STUDY AND DISCUSSION ABOUT ON BACTERIA CONCRETE

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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.. Self-healing concretes are being widely recognized as a remedial technique to improve the durability of concrete. Main reason to prevent cracks or limit crack width is to enhance the durability of the structure. It mostly the general self-healing concrete classification into three categories i.e., natural, chemical and biological processes. 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 remained viable for a period up to 4 months. A continuous decrease in pore size diameter during cement stone setting probably limited life span of spores as pore widths decreased below 1µm, the typical size of Bacillus spores. However, as bacterial cement stone specimens appeared to produce substantially more crack-plugging minerals than control specimens, the potential application of bacterial spores as self-healing agent appears promising.

Keywords: Bacteria, Mortar cube, Concrete, Self healing, CaCO3, Micro cracking.

1. Introduction

Concrete is the most widely used construction material. Despite its versatility in construction, it is known to have several limitations. It is weak in tension, has limited ductility and little resistance to cracking. The development of special concrete considering the speed of construction, the strength of concrete, the durability of concrete and the environmental friendliness with industrial material like fly ash, blast furnace slag, silica fume, metakeolin etc. The way to achieve this could be "Bacterial Concrete".

1.2 Use of bacteria

Bacteria used in the concrete are *Bacillus subtilis* (1A334) use in the present study**.** Researchers with different bacteria proposed different bacterial concretes. In the present study was made by using the bacteria *Bacillus Subtilis*. The main advantage of embedding bacteria in the concrete is that it can constantly precipitate calcite. This phenomenon is called microbiologically induced calcite precipitate (MICP). Calcium carbonate precipitate, a wide spread phenomenon among bacteria, has been investigated due to its wide range of scientific and technological implications. Individual cells of strain were gram positive oval to rods, 0.6-0.8μm in width and 2.0 to 3.0 µm in length, motile and multiplied by binary fission. Various biochemical tests done on the organisms are given .Biochemical characteristics of the pure culture *Bacillus subtilis.*

2 SCOPE AND OBJECTIVES

The Bacterial Concrete can be made by embedding bacteria in the concrete that are able to constantly precipitate calcite. This phenomenon is called Microbiologically Induced calcite precipitation. As per the present investigation it has been shown that under favorable conditions for instance *Bacillus subtilis*, a common soil bacterium, can continuously precipitate a new highly impermeable calcite layer over the surface of an already existing concrete layer.

3 METHODOLOGY

In the present experimental investigation, studies have been carried out on the behavior of fresh and hardened properties of ordinary grade concrete with and without addition of Bacteria. The hardened properties like compressive strength of cement mortar, compressive strength and split tensile strength of concrete, stress-strain behavior of concrete, flexural behavior of concrete and durability aspects of concrete are determined by conducting suitable laboratory tests on concrete in hardened state.

3.1 MATERIALS USED AND THEIR PROPERTIES:

- **CEMENT**

Ordinary Portland cement of 43 grades, available in local market is used in the investigation. The cement used for all tests is from the same batch. The cement used has been tested for various properties as per IS : 4031-1988 and found to be conforming to various specifications of IS:8112-2000

- **ENNORE SAND**

Ennore sand as per BIS specifications is used to find the compressive strength of cement mortar cubes and concrete cubes.

- **COARSE AGGREGATE**

Crushed angular granite from local quarry is used as coarse aggregate. The cleaned coarse aggregate is chosen and tested for various properties such as specific gravity, fineness modulus, bulk modulus etc. The physical characteristics are tested in accordance with IS : 2386 – 1963.

- **FINE AGGREGATE**

The locally available river sand is used as fine aggregate in the present investigation. The cleaned fine aggregate is chosen and tested for various properties such as specific gravity, fineness modulus, bulk density, etc. in accordance with IS : 2386-1963.

- **WATER**

Water used for mixing and curing is fresh potable water, conforming to IS : 3025 – 1964 part-22, part-23 and IS : 456 – 2000.

- **BACTERIA**

Bacillus subtilis , a laboratory cultured bacterium is used.

