Effect of *Bacillus Subtilis* on Concrete with Steel Fibers and Fly Ash

Vijaya Laxmi Govindugari[1*], Srinivas Reddy P[2]. Madhavi Latha D[3] .Sandeep Reddy A[4].

* [1]Assistant Professor, Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India

[2]Assistant Professor, Department of Civil Engineering, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India

[3]Department of Biotechnology, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India.

[4]P.G student, (M.E, Structural Engg.) Department of Civil Engineering, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India

Abstract *- Over more than a decade, microbially induced carbonate precipitation is in use to increase mechanical properties of concrete. The use of self protected bioconcrete has become a topic of thrust area of research worldwide, especially employing bacterial strains. Concrete has an ultimate load bearing capacity under compression but the material is weak in tension. The steel reinforcement have the capacity to take the tensile load, while the concrete cracks under tension. On the other hand, the concrete protects the steel reinforcement from the environment and prevents corrosion. However, the cracks in the concrete cause a major problem which affects the durability of the structures. Here the ingress of water and chloride ions takes place and deterioration of the structure starts with the corrosion of the steel. The conventional methods to increase the strength and durability of the structure and seal the cracks formed in the structure, epoxy injections or latex treatment are used. Unfortunately, the chemicals used in these treatments are expensive and cannot reach the deeper portions of the cracks in the structure. Hence, the present study was aimed to attempt a self healing concrete with increased durability and tensile strength using steel fibres and fly ash. M-30 grade concrete has been tested in the present study with various concentrations of fly ash and steel fibre (1.5%) along with and without bacteria i.e. Bacillus subtilis, to evaluate various mechanical properties of the concrete mix. Concrete mix with bacterial concentration of $1x10^5$ cells per ml. along with 1.5% steel fibre and 20% fly ash was found to show more split tensile and compressive strength (50.13 MPa.) compared to mix without bacterial cells (45.50 MPa) after 28 days of curing. Mix with $1x10^5$ cells/ml bacteria, 1.5%steel fiber and 0% fly ash has less weight loss and strength loss compared to other mix grades devoid of bacteria*

Keywords : *Concrete, Self healing, Fiber, Fly Ash, Bacillus subtilis, Mechanical properties*.

INTRODUCTION

In the past few decades the microbes were known to have deterioration of construction material in the form of leaching, discoloration, internal pressures mechanical erosion etc.[1]. The studies on beneficial effects of microorganisms on building materials specially microbially induced calcium carbonate, ($CaCO_3$, calcite) precipitation in concrete as crack remediation is challenging since more than a decade [2]. Several bacteria are known to precipitate $CaCO_3$. The physical and mechanical properties of calcite are closely similar to those of hardened concrete and hence it can be used as a crack filling agent for concrete structures. It is produced biologically and does not possess any harmful chemicals. Also, its production is self-sustained, happens without any human support and has the potential to remediate every minute crack. These properties of Calcite make it a perfect filling material of cracks developed in concrete [3]. The bacteria are incorporated into the concrete while mixing. This type of concrete is called Bacterial Concrete. It is also called self-healing concrete as the healing process is independent and autonomous [4]. The bacteria become active as soon as they get in contact with water and precipitate Calcite eventually, facilitating the filling of minute voids generated on the account of the physical structure of the constituents of concrete. This process takes place till there is an availability of water. Once the concrete hardens, the water supply is cut off, the bacteria becomes inactive and remains dormant till there is any further supply of water. Hence, it increases the strength of concrete and also repairs the cracks formed, protecting the structural integrity [5] and durability of the concrete [6, 7]. Bacterial spores are specialized cells which can endure extreme mechanical and chemical stresses and spores of this specific genus are known to remain viable for up to many years. Spores are dormant but viable bacterial spores immobilized in the concrete matrix will become metabolically active when revived by water entering freshly into the concrete. *Bacillus subtilis*, a gram-positive, rod shaped bacteria exists naturally in soil and vegetation, known to produce urease enzyme. Urease catalyzes urea to produce CO_2 and ammonia, resulting in an increase of pH in the surroundings where ions Ca^{2+} and CO_3^{2-} precipitate as $CaCO_3$. Possible biochemical reactions in medium to precipitate $CaCO_3$ at the cell surface that provides a nucleation site can be summarized as follows.

