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Effect of Bacteria Inoculums on Compressive Strength

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ABSTRACT

The use of bio-concrete is increasing in the present day context and researchers are working on strength and durability characteristics of concrete using bacteria species which have shown calcite precipitation. Three different species of bacteria namely P. Fluorescence, B. Pumilis and B. Subliis that have calcite precipitation properties have been investigated in this study. The investigations were carried first on cement mortar (CM) cubes using these three bacteria species suspension of 20%; 40% and 60% having colony forming units *P. Fluorescence* (10^8 CFU/ml), *B. Pumilis* (10^6 CFU/ml) and *B. Subtilis* (10^8 CFU/ml) respectively. The 40% suspension in all the three cases has shown increased compressive strength as compared to 20% and 60%. The compressive strength measured showed increase (CS) of 18%; 12% for *P. Fluorescence*; *B. Subtilis* and decrease of 35% with *B. Pumilis* respectively. *B. Subtilis* with optimized 40% suspension having CFU10×10^8/ml showed 4.32% ; 5.56%; and 3.81% increase in CS of CC cubes with 3 days; 7 days and 28 days respectively and 5.92% overall increase in CS of CC cubes as compared to the 3 days CS of control cube.

ABBREVIATIONS

SDW: Sterile Distilled Water; SHC: Self-Healing Concrete; PCR: Polymerase Chain Reaction; BC: Bacterial Concrete; CP: Calcite precipitation; CS: Compressive Strength; CC: Cement Concrete; CM: Cement Mortar; MTCC: Microbial Type Culture Collection; CFU: Colony Forming Unit/ml

1. Introduction

The concrete is the modern material used globally to fulfill the needs of infrastructure development. The research on this material is as old as the invention of cement itself. The maintenance of concrete structures is of significance to have a long structural life [1]. Therefore, CS and durability studies are important in the shelf-life of concrete.

Bacteria Concrete can be made by embedding bacteria in the concrete that are able to constantly precipitate calcite [2]. This phenomenon is called microbe (bacteria) induced calcite precipitation [3-4]. These bacteria are able to produce extracellular urease enzymes. The hydrolysis of urea to carbon dioxide and ammonia can be catalyzed by urease enzyme. The hydrolyzed ammonia and carbon dioxide increases the pH and carbonate concentration in the bacterial environment, respectively [5, 6]. Based on the calcite precipitation of bacterial several researchers have employed various bacteria species for the CS studies *B. Thermusthermophilus* [7]; *B. Pasteurii* [8]; *Escherichia Coli* [8]; *Pseudomonas Aeruginosa* [8]; *B. Flexes* [9] *B. Sphaericus* [10, 11, 12]; *B. Cereus* [13]; *B. Cohni*, *E. Coli* [14]; *B. Subtilis* [3, 7, 10, 14, 15-18]; *Bacillus Sphaericus*, *Bacillus Pasteurii* [10, 12, 13].

Micro-cracks are the main cause to the ingress of moisture and deterioration leading to structural failure [1-19]. It has been reported in literature that mineral-producing bacteria could be used for sand consolidation

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as well as for the repair of limestone monuments [20, 21, 22]. The pores and crack filling were extensively studied for the maintenance; leakages and durability point of view [23, 24]. The strain *B. Subtilis* was used by many researches in the formation of calcium silicate hydrated gel by means of adsorbing silicate using chemically modified *B. Subtilis* (CMBS) [25, 8]. The Reports say 28% improvement in the CS of CMBS incorporated concrete compared to control concrete with optimum concentration of 10^6 cells/ml. The 7 day, 14 day and 28 days CS, was carried out using portland cement with mortar cubes and concrete cubes with cell concentration of *E.Coli* from 10^1 to 10^7 when compared with control (without E.Coli) did not show any change [8].

Similarly *B. Subtilis* showed no improvement in CS when used with concentration of 10^5[3]. However, inoculation with B. Sphaericus result in increase of CS as well as improvement of split tensile test strength. It has been reported that CS has increased by 30.76%, 46.15%, and 32.21% respectively on Day 3, 7 and 28 respectively with B. Sphaericus inoculation, and during the same period split tensile test strength has been increased by 13.75%, 14.28% and 18.35% respectively [26]. The increase in 28 days CS, Split Tensile Strength, Flexural Strength are 16.18; 17.74; 14.42; 13.75; 30.56; 26.51% when inoculated with *B. Subtilis* for M20 & M40 Grade respectively [27]. The increase in CS at 7 and 28 days were found to be 28.53% and 17.21%, using *Spirulina* and 33.99% and 24.67%, using *B. Subtilis* respectively, when compared to control concrete without bacteria [28, 29]. The literature review shows that there is a further scope to work in the direction of obtaining durable concrete using bacteria inoculation by conducting pin pointing experiments using different bacteria species which are known to precipitate calcite.

