

2.2 Isolation by serial dilution and maintaining cell concentration.

The *E.Coli, B. subtilis* and *P. aeruginosa* cultures were taken from Luria broth media, differention agar (mannitol fermentation), and cetrimide selective media respectively from the flasks and mixed thoroughly to make a composite sample for microbiological analysis..Stock was prepared by dissolving 1 ml of *E.Coli, B. Subtilis* and *P. aeruginosa* in 9 ml of distilled water in one test tube and 9 ml of



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water in next 5 test tubes. With the help of micro-pipette, 1 ml of solution was taken from the first test tube labeled i.e. 10^{-1} and added into second test tube labeled i.e. 10^{-2} . So as 1 ml of 10^{-2} sample taken and added to third test tube labeled i.e. 10^{-3} , same was done for a another 4 test tubes and at last 1 ml sample was discarded from 6^{th} test tube labeled i.e. 10^{-6} . From the 6^{th} test tube i.e. 10^{-6} 1 ml of sample was taken with the help of micro-pipette and was poured into the petri-plate containing nutrient agar. Figure 2 shows the proper dilution of bacterial sample.

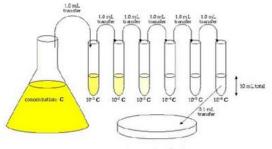


Figure 2.2: Dilution of bacterial sample

The samples were spread over the media with help of spreader in a petri-plate. The sample was allowed to absorb completely on the surface of media for about 10-15 minutes. After that petri-plate was put into incubator and allowed to incubate at 37° for 24 hrs. Fig 3 shows the growth of *E.Coli*, *P.Aeruginosa* and *B.Subtilis* bacteria respectively on petri-plate after incubation.



Figure 2.3: E Coli, P. Aeruginosa and B. Subtilisculture plates.

Determination of colony forming unit (CFU)/ml:

CFU/ml = Number of colonies counted / concentration of sample \times Dilution factor CFU/ml= 250/1 ml \times 10⁻⁶ = 2.5 \times 10⁻⁴/ml

2.3 Production of media for concreting

800 ml of bacterial media was prepared by proper autoclave at 121° for 15 to 20 minutes; the sample was the left to cool down and inoculated with *P.aeruginosa* strain in a laminar air low, the sample was later put in incubation at 37° for 24 hours for bacterial growth in the media which was used for concreting at various proportions. Figure 4 shows 800 ml of *P.aeruginosa*, *B. Subtilis and E.coli*culture media prepared for concreting.



Figure 2.4: 800 ml P. Aeruginosa, B. Subtilis and E. coliculture media for concreting

Table 1, shows the nutrients and their quantities used for growth media with composition of nutrient broth

Table 1: Nutrients and their quantities

Nutrient	Quantity	
Beefextract	1.50 gm	
Yeast extract	1.50 gm	
Peptone	5.0 gm	
NaCl	5.0 gm	
Agar	1.5 gm	
Distilled water	1000 ml	



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2.4 Batching:

Accurate batching of the materials was done using an electronic weight balance to cast 9 cubes in a mould of 150mm×150mm×150mm using bacteria and 3 cubes using conventional practice. Table 2 shows the materials used for casting.

Table 2.2: Materials used for bacterial and conventional concrete

Composition per cube							
Bacterial Replacement Proportion in percent	Cement in Kg's	Fine aggregate in Kg's	Calcium lactate in gm's	Silica Gel ingm's	Natural Coarse Aggregate in kg's	Water in liters	Bacterial culture media in liters
10	1.523	2.512	15	15	4.958	.688	.076
20	1.523	2.498	22	22	4.958	.612	.152
30	1.523	2.482	30	30	4.958	.535	.229

2.5Mixing: Proper mixing was done and the portion of fine aggregate was replaced by calcium lactate and silica gel. The bacterial media was impregnated at this time replacing mixing water by 10%, 20% and 30%. For conventional concrete cement, sand, coarse aggregate and water was used Figure 2.5 and 2.6 shows materials before mixing and addition of bacteria in concrete.



Figure 2.5: Material before mixing Figure 2.6: Addition of bacteria in concrete

2.6 **Casting:** Casting is a manufacturing process in which a fresh concrete is usually poured into a mold, which contains a hollow cavity of the desired shape, and then allowed to solidify. The solidified part is also known as a casting, which is ejected or broken out of the mold to complete the process. The fresh concrete with different proportions of different bacterial media was poured in molds of size 150mm×150mm×150mm and then was allowed to solidify for 24hr and later on demolded for the process of curing.

