

# Bacillus Subtilis Bacteria Impregnation in Concrete for Enhancement in Compressive Strength

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**Abstract** - To avoid micro-cracks in concrete, bacteria 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 are 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 its Rita produces the precipitate of calcium carbonate (lime stone) which ultimately seals the cracks and enhances compressive strength of concrete. As pores from concrete gets reduced and filled with calcium carbonate.

**Key Words:** Bacillus Subtillus, Compressive strength, Bacterial count, Cell structure, Ecology

## 1. INTRODUCTION

Concrete is a versatile material having lower tensile & shear strength. Basically concrete is brittle material and contains thousands of micro-cracks present in the body. The elasticity of concrete is relatively constant at low stress levels but starts decreasing at higher stress levels as matrix starts cracking. Concrete has a very low coefficient of thermal expansion, and as it matures concrete shrinks. All concrete structures will crack to some extent, due to shrinkage and tension. Concrete which is subjected to long-duration forces is prone to creep.

Now natural & artificial fibre are used to improve performance and augment the weakness of concrete for high performance. However all cracks concrete can not be sealed by presently available methods like epoxy and other polymer 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.

## 2. BACILLUS SUBTILIS

### 2.1 Description and Significance

Originally named *Vibrio subtilis* in 1835, this organism was renamed *Bacillus subtilis* in 1872. Other names for this bacterium also include *Bacillus uniflagellatus*, *Bacillus globigii*, and *Bacillus natto*. *Bacillus subtilis* bacteria were one of the first bacteria to be studied. These bacteria are a good model for cellular development and differentiation (Entrez Genome Project).

*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.

### 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).

### 2.3 Ecology

The main habitat of endospore forming Bacillus organisms is the soil. Likewise Bacillus subtilis is most commonly found in soil environments and on plant undergrowth. These mesophilic microbes have historically been considered strict aerobes. Consider how this organism functions in a competitive microbial community: when carbon-, nitrogen- and phosphorus-nutrient levels fall below the bacterium's optimal threshold, it produces spores. Scientists have demonstrated that Bacillus subtilis concurrently produces antibiotics and spores. Antibiotic production increases Bacillus Subtilis's chance at survival as the organism produces spores and a toxin that might kill surrounding gram positive microbes that compete for the same nutrients.

Bacillus subtilis supports plant growth. As a member of Bacillus, this bacterium often plays a role in replenishing soil nutrients by supplying the terrestrial carbon cycle and the nitrogen cycle. Bacillus subtilis bacteria form rough biofilms, which are dense organism communities, at the air and water interface. Bacillus subtilis biofilms are beneficial. They allow for the control of plant pathogen infections. Bacillus subtilis biofilm communities form a mutualistic interaction with plant rhizome systems. The plant benefits because Bacillus subtilis provides preemptive colonization. Preemptive colonization prevents other pathogens from infecting the plant because Bacillus subtilis has the advantage of being at the site first. The biofilm communities form a mutualistic interaction with plant rhizome systems. Bacillus subtilis biofilms found in the rhizosphere of plants promote growth and serve as a biocontroller. In this sense, Bacillus subtilis biofilm communities form a mutualistic interaction with plant rhizome systems. The plant benefits because Bacillus subtilis provides preemptive colonization. Bacillus subtilis benefits by deriving nutrients and surface area for biofilm formation from the plant's root structure. Bacillus subtilis strains can act as biofungicides for benefiting agricultural crops and antibacterial agents. Bacillus subtilis also reduces mild steel corrosion (Morikawa 2006). Bacillus subtilis bacteria are non-pathogenic.

### 3. BACTERIA IMPREGNATION PROCESS

Bacillus subtilis is brought up in its full grown stage in concreting site in liquid / aqueous state. This growth stage is having bacterial concentration  $56 \times 10^6$  bacteria/ml. This aqueous stage bacteria is poured in water which will be used for concreting so now bacterial count gets reduced. By using the following formula bacterial count can be calculated.