2. Materials and Methods

2.1. Isolation and Classification of Strains

The isolation and maintenance of pure culture for the selected bacterial species *B. Subtilis, P. Fluorescence, B. Pumilis* by dilution pour plate method as per the standard procedures outlined [29, 17, 30] and were regularly subcultures at regular interval for the purity and maintained at the department of Biotechnology KUD Dharwad.

2.2. Bacterial Species

B. Subtilis, P. Fluorescence, B. Pumilis are used which were procured from Department of biotechnology and microbiology KUD, Dharwad. The bacterial species in suspension was brought to the laboratory with concentration as given in Table 1 & 2.

Table 1: Bacterial species in suspension

Species	Concentration in CFU/ml
B. Subtilis	10 X 10^8
P. Fluorescence	10 X 10^8
B. Pumilis	10 x 10^6

Table 2: Concentration of bacteria suspension and water

Concentration (%) of bacterial inoculums	Total	Quantity of water in ml	Bacteria Saline suspension in ml
20	270ml	216	20% of 270=54
40	270ml	162	40% of 270=108
60	270ml	108	60% of 270=162
Note: The above concentrations were used for all the three bacterial species.			

2.3. Materials for Cement Mortar and Concrete

Cement with consistency 33%; SpGr-3.15; Coarse aggregate with SpGr-2.71; Fine aggregate Sp Gr-2.6); water were used as per IS: 4031-1988; IS: 2386-1963; IS-383 zone II; IS 456 respectively.

2.4. Cement Mortar (1:3)

Mortar cubes (70.6mm×70.6mm×70.6mm) were prepared as per standards specified by IS: 4031 part 6. The compressive strength of the mortar cubes at 3 days, 7 days and 28 days was determined. Mortar cubes with and without additions of bacteria were cast.

2.5. Concrete Cubes

The concrete mix cubes of M25 grade were cast in accordance with IS 10262-2012 of dimensions (150*150*150) mm respectively. The bacteria inoculums were 40% of the total water content i.e. 300 ml. Slump test is carried out as per the procedure outlined in IS 456-2000. Similarly a slump of 75mm for M30 grade concrete is recorded using *B. Subtilis* [7]. Curing was carried out in accordance with IS 10262:2012. CS is measured at 3, 7 & 28 days.

3. Results and Discussion

3.1. Compressive Strength of Mortar Cubes

The 3 day CS for CM cubes casted with *P. Fluorescence* showed decreasing values, where as the 7 and 28 days CS have shown increased values for all the three 20%; 40% and 60% bacteria suspension respectively as compared to the control CM cube CS Fig. 1. The CS values for 40% and 60% suspensions have almost the same values resulting in overlapping of trends.

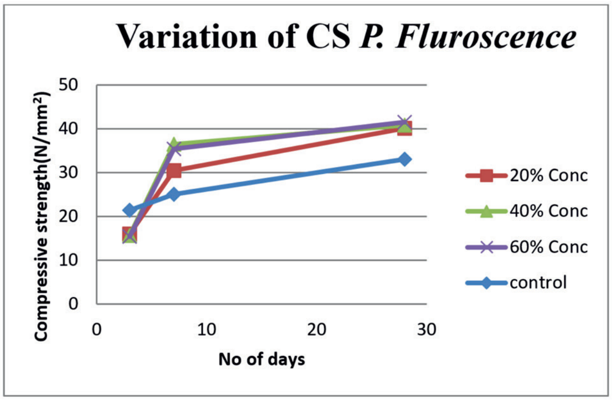

Figure 1: Variation in CS with *P. Fluorescence* and control

The 3, 7 and 28 days CS for CM cubes casted with *B. Subtilis* have shown increasing values as compared to the control CM cube Fig. 2. The 3, 7 and 28 days CS for CM cube casted with *B. Pumilis* have shown decreasing values as compared to control CM cube for all the 20%, 40% and 60% bacteria suspension Fig. 3. The compressive strength measured showed increase CS of CM cubes 18%; 12% for *P. Fluorescence*; *B. Subtilis* and decrease of 35% with *B. Pumilis* respectively.

