Self-healing Concrete to remediate cracks using Bacteria under Optical Microscope

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Abstract: Concrete structures are vulnerable to cracks due to seepage of water and structural instabilities etc. These cracks can be closed by external sealants. But now incorporating bacteria in concrete helps in healing the cracks effectively. The Paper featuring the innovative technique in sealing the cracks autonomously along with that strength and durability parameters were analyzed. The Production of calcium carbonate was observed under optical microscope in order to show the bonding capability with concrete matrix. Compare to conventional concrete, self-healing concrete enhances the strength up to the addition of 0.5% bacteria and nutrient to the cement weight. But quick healing capabilities were observed when addition of bacteria and nutrient increases. Resilient features of bacteria were observed when it subjected up to the heat of 75^{o}c.

Keywords- Bacillus subtilis, Calcium Lactate, Compressive strength, oven heating, Water absorption, Optical Microscope.

I. INTRODUCTION

Self-healing concrete used to remediate cracks autonomously in which bacteria and nutrient supplied along with it plays a vital role in forming calcium carbonate around the open crack. Utilizing this technique we can mitigate concrete problems viz., cracks and porous nature of concrete. In a metabolic process, some bacteria produces enzyme urease which catalyzes the hydrolysis of urea to generate carbonate ions without an associated production of protons which leads to $CaCO_3$ Precipitation in presence of calcium ions [1]. Bacteria cells not only provide a nucleation site for $CaCO_3$ precipitation due to negative charged cell walls, but also create alkaline environment inducing further growth calcite crystals. Formation of $CaCO_3$ depends on the pH value of bacteria because some cannot sustain at high alkaline environment and pH value more than 11 [2]. *Bacillus subtilis* is added in spore form into the concrete. Bacterium gets activated when it's get contacted with water and starts forming calcium carbonate crystals with the help of calcium lactate powder which already exists in cement matrix. The newly produced material performs as a form of bio cement and fills up freshly originated cracks. Spore form of bacteria can survive 45 to 50 years in dry state.

WORKING MECHANISM OF CALCIUM CARBONATE PRECIPITATION

Microorganisms such as *Bacillus subtilis* are able to produce biominerals through metabolic reaction in the presence of calcium source [3]. These urease positive microorganisms are involved in production calcium carbonate through urea hydrolysis [4-6]. Fundamental reaction to form calcium carbonate precipitation are shown in Eqs. 1 and 2 [7].

$$Ca^{2+} + CO_3^{2-} \Leftrightarrow CaCO_3 \quad (1)$$

$$Ca^{2+} + 2HCO_3^{-} \Leftrightarrow CaCO_3 + CO_2 + H_20 \quad (2)$$

Microbial metabolic activities lead to an increase of carbonate concentration and pH [8]. Increase in pH facilitates transformation of carbon dioxide to carbonate. These metabolic conversions promote calcium carbonate precipitation which plays the role of barrier and blocks ingress of corrosive chemicals into cracks [7, 9]. Through urease activity in the presence of bacteria, one mole carbonic acid (NH_2COOH) and one mole ammonia (NH_3) are produced from urea hydrolysis. As can be seen from Eq. 4, Carbamic acid hydrolysis produces one mole carbonic acid (H_2CO_3) and one mole of extra ammonium simultaneously.

$$CO\ (NH_2)_2 + H_20 \rightarrow NH_2COOH + NH \quad (3)$$

$$NH_2COOH + H_20 \rightarrow NH_3 + H_2CO_3 \quad (4)$$

According to Eqs. 3 and 4, reaction of hydroxide ion (which is already produced from reaction of water and ammonia) and carbonate acid produces carbonate (CO_3^{2-}) [10]. As can be seen in Eq. 7, positively charged calcium ions can be bind to negatively charged bacterial cell.

$$2NH_3 + 2H_2O \rightarrow 2NH_4^{+} + 2OH^{-} \quad (5)$$

$$2OH^{-}\ H_2CO_3 \rightarrow CO_3^{2-} + 2H_2O \quad (6)$$

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \quad (7)$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3 \quad (8)$$

To complete the last reaction (Eq. 8), calcium ion can be proved either by internal sources that are available in cement structure or by adding chemicals such as calcium chloride, calcium nitrate or calcium lactate externally [11]. Utilization of calcium as a calcium source may cause chloride ion attack consequently degradation of reinforcement bars. Thus, application of calcium lactate is recommended. Precipitation of calcium carbonate crystals by B. subtilis are shown in Fig: 4.