2.7Compaction: In this study a table vibrator was used. A table vibrator consisting of a rigidly built steel platform mounted on flexible springs and is driven by an electric motor was used for compaction. The normal frequency of vibration of a vibrating table is 4000 rpm at an acceleration of 4g to 7g. The moulds were rigidly clamped on the platform to enable the system to vibrate in unison.

2.8 Curing: In order to make a concrete economical and to provide appropriate water for hydration reaction jute bag curing was done for 28 days to clinch maximum strength. Fig. 2.7 shows the jute bag curing.



Figure 2.7: Jute bag curing of concrete cubes



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3. Tests and Results:

3.1 Physical observation of concrete cubes

White colored lime appeared on the cubes after 1 hr of casting which gradually increased till 28th day which conformed the generation of calcium carbonate by the bacteria. The quantity of calcium carbonate seemed to be equal for all the specimens, the specimens of Pseudomonas aeruginosawith 10% replacement proportion with mixing water seemed to produce more calcium carbonate as compared to other two replacement proportions. **Figure** shows 3.1 generation of lime on specimens.

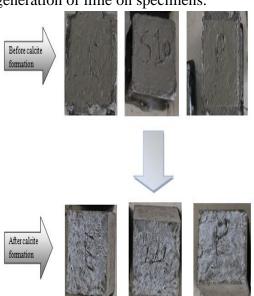


Figure 3.1: Physical observation of concrete cubes

3.2 Compression Testing

An affirmative growth was observed in the compressive strength of concrete after 7, 14 and 28 days after replacing the defined proportions of water by various bacterial cultures and by replacing the defined small quantity of fine aggregate by calcium lactate and silica gel.

As per IS Specifications Compressive strength is calculated by formula given below:

Compressive strength= Load/ Area of specimen

Table 4.3 shows the compressive strength for replacement of water by *E.Coli* bacterial culture and conventional concrete after 7, 14 and 28 days.

Table 4.3: Compressive strength for E.Colibacterial Concrete, Conventional concrete vs. age

Curing period	Compressive strength in N/mm ²				
		Conventional concrete			
	10% E.coli media	20% E.coli media	30% E.coli media		
7 days	20.5	22.6	19.7	15.2	
14 days	26.2	28.4	24.6	21.3	
28 days	27.2	29.7	26.3	24.1	

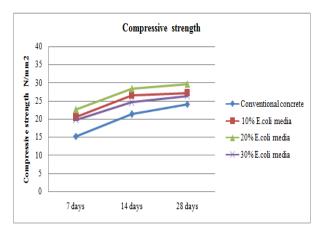


Figure 3.2: E.Coli bacterial concrete, conventional concrete vs. age

Table 4.4 shows the compressive strength for replacement of water by *B. subtilis* bacterial culture and conventional concrete after 7, 14 and 28 days.

 $Table\ 4.4:\ Compressive\ Strength for\ {\it B. subtilis bacterial}\ Concrete, Conventional\ concrete\ vs.\ age$

Curing	Compressive strength in N/mm ²					
period		Conventional concrete				
	10% <u>B. subtilis</u> media	20% B. Subtilismedia	30% <u>B subtilis</u> media			
7 days	25.3	21.6	20.3	15.2		
14 days	37.8	25.2	23.3	21.3		
28 days	39.2	26.3	25.3	24.1		



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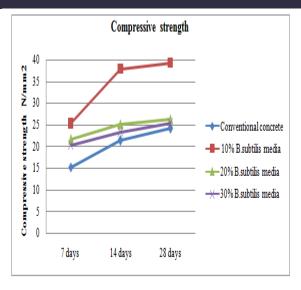


Figure 3.3: B. Subtilis bacterial concrete vs. conventional concrete

Table 4.5 shows the compressive strength for replacement of water by *P.aeruginosa*bacterial culture and conventional concrete after 7, 14 and 28 days.

Table 4.5: C.S. of P. aeruginosabacterial Concrete, Conventional concrete vs. age

Curing period	Compressive strength in N/mm ²				
		Conventional concrete			
	10% P.	20% P. aeruginosa	30% P.		
	aeruginosamedia	media	aeruginosamedia		
7 days	20.5	24.6	20.8	15.2	
14 days	30.2	40.3	31.2	21.3	
28 days	33.3	43.3	30.2	24.1	

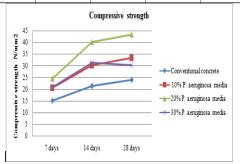


Figure 3.4: P.aeruginosabacterial concrete, conventional concrete vs. age

3.3 Interpretation of Results

1. After 28 days, concrete with *Escherichia Coli* culture reported compressive strength of 29.7 N/mm²on 20% replacement of water by bacterial media, the strength attained was more than that of conventional concrete i.e. 24.1

N/mm². However the strength on 10% and 30% replacement of mixing water by the same culture was 27.8 N/mm² and 28.5 N/mm² respectively and was less than the 20% replacement proportion but was higher than conventional concrete.

