Study of Self Healing Mechanism and its Impact on Bacillus Bacteria Impregnated Concrete

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Abstract - *Concrete is not only a structural component but also used as a shielding material where cracks can not be permitted. The cracks in concrete affect the durability. Although, the remedial measures is not giving the hundred percent results. Self-healing is characterized by regaining performance after a defect occurs. Damage targeted in bacteria-based self-healing concrete particularly relates to increased durability and leakage prevention and extending service life of concrete structures. So study was carried out by various researchers in preventing the cracks formation in concrete. So as per necessity of crack prevention the idea of self healing of cracks in concrete develops. To avoid these micro-cracks now a days bacterias can be effectively used which is called as bacteria impregnated concrete which is recent advancement in concrete technology. In this technique bacteria from bacillus family is impregnated in concrete which are having calcium as their food from concrete and when these bacteria gets in contact with atmosphere they use water and carbon dioxide from surrounding environment and precipitates calcium carbonate (lime stone) which ultimately seals the cracks and makes concrete crack proof. This is called as microbiologically induced calcite precipitation (MICP). In this paper an attempt to study the feasibility of impregnation of bacillus subtilis for crack healing and its impact on concrete is carried out.*

Key Words: Bacillus Subtilis, Compressive strength, Bacterial count, Cell structure, MICP Ecology.

1. INTRODUCTION

Crack formation is also a typical phenomenon related to durability. Percolated cracks may lead to leakage problems or ingress of harmful materials, which can cause deterioration of the concrete matrix or reinforcement corrosion. Durability can be enhanced by preventing further ingress of water and other substances. Self-healing is characterized by regaining performance after a defect occurs. Damage targeted in bacteria-based self-healing concrete particularly relates to increased durability and leakage prevention and extending service life of concrete structures. Jonkers (2007) introduced a two-component healing agent to be added to the concrete mixture, consisting of bacteria and a mineral precursor compound. Upon cracking the system is activated by ingress water. Bacteria convert the mineral precursor compound into the mineral calcium carbonate, better known as limestone. Precipitation of the limestone on the crack surface enables sealing and plugging of the cracks, making the matrix less accessible to water and other deleterious materials. In the case of nuclear power plants concrete is used not only as a structural material but also for shielding purposed where crack in the body of the concrete can not be permitted. Fibre reinforced concrete uses fine fibres distributed throughout the mix or larger metal or other reinforcement elements to limit the size and extent of cracks. Water tanks and highways are examples of structures requiring crack control. So, to overcome with these drawbacks the idea of self healing is invented which heals the cracks in concrete when bacillus bacteria is impregnated without any external treatment. In this study bacteria called bacillus subtilis was impregnated in concrete and study was focused on its self healing mechanism and its impact on concrete properties such as compressive strength, surface velocity.

2. BACILLUS SUBTILIS

Bacillus subtilis was originally named as *Vibrio subtilis* and renamed in 1872, this organism has other names as *Bacillus uniflagellatus*, *Bacillus globigii*, and *Bacillus natto*. *Bacillus subtilis* bacteria were one of the first bacteria to be studied. *Bacillus subtilis* is also known as *hay bacillus* or *grass bacillus.*

Bacillus subtilis cells are rod-shaped, Gram-positive bacteria that are naturally found in soil and vegetation. *Bacillus subtilis* grow in the mesophilic temperature range. The optimal temperature is 25-35 degrees Celsius (Entrez Genome Project). Stress and starvation are common in this environment, therefore, *Bacillus subtilis* has evolved a set of strategies that allow survival under these harsh conditions. One strategy, for example, is the formation of stress-resistant endospores.

Bacillus subtilis has historically been classified as an obligate aerobe, though evidence exists that it is a facultative aerobe. *Bacillus subtilis* is considered the best studied Grampositive bacterium and a model organism to study bacterial chromosome replication and cell differentiation.

2.2 Cell Structure and Metabolism

Bacillus subtilis are rod-shaped bacteria that are Gram-positive (Perez 2000). The cell wall is a rigid structure outside the cell. It is composed of peptidoglycan, which is a polymer of sugars and amino acids. The peptidoglycan that is found in bacteria is known as murein. Other constituents that extend from the murein are teichoic acids, lipoteichoic acids, and proteins. The cell wall forms the barrier between the environment and the bacterial cell. It is also responsible for maintaining the shape of the cell and withstanding the cell's high internal turgor pressure (Schaechter 2006).