II. EXPERIMENTAL PROGRAM

Study on Bacteria

The bacteria strain morphology was determined by gram strain method, after staining the bacterial smear slide with crystal violet for 1-2 min, it was flooded iodine for 1-2 min to remove color, slide was slowly washed with acetone for 2-3s. After depolarization, the slide was rinsed with water and then flooded with safranin counter strain for 2min. The bacterial samples is observed using microscope.

Survival of bacteria on Ph. 11 is examined using nutrient agar supplemented with 0.5% peptone, 0.3% beef extract, 1.5% agar, 0.5% nacl is prepared by adding 28gms of nutrient agar in 1 liter of water. Nutrient agar is autoclaved at 121°c for 15min (15 psi) and poured into series petri plates and inoculated with bacillus subtilis. After 48hrs of incubation the growth of bacteria is observed.

Materials

Ordinary Portland Cement (OPC) of 53 grade confirming to IS: 12269-1987 and physical properties is shown in Table: 1. Coarse aggregate with nominal size of 12.5mm and fine aggregate confirming ZONE- II. Bacteria having cell concentration of ($1.06*10^7$) determined using haemocytometer and calcium lactate supplied as nutrient is used in experimental work.

Description of test	Specific Gravity	Fineness of cement	Standard Consistency	Intial setting time	Final setting time
Test Result	3.12	7.8%	33%	30min	275min

Table: 1 Physical Properties of cement

Mix Composition

Concrete having cube strength of 32Mpa for 28 days of curing is adopted. Bacterial culture ($1.06* 10^7$ cfu/ml) and calcium lactate is added in equal proportion (0.25%, 0.5%, 1%, 3%, 5%) to the weight to cement additionally. Six mix proportions were made. First one was conventional mix (with bacteria and nutrient) and from next mix subsequently bacteria was gradually added to the cement weight.

Concrete cubes size of 100mm*100mm*100mm were casted for compressive strength, water absorption and samples were subjected to heating. The samples were casted with mix proportion of 1: 1.67: 2.37: 0.43. The casted samples were placed in molds for more than a day. After demolding the samples were placed for curing. The specimens were tested after 7 days and 28 days of curing period.

Concrete testing

The compressive strength was determines as per IS 516 (1959). Water absorption were measured as per IS 1124 (1974). Survival of bacteria subjected to heating is correlated with autoclave process. Optical microscope Fig: 1 (NIKON ECLIPSE LV 100NPOL) test is performed on healed bacterial sample by mounting thin specimen using thin cutting machine.

Fig: 1 Optical Microscope

III. RESULTS AND DISCUSSIONS

Compressive Strength

From the different mixes shows in Table: 2 0.5% bacterial set shows increase in cube strength compared to conventional set for both 7dyas and 28dyas of curing period. It was observed that increase in calcium lactate content leads to decrease in compressive strength and retard the setting time. Bacillus bacteria and lactate doesn’t involve in the process of hydration directly. Calcium carbonate helps in filling the pores and healing of the concrete cracks shown in Fig: 2.

Concrete Type	Compressive strength for 7 days (MPa)		Compressive strength for 28 days (MPa)	
	Average	% Variation	Average	% Variation
CS	20.6	0	31.8	0
0.25%BS	21.3	3.39	32.5	2.2
0.50%BS	**24.9**	**20.89**	**35.1**	**10.37**
1%BS	22.4	8.73	33	3.77
3%BS	18.3	-11.16	28.2	-11.32
5%BS	15.1	-26.69	24.1	-24.21

Table: 2 Compressive strength for 7 days and 28 days

Fig: 2 Bacterial set before and after healing

Water absorption

Water absorption of concrete is correlated to compressive strength and it is illustrated in Fig: 3 respectively. The test was performed after 28dyas of curing. The rate of water absorption decreases gradually when the percentage of bacteria increases. It is due to the fact that bacteria in retard state absorbs less water and in certain cases strength decreases due to non-hydrated particles present in the mortar.