$Ca^{2+} + Cell \rightarrow Cell\text{-}Ca^{2+}$ . . . . (1)

$Cl^{-} + HCO_3^{-} + NH_3 \rightarrow NH_4Cl + CO_3^{2-}$ . . (2)

$Cell\text{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3\downarrow$ . . . (3)

Concrete is one of the most extensively used construction materials in the world, with about two billion tons of utilization worldwide each year. Concrete has an ultimate load bearing capacity under compression but the material is weak in tension. The steel reinforcement takes the load when the concrete cracks under tension. On the other hand the concrete protects the steel reinforcement from the environment and prevents corrosion. Durability is one the most important property to be considered in the design of reinforced concrete structures exposed to aggressive environments. However, the cracks in the concrete cause a major problem which affects the durability of the structures. Here the ingress of water and chloride ions takes place and deterioration of the structure starts with the corrosion of the steel. When reinforced concrete structure is subjected to severe environmental conditions its properties are affected adversely depending on the type of exposure. Chemical admixtures are used in concrete to reduce or fill the cracks. However, environmental concerns, stemming from high-energy expense and CO_2 emission associated with cement manufacture, have brought pressures to reduce consumption through the use of supplementary materials [8]. Later some attention has been given to the use of pozzolana, micro silica as partial replacement to portland cement which helps in increasing durability of structures. In plain concrete and similar brittle material, structural cracks (micro cracks) develop even before loading, particularly due to drying shrinkage or other causes of volume change. The width of these initial cracks seldom exceeds a few microns. When loaded these micro cracks propagate and open up and due to stress concentration, additional micro cracks are formed. Ter Herde and his co workers studied on autogenous healing of micro cracks. The micro cracks are the main cause for elastic deformation in concrete. Fibre reinforced concrete helps to overcome these problems. This gives the cement-based materials maximum ductility overcoming its low tensile strength properties [8,9]. Cracking is an important issue that has to be considered as it leads to irregular stress distribution across the section and also renders the section useless to carry any further load. Hence, they are to be avoided as much as possible or remedied as efficiently as possible. Another type of problem with treatment of cracks is that some cracks are formed in deeper portions of an element of the structure which cannot be accessed easily for treatment. If we can find a reliable method or material that repairs cracks in concrete automatically (self healing) which can address the disadvantages mentioned , then the durability of the structure would be enormously increased. A bacterium is one such component which helps in treatment of cracks automatically i.e. self healing.

Cracks in a concrete structure reduces the life and cracks of dimension smaller than that of cement (micro cracks) cannot be filled with chemicals. Crack treatment processes involve harsh materials such as cement and chemicals adding to the pollution of environment. There have been instances of allergic reactions due to chemicals faced by the inhabitants and handlers. Tensile strength of concrete is very less. The effect of egg shell powder and silica fume in concrete mix was also studied in increasing the compressive strength and split tensile strength [10]. Literature indicates using fibers would increase the tensile strength of the concrete. The environmental impacts of poor disposal of mineral wastes need to be addressed which can be achieved by reusing their mineral properties. The use of fly ash in the manufacture of concrete reduces its interaction with the environment as well as gives strength to the concrete. As there is limited literature available on the study of properties and characteristics of such combination, the present study aims to investigate in this direction.

MATERIALS AND METHODS

Materials: Cement:

Ordinary Portland cement of 53 grade available in local market conforming to specifications of IS: 12269-1987 having specific gravity of 3.03 is used.

Fine Aggregate:

Locally available clean, well-graded, natural river sand conforming to specifications of IS 383-1970 having specific gravity of 2.63 is used.

Coarse Aggregate:

Crushed granite angular aggregate of size 20 mm nominal size from local source with specific gravity of 2.70 is used.

Water

Locally available potable water conforming to IS 456 is used.

Fibers

Steel Fiber of hooked end type with aspect ratio of 80 is used.

Fly Ash

Fly Ash of class 'F' with 1.91 specific gravity, procured from KTPS, Warangal is used.

Microorganisms

Bacillus subtilis of $1x10^5$cells/ml of nutrient broth was used to prepare the concrete mix in the present study.