Eg. If one litre of liquid state bacteria is poured in nine litres of water then bacterial count will be as follows.

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

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

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

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

### 4. TEST FOR COMPRESSIVE STRENGTH OF CONCRETE

Compression test is the important test conducted on hardened concrete because most of the desired characteristics of concrete are qualitatively related to compressive strength. 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 an idea about correction to be made in further mixes. Cubes of standard sizes  $150 \times 150 \times 150$  mm 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 stripped and immersed in water for curing. These cubes are then tested at the age of 7 days & 28 days.

After curing, these cubes were tested on Compression Testing machine at 7 and 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,

$$\text{Compressive strength (MPa)} = \frac{\text{Failure load}}{\text{cross sectional area}}$$

**Table 1 - Compressive Strength (MPa) Results After 7 Days Curing**

Sr. No.	Cross-Sectional Area (mm <sup>2</sup> )	Load (KN)	Compressive Strength (N/mm <sup>2</sup> )	Average Compressive Strength (N/mm <sup>2</sup> )
For Normal M30 grade Concrete				
1.	22500	500	22.22	22.21
2.	22500	490	21.77	
3.	22500	510	22.66	
For bacteria count 1.5 x 10 <sup>6</sup>				
4.	22500	560	24.88	25.03
5.	22500	570	25.33	
6.	22500	560	24.88	
For bacteria count 2 x 10 <sup>6</sup>				
7.	22500	580	25.77	26.21
8.	22500	600	26.66	
9.	22500	590	26.22	
For bacteria count 4 x 10 <sup>6</sup>				
10.	22500	580	25.5	26.35
11.	22500	600	26.00	
12.	22500	620	27.55	
For bacteria count 6 x 10 <sup>6</sup>				
13.	22500	600	26.66	27.55
14.	22500	620	27.55	
15.	22500	640	28.44	

For bacteria count $56 \times 10^6$				
16.	22500	810	36.00	35.40
17.	22500	790	35.11	
18.	22500	790	35.11	

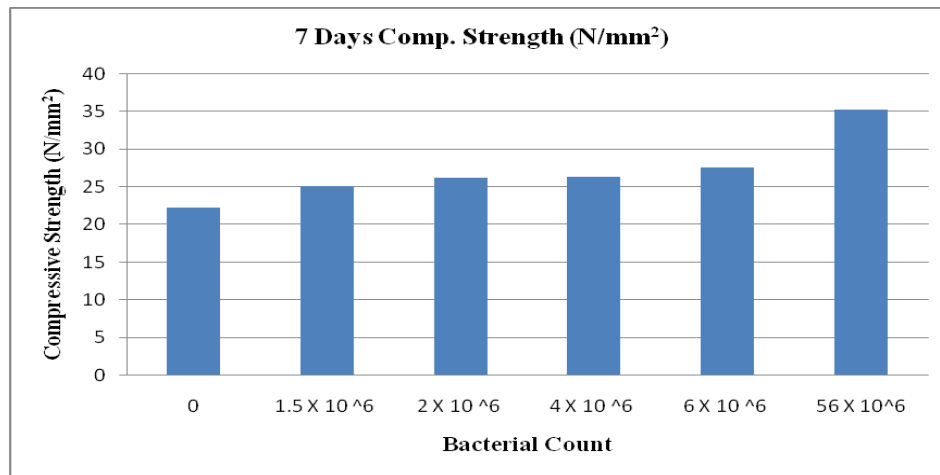


Chart 1 - Varying results of compressive strength as varies bacterial count for 7 days curing

Table 2 - Compressive Strength (MPa) Results After 28 Days Curing

Sr. No.	Cross-Sectional Area (mm <sup>2</sup> )	Load (KN)	Compressive Strength (N/mm <sup>2</sup> )	Average Compressive Strength (N/mm <sup>2</sup> )
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 $1.5 \times 10^6$				
4.	22500	830	37.33	37.18
5.	22500	840	36.88	
6.	22500	830	37.33	
For bacteria count $2 \times 10^6$				