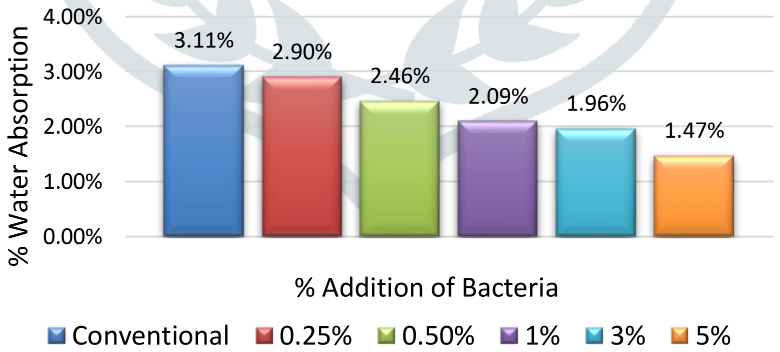

Fig: 3 Rate of water absorption

Survival of bacteria- Hear Exposure

The survival of bacteria is studied on 0.5% Bacterial mix as it shows the maximum variation in compressive strength, the process is correlated with autoclave and cubes were kept at different temperatures (50°c, 75°c and 110°c) for 6 hrs. After the heat exposure the cubes were subjected to partial compressive loading in order to form cracks on cubes. At 50°c and 75°c 0.5% bacterial mix was survived and healing of crack starts from 7th day. At 110°c bacterial mix hasn't survived as the exterior part of specimen was exposed to high temperature and bacteria couldn't able to survive and healing of the specimen wasn't seen as the bacteria became diminished due to high heat exposure.

Optical Microscope analysis

Fig: 4 and Fig: 5 shows deposition of calcium carbonate in between the pore and bonding between concrete matrix and carbonate was captured using NIKON ECLIPSE LV 100NPOL. For this a thin section of healed sample was prepared using Geoform cutting machine and polished using Forcipol. Thickness of thin specimen preferred in between 20-40 microns.

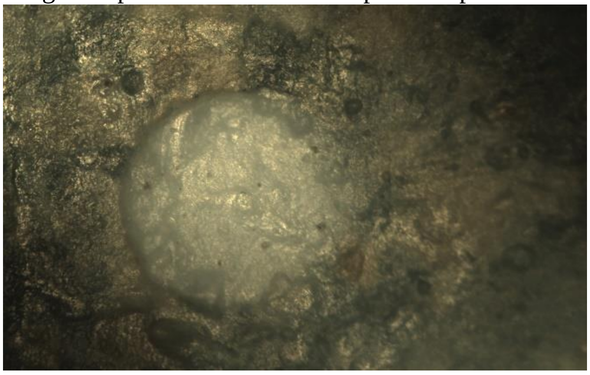

Fig: 4 Deposition bacteria in the thin specimen pore

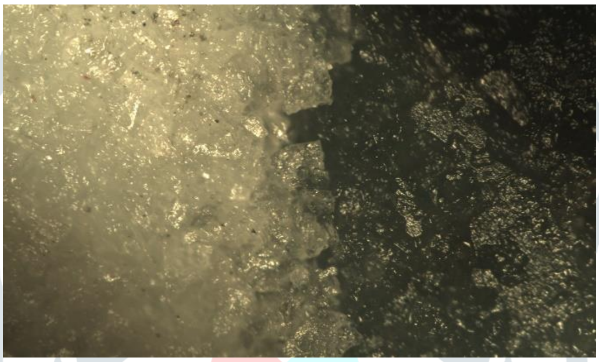

Fig: 5 Bacteria bonding with cement matrix

IV. Conclusions

- It is observed that bacillus subtilis can survive in alkaline concrete like environment and can produce calcium carbonate. Addition of bacteria alone cannot improve the properties of concrete. Bacteria requires urea and a source of calcium to produce calcium carbonate.
- Compressive strength (at 7days and 28 days) of cement cube is found to be increasing with increase of bacteria cell concentration up to 1% of cement weight. However, it is found that further increase of bacteria concentration the compressive strength of cement mortar.
- Bacillus Subtilis is found to be retard the setting time of the concrete.
- The decrease in percentage of water absorption was seen when the dosage of bacteria increases in concrete.
- The bacterial when it is exposed to heating has survived up to 75^{o}c.

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