**ABSTRACT :**

The objective of the present investigation is to study the potential application of bacterial species i.e. B.sphaericus to improve the strength of cement concrete. Here we have made an attempt to incorporate dormant but viable bacteria in the concrete matrix which will contribute to the strength of the concrete. Water which enters the concrete will activate the dormant bacteria which in turn will give strength to the concrete through the process of metabolically mediated calcium carbonate precipitation. Concrete, however, is due to its high internal pH, relative dryness and lack of nutrients needed for growth, a rather hostile environment for common bacteria, but there are some extremophilic spore forming bacteria may be able to survive in this artificial environment and increase the strength and durability of cement concrete. In this study we found that incorporation of spore forming bacteria of the speceies Bacillus will not negatively affect the compressive and split tensile strength of the cement concrete.

Keyword : Concrete, B.sphaericus, calcium carbonate , compressive and split tensile strength.

**INTRODUCTION:**

Concrete is the most commonly used building material, but the cracks in concrete create problem. Cracks in concrete occur due to various mechanisms such as shrinkage, freeze-thaw reactions and mechanical compressive and tensile forces. Cracking of the concrete surface may enhance the deterioration of embedded steel bars as ingression rate of corrosive chemicals such as water and chloride ions in to the concrete structure increased. Therefore a novel technique has been developed by using a selective microbial plugging process, in which microbial metabolic activities promote calcium carbonate (calcite) precipitation., this technique is referred as Microbiologically Enhanced Crack Remediation (MECR).

In this technique urolytic bacteria are used hence the concrete is called Bacterial concrete. The “Bacterial concrete” can be prepared by adding spore forming bacteria in the concrete that are able to continuously precipitate calcite, this process of production of calcite precipitation is called Microbiologically Induced Calcite Precipitation (MICP). B.sphaericus is used to induce calcite precipitation in concrete. The basic principle for this process is that the microbial urease hydrolyzes urea to produce ammonia and carbon dioxide and the ammonia released in surrounding subsequently increases pH, leading to accumulation of insoluble calcium carbonate. Bacterial cultures improve the strength of cement sand mortar and crack repair on surfaces of concrete structures.

Calcium carbonate precipitation, a metabolic process which occurs in some bacteria, has been investigated due to its wide range of scientific and technological implications. Calcite formation by Bacillus species is used in making bioconcrete, which can produce calcite precipitates on suitable media supplemented with a calcium source. The basic principles for this application are that the microbial urease hydrolyzes urea to produce ammonia and carbon dioxide and the ammonia released in surroundings subsequently increases pH, leading to accumulation of insoluble calcium carbonate . 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 200 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. These cracks will subsequently be rapidly plugged and sealed through metabolically mediated microbial calcium carbonate precipitation, hampering further ingression of water and other chemicals. As revived bacteria also need a suitable substrate that can metabolically be converted to calcium carbonate, this also needs to be part of the concrete compatibility.

Durability studies carried out in the investigation through acid attack test with 5% sulphuric acid revealed that bacterial concrete is more durable in terms of “Acid Durability Factor” than conventional concrete and bacterial concrete is less attacked in terms of “Acid Attack Factor” than conventional concrete . Previous research has shown that Bacillus sphaericus bacteria are able to precipitate calcium carbonate on their celll constituents and in their micro-environment by conversion of urea in to ammonium and carbonate. The bacterial degradation of urea locally increases the pH and promotes the microbial deposition of calcium carbonate in a calcium rich environment. Through this process, the bacterial cell is coated with a layer of calcium carbonate .

**BACILLUS SPHAERICUS**

B. sphaericus is used primarily as a mosquito larvacide. The larvacide is sold under the Abbott Laboratories commercial tradename VectoLex. It is a naturally-occurring bacteria found in soil. It also has the characteristic of being able to survive in water that is rich in organics. It is currently used to control the particular strain of mosquito prone to carrying the West Nile Virus.

The bacteria, when ingested by a mosquito larva, secretes a binary toxin which causes the larvae to stop feeding and therefore starve. It has no known effect on mature adult mosquitoes or their pupae. There are no reports of any ill-effects of significance from exposure to the Bacillus sphaericus bacteria in either human or animal populations. It may cause a skin rash from prolonged or heavy exposure in some people, based solely on reported effects of related Bacillus.

Soap and water washing is the government Department of Health recommended method of cleansing in the unlikely event of heavy exposure.
B. Sphaericus is also used in probiotic applications, notably as a component of yogurt starter. It has no ill-effects on humans when ingested and is considered as a probiotic ingredient. This relatively little-known probiotic comprises enzymes, amino acids, anti-inflammatory compounds and colostrum. On the other hand, B.

 sphaericus is not shown in Bergey's Manual – considered to be the microbiologist's primary reference – doesn't show B. sphaericus to have any particular probiotic properties. It is part of the probiotic products Microflora, Neoflora and Superflora. B. sphaericus is one of approximately 400 bacteria that live in the human digestive tract. While clinical studies of this particular strain aren't widespread, it seems to have no ill-effects and it is classified as probiotic.
  

**MATERIALS AND METHODS**

The following are the details of the materials used for concrete making. Sand: Natural river sand well graded passing through 4.75mm sieve was used to find the compressive strength of cement mortar cubes and cylinders. Cement: Portland cement of 43 grade available in local market is used in the investigation.

The cement used has been tested for various properties as per IS: 4031-1988 and found to be confirming to various specifications of IS:12269-1987 having specific gravity of 3.15. Aggregate: Crushed aggregates of well graded igneous rocks.