The formation of the endospore occurs in several stages, denoted 0 through VI. Sporulation occurs in the following fashion. First the nucleoid lengthens, becoming an axial filament. Then, the cell forms a polar septum, one-fourth of the cell length from one end, and begins to divide. The smaller product of this division is called the forespore and the larger product is called the mother cell (Perez 2000). The mother cell is responsible for nourishing the newly formed spore. When the septum forms, 30% of the chromosome is already on the forespore side (Schaechter 2006). The remaining 70% of the chromosome enters the forespore in a fashion similar to DNA transfer during conjugation; it is pumped by a protein called SpoIIIE. The mother cell then engulfs the forespore by acting like a phagocyte. This causes the forespore to have two cytoplasmic membranes with a thick murein layer, namely the cortex, between them. A protein spore coat and an exosporium, a membranous layer, form outside of the forespore membranes. At this time, the forespore undergoes internal changes. Lastly, the forespore leaves the mother cell upon lysis of the mother cell (Perez 2000). A mature endospore has no metabolic activity; it is inert. The interior of the endospore, the core, is very dry and resistant to moisture (Schaechter 2006).

3. . Materials

3.1 Water

Water is an important ingredient of concrete as it actively participates in the chemical reaction with cement. Since it helps to form the strength giving cement gel, the quantity and quality of water is required to be looked into very carefully. Water should be free from acids, oils, alkalis, vegetables or other organic impurities. Soft water also produces weaker concrete.

Water has two functions in a concrete mix. First, it reacts chemically with cement to form a cement paste in which the inert aggregates are held in suspension until the cement paste has hardened. Next, it serves as a vehicle or lubricant in the mixture of fine aggregate and cement. Potable water is generally considered satisfactory. In the present investigation, potable tap water was used for both mixing and curing purposes.

3.2 Cement

Cement is a fine, grey powder. It is mixed with water and materials such as sand, gravel, and crushed stone to make concrete. The cement and water form a paste that binds the other materials together as the concrete hardens. The ordinary cement contains two basic ingredients namely argillaceous and calcareous. In the present work 53 grade ACC cement was used for casting cubes and beams for all concrete mixes. The cement was of uniform colour i.e. grey with a light greenish shade and was free from any hard lumps and fulfilling the requirements as per IS 12269 -1987

3.3 Fine Aggregate

The sand used for the experimental works was locally procured and conformed to grading zone II. Sieve Analysis of the Fine Aggregate was carried out in the laboratory as per IS383-1970

3.4 Coarse Aggregate

Crushed basalt stones obtained from local quarries were used as coarse aggregate. The maximum size of coarse aggregate used was 20 mm. The properties of coarse aggregate were determined by conducting tests as per IS: 2386 (Part – III).

3.5 Bacillus Subtilis Bacteria

Bacillus subtilis is brought up in its log phase in concreting site in liquid or aqueous state. This stage is having bacterial concentration 56×10^6 cells/ml. This full grown stage lasts for 2 to 3 hours at room temperature. These bacteria should be impregnated in concrete in its full grown stage.

4. Methods

4.1 Calculation for mix proportion of M30 grade

4.1.1 Target mean strength of concrete:

$$f'_{ck} = f_{ck} + t \times S$$

Where,

f'_{ck} = target average compressive strength at 28 days

f_{ck} = characteristic compressive strength at 28 days

S = Standard deviation

f'ck= 30+1.65x4.0

f'ck = 36.6 MPa

for S refer IS 10262:2009 Cl 3.2.1.2 Table No.1 page no.2

In old IS 10262:1982 t value was taken from tableNo.2 of same IS page no. 6

4.1.2 Selection of water cement ratio:

Referring IS 10262:2009 Cl. 4.1 page no.2

The maximum water cement shall be taken from table No.5 of IS 456:2009 page no.20

From Table 5 of IS 456, max. water cement ratio = 0.45

Adopt w/c ratio 0.42

0.42<0.45, hence O.K.

4.1.3 Selection of water content:

Referring IS 10262:2009 Cl. 4.2 table No.2 page no.3

For 20mm nominal max. size of aggregate water content is 186 lit. for 25 to 50 mm slum range.

Estimated water content for 25-50mm slump is 186 litre.

4.1.4 Calculation of cementitious content:

Water cement ratio = 0.42

Water content = 186 litre

w/c = 0.42

186/0.42 = 442.86 kg

Therefore provided cement = 442.86 kg/ m^3

Which is greater than the minimum cement content of 300 kg/m^3 for moderate exposure condition as per table No.4 of IS 456-2009.

4.1.5 Proportion of volume of coarse & fine aggregate content:

From IS 10262:2009 Cl no. 4.4.1 & table no. 3, page no.3

Volume of C.A corresponding to 20mm size aggregate & sand conforming to zone II for w/c ratio of 0.5 is 0.62

In present case w/c ratio is 0.42. Therefore volume of C.A is required to be increased for degrease in F.A content.