Culture and maintenance of Bacteria

The pure culture of *Bacillus subtilis* was isolated by serial dilutions of the soil sample from Chaitanya Bharathi Institute of Technology campus (CBIT), Hyderabad, Telangana, India, and cultured in the Biotechnology laboratory. *Bacillus subtilis* culture was streaked on nutrient agar slants with an inoculating loop and the slants were incubated at 37°C. After 24 hrs of growth, slant cultures were preserved under refrigeration (4°C) until further use. Whenever required a single bacterial colony is cultured in 100 ml of nutrient medium for 24 hours. It is subjected to a 10 fold dilution and inoculated into 1 litre of autoclaved nutrient broth. The growth condition is maintained at 37°C temperature at 100 rpm for 24 hours. The medium composition required for growth of culture is Peptone: 5 gm/lit., NaCl: 5 gm/lit., Yeast extract: 3 gm/lit. pH 7.4. The culture density of $1x10^5$cells/ml of bacterial cells was confirmed based on spectrophotometer reading of 600nm,. Contamination from other bacteria was checked periodically by streaking on nutrient agar plates.

Experimental procedure

All test samples of concrete mix without and with *Bacillus subtilis* were at least prepared in three replicates and the average values have been recorded for test results. The concrete bars have been made with above mentioned cement and aggregates along 1.5% steel fibre, varying concentrations of fly ash (0-30%) and with or without bacteria (*Bacillus subtilis*) to study the mechanical properties of the concrete mix.

TABLE I : TYPES OF MIX CONSIDERED IN THE STUDY (M-30 GRADE CONCRETE)

Mix	Steel Fiber (%)	Fly Ash (%)
M0	0	0
M1	1.5	0
M2	1.5	10
M3	1.5	20
M4	1.5	30

Tests are conducted for the above mix grades with and without bacteria ($1x10^5$ cells/ml)

Compressive Strength Test

In the present investigation, compressive strength is determined as per the procedure given in IS 516:1959. Compressive strength is determined on 150 x 150 mm cubes using UTM at a loading rate of 140kg/cm ^{2}/min.

Compressive strength = P /A

P - Maximum load applied on the specimen during the test in Newton

A - Cross-sectional area calculated from the mean dimensions of the section

Split Tensile Strength Test

A diametric compressive load will be applied along the length of the sample at a continuous rate until failure occurs. This loading induces tensile stresses on the plane containing the applied load, causing tensile failure of the sample. IS 5816:1999 formed the basis for the development of this procedure. The measured splitting tensile strength of the specimen shall be calculated using the following formula

F_{ct}=2P/Π dl

P = Maximum load applied on the specimen during the test in Newton

l = length of the specimen in mm

d= cross sectional dimension of the specimen in mm.

Durability (Acid test)

The chemical resistance of the concretes was studied through chemical attack by immersing concrete blocks in an acid solution. After 28 days period of curing, the specimens were removed from the curing tank and their surfaces were cleaned with a soft nylon brush to remove weak reaction products and loose materials from the specimen. The initial weights were measured and the specimens were identified with number. The specimens were immersed in 5% HCL for 30 days. The solution was replaced at regular intervals to maintain constant concentration throughout the test period.

RESULTS AND DISCUSSION

Slump

Slump is one of the parameters of workability. Table II shows the slump values for various concrete mixes with bacteria and without bacteria. Fig 1 shows the graph for different slump values.

TABLE II: SLUMP VALUES (in mm)

Mix	Without bacteria	With bacteria
M0	15	19
M1	11	15
M2	14	19
M3	16	21
M4	19	25

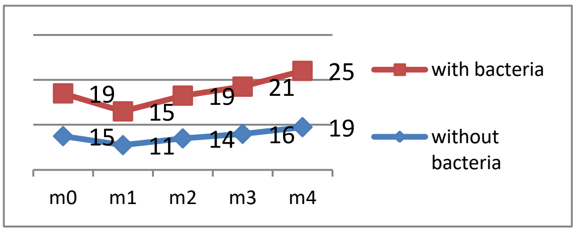

Fig.1: Slump values

Compressive Strength

Effect of bacteria on compressive strength is observed for different concrete mixes. Compressive strength values statistically increased with and without bacterial cells when recorded at 7 days and 28 days curing (TableIII). Fig.4 shows the variation in compressive strength values in the form of graph

TABLE III: COMPRESSIVE STRENGTH AT 7 & 28 DAYS

Mix	Without bacteria		With Bacteria	
	7 days (MPa)	28 days (MPa)	7 days (MPa)	28 days (MPa)
M0	26.90	34.90	35.33	44.60
M1	30.20	39.20	38.66	46.80
M2	31.96	41.70	39.80	47.60
M3	**34.96**	**45.50**	**43.50**	**50.13**
M4	27.20	37.40	36.40	45.14