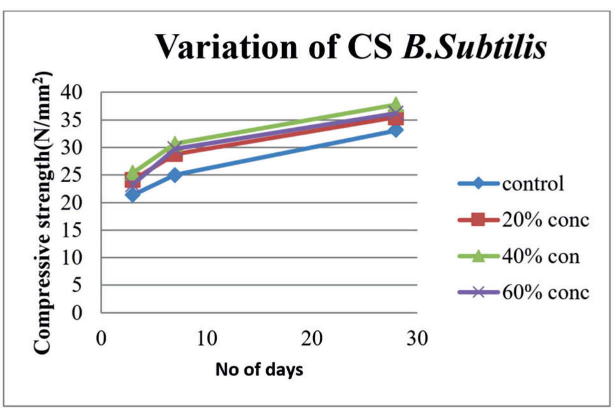

Figure 2: Variation in CS with *B. Subtilis* and control

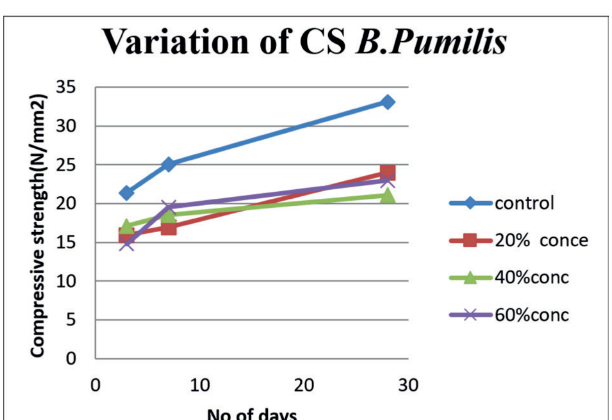

Figure 3: Variation of CS with *B. Pumilis* and control

The order of CS for development CM Cubes is *P. Fluorescence*> *B. Subtilis*> *B. Pumilis*. The highest CS of 28 days in the species is given by *B. Subtilis* 40% bacteria suspension. The 3 days and 7 days early strength development is better with *B. Subtilis* which is of significance in removal of formwork. The *B. Subtilis* species has shown its superiority in developing the CS in CM Cubes and therefore the compressive strength of concrete cubes is further carried out only with *B. Subtilis*. The available literatures of Reddy S P et al. (2012)[3] confirms our study and Seshagiri Rao et al. (2012) [18] have also shown the similar results of increasing CS of 25% in 28 days with *B. Subtilis*. The review also supports the use of *B. Subtilis* species [3, 7, 10, 16, 18, 23, 14, 15]. The strength improvement is due to growth of filler material within the pores of the cement–sand matrix. There is gain in strength after cooling in all grades of controlled and bacterial concretes may be due to the absorption of moisture from the surrounding medium which leads to extra hydration. The moisture content has a significant bearing on the strength of concrete. The CS increase is 18.3% and 12.2% respectively for *B. Sphaericus* and *Sporosarcina Pastuerii*. The increased CS with *B. Subtilis* strains is also reported when used in combination in fiber concrete [10].

3.2. CS of Concrete Cubes with Bacillus Subtilis

Depending upon the results of CS of mortar cubes with selected bacterial speciesand analyzing, high % increase in CS is showed by *B. Subtilis*. Therefore, concrete cubes using *B. Subtilis* only are casted and subjected to CS test and the results are placed in Fig 4. The addition of *B. Subtilis* showed noticeable increase in the compressive strength of concrete *B. Subtilis* with optimized 40% suspension having CFU $10x10^8$/ml showed 4.32%; 5.56%; and 3.81% increase in CS of CC cubes with 3 days; 7 days and 28 days respectively and 5.92% overall increase in CS of CC cubes as compared to the 3 days CS of control cube.

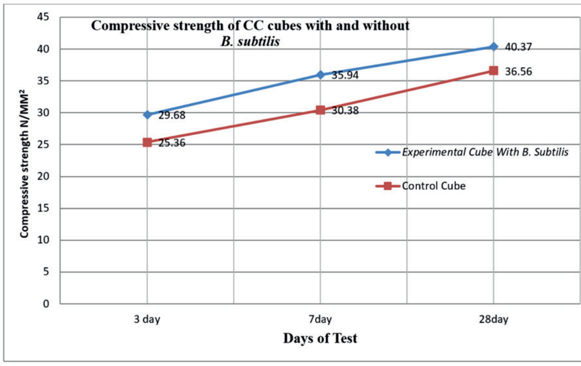

Figure 4: Variation in CS of concrete cubes with *Bacillus Subtilis* and control

The increase in CS is in conformity with the results of [16, 24]. However the studies in bio concrete are at the infant stage requiring more pin pointing experiments to solve the field problems of variability's like temperature, water cement ratio monitoring; compaction etc. The most challenging in bio concrete is to address issues of supplying the designated bacteria right in time & proportion during mixing of concrete and practicability of handling the bacteria.

4. Conclusion

The 40% suspension in all the three cases has shown increased compressive strength as compared to 20% and 60%. The compressive strength of CM 1:3 proportion showed an increase (CS) of 18%; 12% for *P. Fluorescence*; *B. Subtilis* and decrease of 35% with *B. Pumilis* respectively. *B. Subtilis* with optimized 40% suspension having CFU $10x10^8$/ml showed 4.32%; 5.56%; and 3.81% increase in CS of CC cubes with 3 days; 7 days and 28 days respectively, and 5.92% overall increase in CS of CC cubes as compared to the 3 days CS of control cube. Therefore it is concluded that *B. Subtilis* has shown its superiority in CS indicating it has produced more calcite to fill up the pores and give better bond between the ingredients of CC.

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