Correction to be made at the rate of ± 0.01 for every ± 0.05 change in w/c ratio.

Therefore the corrected proportion of volume of C.A for the w/c ratio 0.42 is +0.016

Volume of C.A = 0.636

Volume of F.A = 1-0.636= 0.364

4.1.6 Mix calculation:

a) Volume of concrete = 1 m^3

b) Volume of cement = (Mass of cement/ Specific gravity of cement) x (1/1000)

= (442.86/3.15)x(1/1000)

= 0.1406 m^3

c) Volume of water = (Mass of water/ Specific gravity of water) x(1/1000)

= (186/1)x(1/1000)

= 0.186 m^3

d) Volume of all aggregate = [a- (b + c)]

= [1-(0.1406+0.186)]

= 0.6734 m^3

e) Mass of C.A = [d] x vol of C.A. x specific gravity of C.A x 1000

= 0.6734x0.636x2.867x1000

= 1226.13 kg

f) Mass of F.A = [d] x vol of F.A. x specific gravity of F.A x 1000

= 0.6734x0.364x2.571x1000

= 630.19 kg

4.1.7 Mix proportions

Cement = 442.86 kg/ m^3

Water = 186 kg/ m^3

F.A = 630.19 kg/ m^3

C.A = 1226.13 kg/ m3

4.2 Mix Design Stipulation

Table 1 Concrete Mix Design Stipulation

Grade designation	30
Type of cement	OPC 53 Grade
Maximum nominal size of aggregate	20
water-cement ratio	0.42
Workability	Medium
Exposure condition	Sever
Method of concrete placing	Manual
Degree of supervision	Good
Type of aggregate	Crushed angular aggregate
Proportion of Cement : Sand : Aggregate	1 : 1.42 : 2.77

4.3 Bacteria Impregnation process and its bacteria/ml. calculation

Bacillus subtilis was grown to log phase at density of 56×10^6 bacterias/ml in nutrient broth under optimum conditions. The bacterial culture was diluted by mixing in water and was used for concrete preparation. By using following formula bacterial count can be calculated. Eg. If one litre of liquid state bacteria is poured in nine litre of water then bacterial count will be as follows.

$$\text{Bacterial count} = \frac{\text{Full grown no.(no./ml) x solution used(ml)}}{\text{Total solution used (ml)}}$$

$$\text{Bacterial count} = \frac{56 \times 10^6 \text{ bacterias/ml. x 1000 ml.}}{10000 \text{ ml.}}$$

$$\text{Bacterial count} = 5.6 \times 10^6 \text{ bacterias}$$

As bacterial count changes results varies. This full grown stage lasts for 2 – 3 hours at room temperature. These bacteria should be impregnated in concrete in its full grown stage.

5. Experimental Procedure

5.1 determination of compressive strength of concrete

The main objective of the test is control of quality and to check that the concrete at site has developed required strength. It gives us idea about correction to be made in further mixes. Cubes of standard sizes 150x150x150mm as per I.S. 516 are cast. Three cubes each of 7 days & 28 days test are cast. The surface of the moulds are covered with oil in order to avoid the development of bond between the mould and concrete and also on the contact surface at the bottom of mould and the base plate so that water does not escape during filling. Cube specimen is filled soon after mixing. Cube is filled in three layers and each layer is well compacted. Compaction is done by hand or by vibration. After compaction, the top surface of the concrete is properly finished with the help of trowel. The cube is stored undisturbed for 24 hours at 50% humidity and then striped and immersed in water for curing. These cubes are then tested at the age of 28 days.

After curing, these cubes were tested on Compression Testing machine at 28 days. The failure load was recorded. In each category three cubes were tested and their average value is reported. The compressive strength was calculated as follows.

Compressive strength (MPa) = Failure load / cross sectional area.

Figure 1: Experimemental setup of compression testing machine

5.2 Ultrasound pulse velocity test for crack depth and surface velocity measurement

For this test ultrasonic testing machine is used. This instrument is manufactured by TICO and called as PROCEQ testing instrument made in Switzerland.