7.	22500	850	37.77	38.06
8.	22500	850	37.77	
9.	22500	870	38.66	
For bacteria count $4 \times 10^6$				
10.	22500	850	37.77	39.09
11.	22500	880	39.11	
12.	22500	875	39.33	
For bacteria count $6 \times 10^6$				
13.	22500	910	40.44	40.14
14.	22500	880	39.11	
15.	22500	920	40.88	
For bacteria count $56 \times 10^6$				
16.	22500	1130	50.22	49.77
17.	22500	1130	50.22	
18.	22500	1100	48.88	

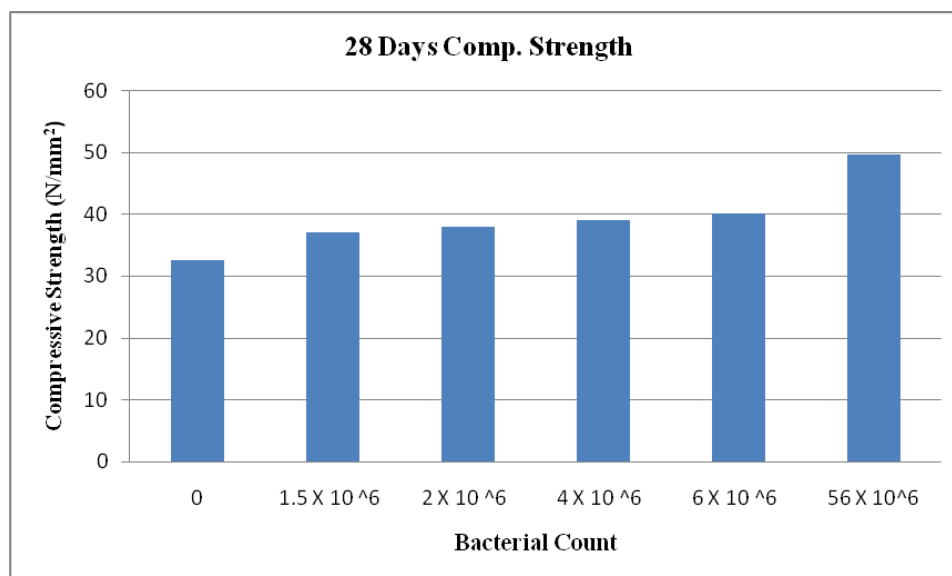


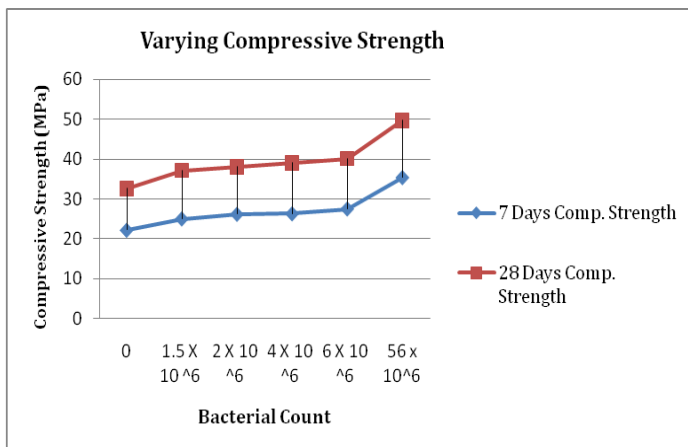
Chart 2 - Varying results of compressive strength as varies bacterial count for 28 days curing

## 5. RESULTS

1. Bacillus subtilis has positive impact on concrete's compressive strength.
2. Change in compressive strength as bacterial count changes is as follows

Change in Bacterial Count	Percentage Change in Compressive Strength
$1.5 \times 10^6$	23.93
$2 \times 10^6$	26.86
$4 \times 10^6$	30.30
$6 \times 10^6$	33.80
$56 \times 10^6$	52.71

3. Comparitive varying diagram of 7 days and 28 days compressive strength is as follows



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