3.2 MATERIAL AND PROPERTIES

Sl. No.	Properties	Test Results	Requirements as per IS :12269-1987
1	Normal consistency	32	--
2	Specific gravity	3.00	--
3	Initial setting time	55min	Not less than 30m
	Final setting time	240min	Not more than 600 min.
4	Soundness by Le Chatelier	1.5mm	Not more than 10 mm
5	Fineness of Cement	2%	Less than 10%

Table 1: Materials and properties of ingrediants

3.3 PROPERTIES OF AGGREGATE

S.o.	Property		Fine Aggregate	Coarse Aggregate
1	Specific gravity		2.64	2.70
2	Bulk density	Loose density	1439 kg/m^3	1439 kg/m^3
		Rodded density	1622kg/m^3	1610 kg/m^3

Table 2: Properties of fine and coarse aggregate

3.4 GROWTH OF BACTERIA - *Bacillus subtilis*

The pure culture was isolated from the soil sample and is maintained constantly on nutrient agar slants. It forms irregular nutrient broth of 100ml, in a conical flask 250ml and the growth conditions are maintained at 37°C temperature and placed in 125 rpm orbital shaker**.** Micro-organism *Bacillus subtilis (1A334)* were obtained from **Department of IMTECH, "MTCC" Chandigarh, India**. Purebacteria cultured was isolated from

Microbiology department (Faculty of Agri) PG research lab, Annamalai University. Isolate was maintained constantly on nutrient agar slants.

S.no	NA Broth test	Quantity
1	Dextrose	5g/Lt
2	Yeast extract	3g/Lt
3	Peptone	5g/Lt
4	Sodium chlorides	5g/Lt
5	Distilled water	1000ml
6	Agar	15gm
7	Ph	6.8 – 7.0

Table 3: Preparation for NA broth test

3.8.2 Chloride diffusion:

Diffusion is the process by which matter is transported from one part of a system to another due to concentration gradient.

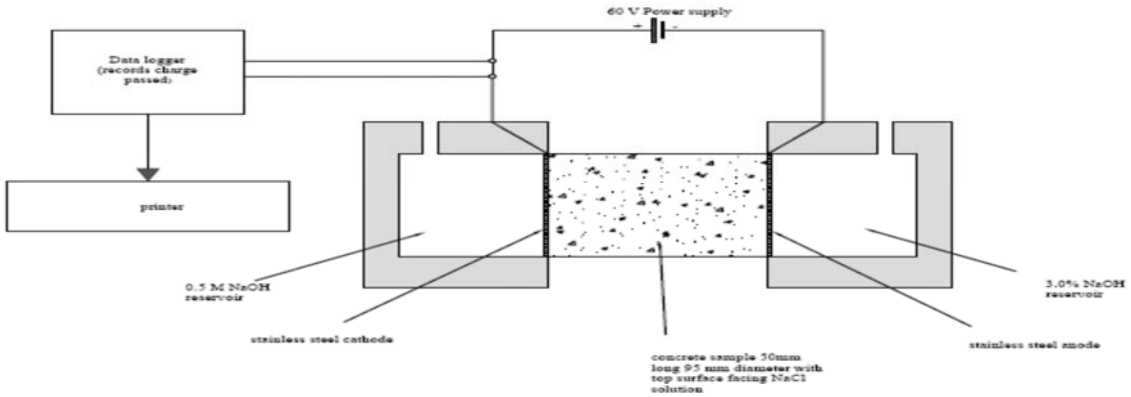

Fig 1: Line diagram of RCPT

The resistance to chloride penetration is one of the simplest measurements to determine the durability of concrete. Separate specimens of similar size were cut from the same cylinder for conducting RCPT and sorptivity tests and the test duration was also same (6h), so that the coefficient of sorptivity and the chloride transport can be compared for each mix. The RCPT was conducted on saturated and surface dry specimens as per ASTM C1202 – 10

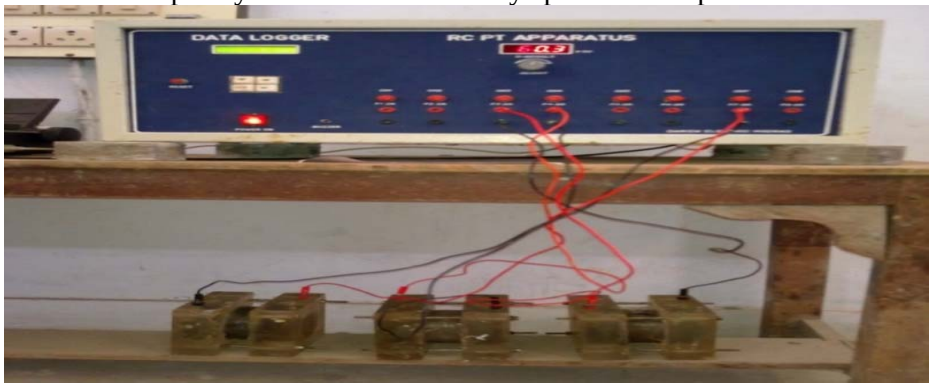

Fig 2: RCPT test setup for specimen

CALCULATION:

$Q = 900 [I_O + 2I_0 + 2I_{60} + \ldots\ldots\ldots\ldots + 2I_{330} + 2I_{360}$

Where,

Q = Charge passed (columbs)

I = Current (Amperes) immediately after voltage is applied

I_t = Current (amperes) at t min voltage is applied

$Q = Q \times (95/X^2)$

Where,

Q_S = Charge passed (columbs) through a 100mm diameter specimen.