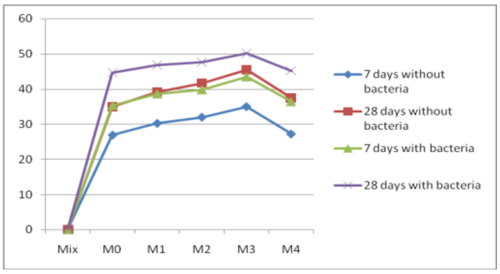

Fig.2: Comparison of compressive strength

Split tensile Strength

Split tensile strength is a parameter of mechanical property. Split tensile strength values observed at 7 days and 28 days curing with and without bacteria are shown in Table IV. Fig.5 shows the variation in compressive strength values in the form of graph.

TABLE IV: SPLIT TENSILE STRENGTH AT 7 & 28 DAYS

Mix	Without bacteria		With Bacteria	
	7 days (MPa)	28 days (MPa)	7 days (MPa)	28 days (MPa)
M0	2.32	2.99	2.41	3.08
M1	2.63	3.01	2.97	3.21
M2	3.00	3.18	3.19	3.38
M3	**3.04**	**3.27**	**3.12**	**3.49**
M4	2.48	2.86	2.78	3.06

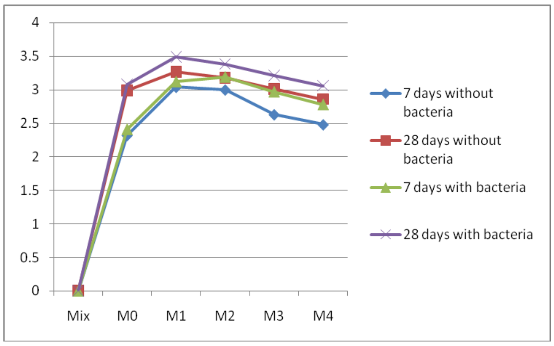

Fig. 3 split tensile strength values for different mixes

Durability: Strength loss

Durability of the concrete is measured in terms of strength loss. Table V and Table VI show loss in strength for different mixes with bacteria and without bacteria after immersion in acid for 30 days after 28 days of curing. Fig.6 shows the comparison between concrete mix with bacteria and without bacteria (Table VII).

TABLE V STRENGTH LOSS (WITHOUT BACTERIA)

Mix	Initial strength (Mpa)	Final strength (Mpa)	Strength loss(%)
M0	38.02	33.18	12.73
M1	39.00	33.70	13.60
M2	42.00	34.90	16.70
M3	45.80	37.40	18.30
M4	37.30	27.75	25.60

TABLE VI STRENGTH LOSS (WITH BACTERIA)

Mix	Initial strength (Mpa)	Final strength (Mpa)	Strength loss (%)
M0	44.27	40.69	8.08
M1	46.30	42.00	9.30
M2	47.80	41.73	12.70
M3	49.80	42.70	14.26
M4	45.20	36.20	19.90

TABLE VII COMPARISON OF STRENGTH LOSS

Mix	Strength loss %(without bacteria)	Strength loss %(with bacteria)
M0	12.73	8.08
M1	13.60	9.30
M2	16.70	12.70
M3	18.30	14.26
M4	25.60	19.90

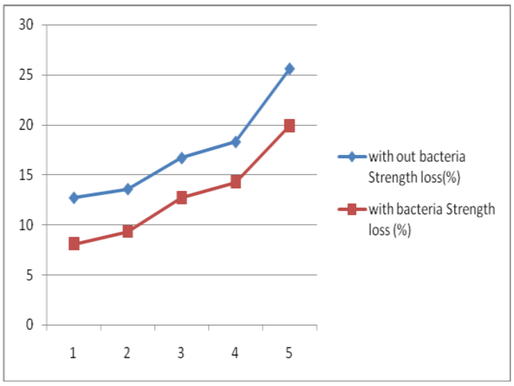

Fig.4 Comparison of Strength loss

Weight loss

The cube weights and weight loss percentage for various concrete mixes with bacteria and without bacteria have been tabulated and the values obtained were recorded (Table VIII, Table IX, Table X & Fig 5). All the mix proportions with bacteria are found to have less weight loss value compared to mixes without bacteria.