5.2.1 Procedure for finding out crack depth:

1. Connect transducers to the unit and apply coupling paste.
2. Measure distances b and 2b on the test object and identify.
3. Input distance b on the unit with keys ↑↓←→.
4. Press the "Start" key: the distance is displayed under b in the measurement image, at the same time the sound pulses are emitted.
5. Press transducers on the object and carry out measurement b to b: as soon as the measurement value is stable for 3 seconds, a beep is heard and the transmission time is displayed as t1.
6. By pressing the "Store" key, the value is stored and the unit is switched to t2 for the measurement.
7. Press transducers to distance 2b. After a stable display for 3 seconds, a beep is heard after which the "Store" key is pressed. The crack depth is now displayed at C.
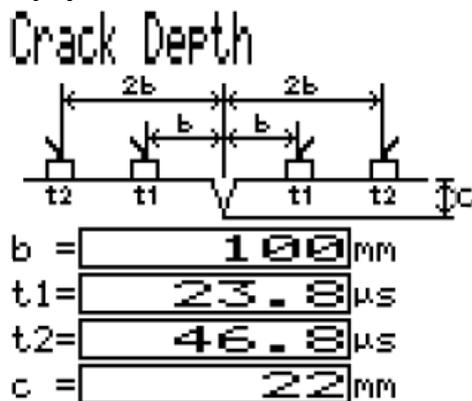
Figure 2 Crack depth measurement display

5.2.2 Procedure for finding out surface velocity by direct transmission:

1. In menu key select distance and then press 150 by using the keys ←→↑↓ and then press "End" key.
2. Apply coupling paste to contact surfaces of the transducers and to the points on the object to be measured (thin coat for fine concrete surface, thicker coat for rough surface.
3. Press "Start" key.

4. Position transducers exactly on the measurement points and press down.
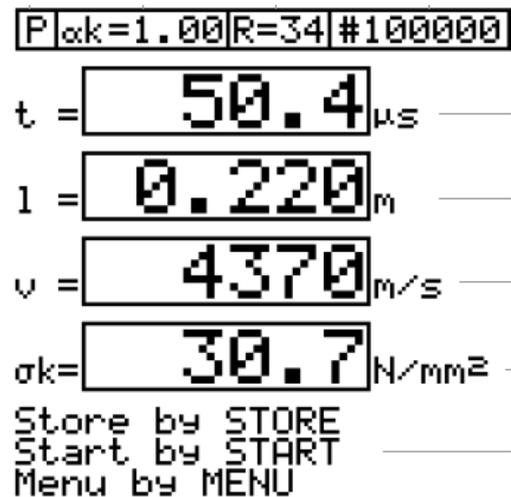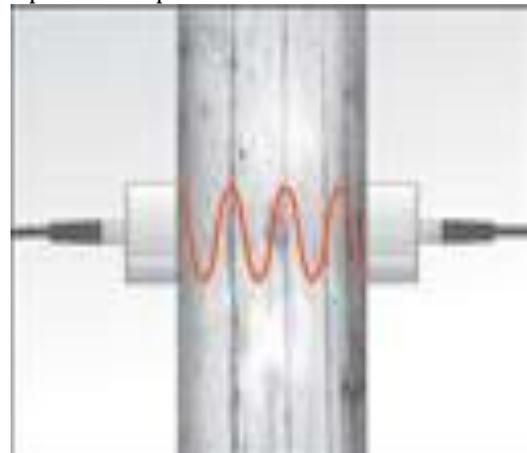
Figure 3 Direct transmission concept and surface velocity display

5.3 Effect of temperature variation on concrete:

To find the effect of temperature variation on concrete two tests are carried out. In first test 3 concrete cubes of 150 x 150 x 150mm size are heated for 7 days at 65^0c in oven. In second test concrete cubes of 150 x 150 x 150mm size are cooled down for 7 days to 4^0c in refrigerator. All these specimens are then subjected to compressive testing machine for checking its compressive strength as per the procedure described in 5.1