X = Diameter of the non-standard apecimen

4. METHODS

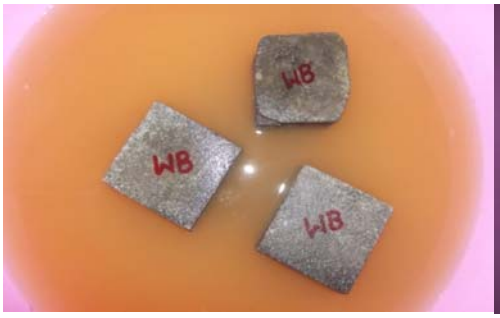

Fig 3: Immersion of samples

4.2 BACTERIA TREATMENT:

The mortar specimens after de-molding were immersed in triplicates in respective bacterial solution grown overnight separately for 24hrs. After 24hrs the mortar cubes were wiped with a blotting paper to remove any surface bacteria and cured in corresponding calcite precipitation media (Calcium source 49gm/Lt + Urea 20g/Lt) at room temperature (Figure: 1) until compression testing at the intervals of 7, 14 and 28 days. Media were replaced at a regular interval of 7 days. 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 1mol of urea is hydrolyzed intracellular to 1mol Calcium carbonate and 1mol of ammonia and carbonic acid. These products subsequently from 1mol of bicarbonates and 2mol of ammonia of hydroxide ions. The last 2reactions give rise tom a Ph increase, which in turns shifts the bicarbonates equilibrium equilibrium, resulting in the formation of carbonate ions.

4.1 IMMERSION METHOD

The mortar specimens were immersed in the respective bacteria solution for 24hrs. which later was immersed in the different precipitation media at room temperature till they were tested for compressive strength. After 3days the cubes with isolate immersed in calcite precipitate media started showed white precipitate on the surface, whereas *Bacillus subtilis* white precipitate after 6days. The amount of precipitate in calcium chlorides source was comparatively higher than calcium nitrate. The white precipitate was due to calcite formation by bacteria. The precipitation media was replaced every 7days in order to maintain the pH of the media.

Fig 4: Urea + Calcium

4.3 ISOLATING AND IDENTIFICATION OF CALCITE PRECIPITATE BACTERIA-GRAM STAINING AND END SPORE STRAINING

Gram staining and endospore straining was conducted to determine the **gram** reaction and morphology of strains of bacteria used for investigation. The strains were found to be gram positive and endospore forming.

4.3.1 UREA TEST:

The strains of bacteria were tested for urease activity. The change of the color of the media from yellow to pink indicated that it is urease positive. The strain were urease positive.

Fig 5: Calcite precipitation

4.3.2 EFFECT OF P^H ON THE GROWTH OF BACTERIA

Growth and survival of microorganisms are greatly influenced by the pH of an environment especialy of the environment especially high alkaline environment of the cement mortar. The bacteria were tested for optimum Ph its growth. It was observed that isolate had optimum growth in pH range 7.5-9.0 however the growth in pH upto 12. Whereas, bacillus subtillis had optimum growth in pH range 7-9.

4.3.3 GENERATION TIME

Generation time is the time required for the microbial population to double under standard condition. The generation time of isolate bacillus subtillis was 20min, 90min, 120min respectively.

4.3.4 UREASE ASSAY

The ability to precipitate calcium carbonate (calcite) is directly related to the amount of the enzyme urease produced by the bacteria. Bacteria urease activity after 24hrs of incubation at room temperature of 28ºC for isolate, *Bacillus subtillis* was 14.2, 9.0 and 10µg/ml/minute respectively