- 2. On 10% replacement of water by bacterial media in case of Concrete with bacillus subtilis culture reported maximum compressive strength among the three replacement proportions of 39. N/mm²after 28 daysand was almost double than the conventional concrete i.e. 24.1 N/mm². However the strength on 20% and 30% replacement of mixing water by the same culture was 26.2 N/mm² and 25.3 N/mm²respectively and was less than 10% replacement proportion but was higher than the conventional concrete.
- Concrete with Pseudomonas *aeruginosa*culture showed maximum compressive strength of 43.3 N/mm² after 28 days on 20% replacement of water by bacterial media, which was than the conventional concrete i.e. 24.1 N/mm². However the strength on 10% and 30% replacement of mixing water by the same culture was 33.3 N/mm² and 30.2 N/mm²respectively and was less than 20% replacement proportion but was higher than the conventional concrete.

4 Discussion

- 1. *E.Coli* bacterial culture media in concrete acts as a strength enhancer, on addition of 10% and 20% of the media has shown an increase in strength by 23.2% while the same in 30% has shown a minute decrease in the compressive strength of concrete at cell concentration of 10⁻⁶ cells/ml.
- 2. P. aeruginosabacterial culture in concrete proved to be the most feasible



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culture in concrete as 79.6% increase in the strength was observed. The strength was maximum on addition of 20% of bacterial media and decrease in strength was observed for 30% replacement at cell concentration of 10⁻⁶cells/ml.

3. 61.5% increase in compressive strength was observed from the results on addition of *B. Subtilis* bacteria media in concrete. However the increase in compressive strength of concrete using *B.Subtilis* culture is considerably more than *E.Coli* bacterial media hence its use can be preferred over *E.Coli* culture media.

4.1 Advantages and Disadvantages

4.1.1 Advantages of calcite precipitating bacteria in concrete

- 1. Significant increase in compressive strength when compared to normal concrete.
- 2. The method is eco friendly as there is no involvement of admixtures, which during their manufacturing produce lots of harmful by products.
- 3. The method is cost effective due to the replicating property of bacteria since huge quantity of the bacterial media can be produced by inoculating large quantity of distilled water by trifling quantity of bacterial culture.
- 4. Carbon dioxide is an essential element in the process of corrosion of steel and when the bacterial activity has consumed it all it increases the durability of steel reinforced concrete constructions.
- 5. The filling up of micro pores in concrete helps in reduction of permeability.

4.1.2 Disadvantages

- 1. Growth of bacteria is not good for environment
- 2. There is no IS design code available for bacterial concrete.

3. Investigations and study on calcite precipitation can be costly.

5Conclusion

The method of microbiologically induced calcite precipitation (MICP) proved to be an eco friendly technique to increase the strength and durability of concrete, the only snag in the technique is that extra care needs to be taken as bacteria before impregnation can be pathogenic but after impregnation in concrete proved to be safe innocuous. There is a reorientation in the whole production bacterial process of concrete than which include conventional concrete additional tread of selection and cultivation of bacteria and its impregnation during mixing, the process somehow can be frenetic by the addition of these two treads but in future these can be turned down into facile by certain measures with the new and advanced techniques. These bacteria are easy to cultivate hence can be produced abundantly when needed. There are advances being made which include the direct application of the bacterial media after cracking in form of spray or grout which can also produce the calcium layer on the concrete helping the concrete in increased level of penetration.Such bacteria are harmless to environment and do not affect the human health.It can provide ways for low cost and durable roads, High strength buildings with more bearing capacity, long lasting river banks and erosion prevention of loose sands. From enhancement in durability cementious materials to improvement in sand properties, from repair of limestone monuments, microbial concrete has been successful in one and all. This new technology can be used in sectors such as



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tunnel-lining, structural basement walls, highways, bridges, concrete floors, marine structures etc. Use of MICP in concrete can trivialize the use of super plasticizers, high grade of cement in conventional concrete, GBBS, fly ash and many more techniques of improving strength of concrete. The prioritizing sequence of using calcite precipitating bacteria in concrete as per the study can be, *Pseudomonas Aeruginosa*, *Bacillus Subtilis* and *Escherichia Coli*.

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