TABLE VIII WEIGHT LOSS (WITHOUT BACTERIA)

Mix	Initial weight (kg)	Final weight (kg)	weight loss(%)
M0	2.47	2.40	2.83
M1	2.51	2.43	3.18
M2	2.47	2.34	5.26
M3	2.40	2.27	5.41
M4	2.39	2.23	6.69

TABLE IX WEIGHT LOSS (WITH BACTERIA)

Mix	Initial weight (kg)	Final weight (kg)	weight loss(%)
M0	2.51	2.47	1.59
M1	2.50	2.45	2.00
M2	2.48	2.37	4.43
M3	2.47	2.35	4.85
M4	2.42	2.30	4.96

TABLE X COMPARISON OF WEIGHT LOSS FOR DIFFERENT MIXES WITH AND WITHOUT BACTERIA

Mix	Weight loss % (without bacteria)	Weight loss % (with bacteria)
M0	2.83	1.59
M1	3.18	2.00
M2	5.26	4.43
M3	5.41	4.85
M4	6.69	4.96

In our study, after 28 days of curing period, a portion of crushed test specimen is pasteurized at 70º C for 30 minutes (to inactivate bacteria that might have come in contact with cement during curing process) and placed in the nutrient broth Fig 8. The *Bacillus subtilis* colonies were revived on the agar plates, on streaking the suspension on nutrient agar plates (Fig.9).

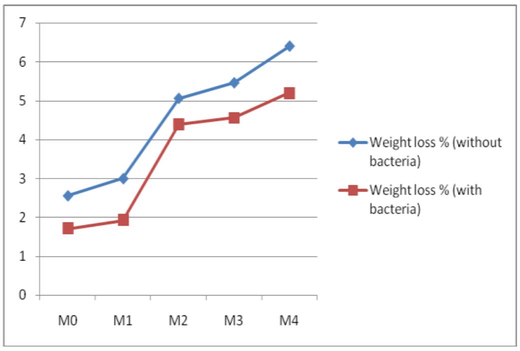

Fig.5 comparison of weight loss

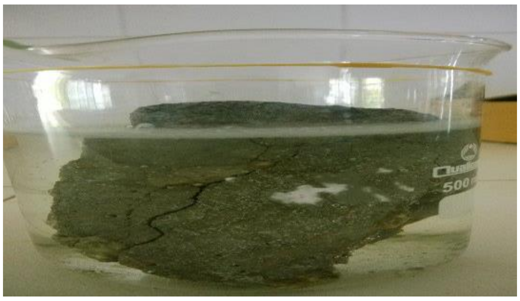

Fig. 6. Crushed test piece after 28 days placed in nutrient broth

Fig. 7. Revived bacterial colonies from concrete sample

Microbial concrete mix was found to be promising in the present study with respect to increase in compressive strength (50.13MPa) and tensile strength(3.49 MPa). Bacteria have the property of secreting calcium ions and react with CO_2 in the atmosphere to produce calcium carbonate crystals. These crystals fill the micro cracks and reduce permeability of corrosive structures, increase durability of the concrete. Addition of nutrients and yeast extract of bacterial medium in concrete mix, accelerated *B sphaericus* spore germination and promoted bioprecipitation in unfavorable conditions[3]. $CaCO_3$ could be precipitated even in nutrient poor environment, making the self healing mechanism more feasible [13, 14].

Increased concrete density found to increase the compressive strength. In the present study, the increased durability may be due to filling of micro cracks with calcite crystals, and addition of steel fibers in the concrete mix is the cause for increase in split tensile strength of the concrete [5, 6,11]. As the fly ash content is increased, slump is gradually increasing in our study probably due to spherical shape of fly ash particles [12]. Due to this spherical shape the frictional resistance between the particles is reduced thus leading to increased slump values. Mix M3 having $1x10^5$ cells/ml bacteria, 1.5% steel fiber and 20% fly ash content is found to have more split tensile and compressive strength in comparison to other mixes. All Mix grades **with** bacteria are found to have less weight loss and strength loss compared to Mix grades **without** bacteria.

CONCLUSION

Bacillus subtilis, a non pathogenic bacteria in concrete mix is cost effective method in increasing the compressive strength, promotes self healing of micro cracks. Further exploitation of new and genetically transformed bacteria with more calcite producing and corrosion inhibitory property, and sustain in extreme conditions might improve long term durability of the concrete, reduce the labour cost for industrial application and revolutionize the civil engineering process.

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