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ASTM C 642-97 STANDARD TEST METHOD FOR DENSITY, ABSORPTION AND VOIDS IN HARDENED CONCRETE											
Specimen number	Oven dry mass in (gms)	Saturated mass after immersion in (gms)	Saturated mass after boiling in (gms)	Immersed apparent mass (gms)	Absorption after immersion for 48hours in %	Absorption after immersion and boiling	Bulk density dry (g1) Mg/m^3	Bulk density after immersion	Bulk density after immersion and boiling	Apparent density (g2) Mg/m^3	Volume of permeable pore space (voids)%
	a	*B*	*c*	*d*	*((b-a)/a)×100*	*((c-a)/a)×100*	*(a/(c-d))×ρ*	*(b/(c-d))×ρ*	*(c/(c-d))×ρ*	*((a/(a-d))×ρ*	*(c-a)/(c-d)×100*
B1	2510	2545	2548	1524	1.39	1.51	2.45	2.49	2.49	2.55	3.71
B2	2427	2453	2459	1469	1.07	1.32	2.45	2.48	2.48	2.53	3.23
B3	2503	2524	2529	1529	0.84	1.04	2.50	2.52	2.53	2.57	2.60
B4	2476	2494	2497	1523	0.73	0.85	2.54	2.56	2.56	2.60	2.16
B5	2479	2497	2501	1503	0.73	0.89	2.48	2.50	2.51	2.54	2.20

Table 4: Conventional mix results of 28days

ASTM C 642-97 STANDARD TEST METHOD FOR DENSITY, ABSORPTION AND VOIDS IN HARDENED CONCRETE											
Specimen number	Oven dry mass in (gms)	Saturated mass after immersion in (gms)	Saturated mass after boiling in (gms)	Immersed apparent mass (gms)	Absorption after immersion for 48hours in %	Absorption after immersion and boiling	Bulk density dry (g1) Mg/m^3	Bulk density after immersion	Bulk density after immersion and boiling	Apparent density (g2) Mg/m^3	Volume of permeable pore space (voids)%
	a	*b*	*c*	*d*	*((b-a)/a)×100*	*((c-a)/a)×100*	*(a/(c-d))×ρ*	*(b/(c-d))×ρ*	*(c/(c-d))×ρ*	*((a/(a-d))×ρ*	*(c-a)/(c-d)×100*
C1	2442	2502	2505	1497	2.46	2.58	2.42	2.48	2.49	2.58	6.25
C2	2474	2519	2521	1527	1.82	1.90	2.49	2.53	2.54	2.61	4.73
C3	2472	2511	2515	1513	1.58	1.74	2.47	2.51	2.51	2.58	4.29
C4	2462	2500	2502	1512	1.54	1.62	2.49	2.53	2.53	2.59	4.04
C5	2486	2519	2525	1527	1.33	1.57	2.49	2.52	2.53	2.59	3.91

Table 5: Bacteria mix results of 28days

NO. OF MIX	SAMPLE	COMPRESSIVE STRENGTH N/mm²			Average N/mm²
Mix:1	C1	19.5	18	20	19.15
	B1	20	20.75	21	20.58
Mix:2	C2	21	21.5	22	21.5
	B2	22.25	23.5	23.75	23.15
Mix:3	C3	23.5	23.5	23.5	23.5
	B3	25	25.25	25.15	25.45
Mix:4	C4	25.5	26	25	25.5
	B4	26.3	27.25	27.25	26.93
Mix:5	C5	27.5	27	26.5	27
	B5	27	28	29	28

Table 6: Compressive strength 28days after immersion in acid

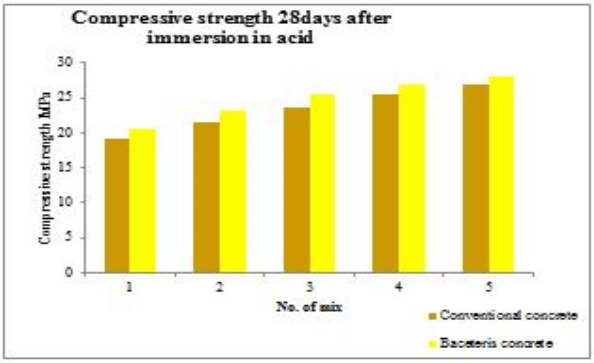

Fig 6 : Compressive strength 28days after immersion in acid

5. CONCLUSIONS

The microbiologically induced *Bacillus subtilis* bacteria improves the compressive strength of cement mortar by 16.15%. At a particular microbial cell concentration i.e. 10^6/ml compressive strength of cement mortar is maximum. Increase in compressive strength of cement mortar is lower at higher microbial cell concentration i.e. 10^7/ml. The addition of *Bacillus subtilis* bacteria improves the hydrated structure of cement mortar. The compressive strength of cement mortar is maximum with the addition of *Bacillus subtilis* bacteria for a cell concentration of 10^5 cells per ml of mixing water.

In ordinary grade concrete, the compressive strength is increased up to 13.93% at 28 days on addition of *Bacillus subtilis* bacteria when compared to conventional concrete. In ordinary grade concrete, the split tensile strength has increased up to 12.60% at 28 days on addition of Bacillus

subtilis bacteria when compared to conventional concrete.

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