6 Results and Discussions

6.1 Comparison of compressive strength of regular M30 concrete with bacterial M30 concrete

Table 2 – Compressive Strength (N/mm^2) Results After 28 Days Curing

Sr. No.	Cross-Sectional Area (mm²)	Load (KN)	Compressive Strength (N/mm²)	Average Compressive Strength (N/mm²)
For Normal M30 grade Concrete				
1.	22500	750	33.33	32.59
2.	22500	730	32.44	
3.	22500	720	32.00	
For bacteria count 2 x 10⁶				
7.	22500	850	37.77	38.06
8.	22500	850	37.77	
9.	22500	870	38.66	
For bacteria count 4 x 10⁶				
10.	22500	850	37.77	39.09
11.	22500	880	39.11	
12.	22500	875	39.33	
For bacteria count 6 x 10⁶				
13.	22500	910	40.44	40.14
14.	22500	880	39.11	
15.	22500	920	40.88	
For bacteria count 56 x 10⁶				
16.	22500	1130	50.22	49.77
17.	22500	1130	50.22	
18.	22500	1100	48.88	
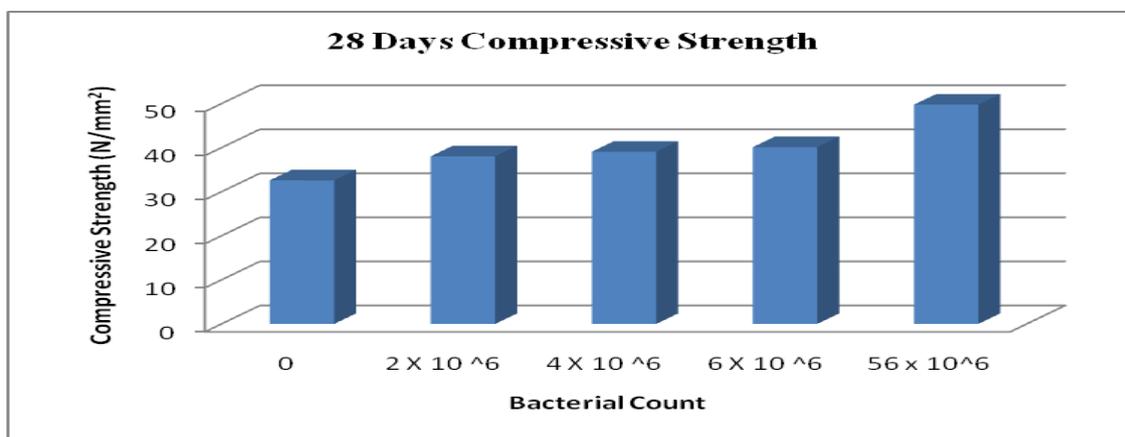
Graph 1 – Varying results of compressive strength as per variation in bacterial count for 28 days curing

From above results it is observed that compressive strength of bacteria impregnated concrete is increased upto 52.71% as per the bacteria number increases to its final count.

Bacterial count	Percentage increase in Compressive strength in 28 days curing
2×10^6	26.66%
4×10^6	28.45%
6×10^6	33.80%
56×10^6	52.71%

6.2 Comparison between surface velocity test by ultrasonic pulse velocity machine before cracking the cube and after healing the crack in cube

Table 3 Surface Velocity Test for M30 Grade of concrete made with bacteria impregnated concrete

Before cracking the cube	t	36.2 μs	After healing of crack in cube	t	36.2 μs
	l	0.150 m		l	0.150 m
	v	4170 m/s		v	4140 m/s

Hence from above results it is observed that surface velocity before cracking the cube and after healing of that crack is nearly same.

6.3 Crack depth measurement by ultrasonic pulse velocity machine after cracking the cube and after healing the crack in cube

Table 4 Crack depth measurement for M30 Grade of concrete made with bacteria impregnated concrete

After cracking the cube	b	25 mm	After healing of crack in cube	b	25 mm
	t_1	21.8 μs		t_1	23.8 μs
	t_2	35.5 μs		t_2	34.4 μs
	d	27 mm		d	5 mm

From above results it is observed that initial crack depth was 27mm and after 75 days of cracking was 5mm. Therefore within 75 days 22mm of crack is filled.

6.4 Effect of change in temperature in bacteria impregnated concrete

Table5 Compressive strength Test at 28th Day for M30 Grade of concrete made up with bacteria impregnated concrete at various temperatures

Sr. No	Cross sectional area (mm²)	Load (KN)	Compressive strength (N/mm²)	Average Compressive strength (N/mm²)
Heating at 65^0c for 7 days for bacterial count 6×10^6				
1	22500	910	40.44	
2	22500	890	39.55	40.14
3	22500	910	40.44	
Cooling at 4^0c for 7 days for bacterial count 6×10^6				
4	22500	880	39.11	
5	22500	890	39.55	39.84
6	22500	920	40.88	

There is no change in compressive strength of bacteria impregnated concrete after 7 days of heating at 65^0c and freezing at 4^0c. Therefore it is proven that bacteria can survive in extreme weather conditions.

7. Conclusion

1. In this study it is found that bacillus subtilis was more suitable bacteria for self healing concrete compared to other bacterias from bacillus family.
2. The compressive strength of concrete having bacteria count 56×10^6 was increased by 52.71% however for compressive strength was increased from 24% to 33% for diluted bacteria count ranging from 1.5×10^6 to 6×10^6.
3. The compressive strength of cement mortar having bacteria count 6×10^6 was increased by 21%
4. As there was no change in compressive strength when concrete is exposed to extreme weather condition, bacteria is alive in any extreme weather conditions.
5. Surface velocity remains unchanged of bacteria impregnated concrete before the crack was induced and after its healing.

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