 Water: Locally available river water confirming to IS 456 is used.

Bacterial strains: Pure cultures were maintained on nutrient agar slants and were preserved under refrigeration untill further use, sub-culturing was carried out for every 30 days. Contamination from other bacteria was checked periodically by streaking on nutrient agar plates. Whenever required, few colonies of the pure culture is inoculated into nutrient broth of 25ml in 100ml conical flask and the growth condition are maintained at 370C temperature and placed in 125 rpm orbital shaker.

**TEST ON MATERIALS**

Tests on Aggregate: The coarse aggregate of 20mm and down size is tested as per IS:2386-1963 and properties like Specific gravity = 2.923, Water absorption = 1.96% and Bulk density = 1.615. C. C. Gavimath, et al. 543

Test on Sand: The sand is tested as per IS:2386(Part III) -1963 and specific gravity 2.92.

Test on Cement: Cement has been tested as per IS: 4031-1988 and properties like specific gravity = 3.15, Consistency = 32%, Initial setting time 95 minutes and Final setting time 215 minutes.

Estimation of Calcium carbonate from Bacterial culture: Using the standard graph our bacterial test sample value was generated by carrying titration with EDTA. This was alkalized by using ammonia buffer. End point was obtained by using EBT indicator, which turns steel blue color from reddish pink color. Confirmation of calcium carbonate precipation in the cultre was done using lased Raman spectroscopy [10]. Preparation of specimen for compressive and split tensile test: The cubes and cylinders were prepared for concrete mix with and without addition of microorganisms. The size of the cubes and cylinders were taken as 100mm x 100mm x100mm and 100mm diameter 200mm height respectively. Cubes and cylinders were prepared in a standard manner according to Indian specifications. The cubes and cylinders were demoulded after 24 hours and subsequently cured in a water bath for 28 days. Total number of 18 cubes and 18 cylinders were casted using bacteria B. sphaericus.

**RESULTS:**

The cubes and cylinders have been tested as per IS specifications. The compressive test and split tensile tests were carried out both on conventional and bacterial concrete specimens. The conventional and bacterial concrete cube specimens after casting were cured for 28 days in the water bath and were tested in compression testing machine. From the tests, it was observed that the concrete specimens prepared by incorporating the micro-organisms yielded higher strength as compared to the conventional concrete. The results of the compressive test on conventional and bacterial concrete is indicated in Table 1. The results of compressive test with and without addition of B.sphaericus is shown in Table 2. From the observation it is revealed that there is an increase in compressive strength of 30.76%, 46.15% and 32.21% at 3rd, 7th and 28th day respectively while using B.sphaericus bacteria compared to conventional concrete.

Split tensile test: The conventional and bacterial concrete cylinder specimens after casting were cured for 28 days in the water bath and were tested in compression testing machine as per Indian Standards. From the tests, it was observed that, the concrete specimens prepared by incorporating the micro-organisms yielded higher tensile strength as compared to the conventional concrete. The result of split tensile test with and without addition of B.sphaericus is shown in Table 3. It can be observed that there is an increase in split tensile strength of 13.75%, 14.28% and 18.35% at 3rd, 7 th and 28 th day respectively when B.sphaericus bacteria are used and compared with conventional concrete.

 

Table 1. Results of the compressive test with and without addition of microorganisms.

|  |  |  |  |
| --- | --- | --- | --- |
| Sr No. | Type of Bacteria | Compressive strength | % increase  |
| 1. | Without bacteria | 23.94 | - |
| 2. | B. sphaericus | 36.28 | +51.54  |

Table 2. Results of the compressive tests with and without addition of B.sphaericus.

|  |  |  |  |
| --- | --- | --- | --- |
| No. of days  | Compressive strength of conventional concrete cubes, N/mm2 | Compressive strength of B.sphaericus concrete cubes, N/mm2 | % increase in strength  |
| 3 | 19.24 | 25.16 | 30.76 |
| 7 | 23.66 | 34.58 | 46.15 |
| 28 | 34.52 | 45.72 | 32.21 |

Table 3. Results of the split tensile test with and without addition of B. sphaericus.

|  |  |  |  |
| --- | --- | --- | --- |
| No. of days  | Split tensile strength of conventional concrete cylinders,N/mm2N/mm2 | Split tensile strength B. sphaericusconcrete cubes, N/mm2 | % increase in strength  |
| 3 | 3.78  | 4.30  | 13.75  |
| 7 | 4.62  | 5.28  | 14.28 |
| 28 | 4.85 | 5.74 | 18.35 |

**DISCUSSION:**

The main objective of this study was to investigate whether bacteria can potentially act as a self healing agent in concrete. The bacteria tested are known to be alkali resistant i.e. they grow in natural environments characterised by a reletively high pH (10-11). In addition, these strains can produce spores which are resting cells with sturdy cell walls that protect from extreme environmental mechanical and chemical stresses. Therefore these specific bacteria may have the potential to resist the high internal concrete pH values (12-13 for Portland cement based concrete), and remain viable for a long time as well, as spore viability for up to 200 years is documented. A dormant (alive but not growing) and viable (capable of working successfully) micro-organism of certain number is induced in concrete during mixing. Bacterial spores immobilized in the concrete matrix will become metabolically active when revived by water and calcium media of concrete. The hollow space (microscopic level) will subsequently be rapidly plugged and sealed through metabolically mediated microbial calcium C. C. Gavimath, et al. 544 carbonate precipitation, hampering further ingression of water and other chemicals. The microorganism hydrolyzes urea to produce ammonia and carbon dioxide, resulting in an increase of pH in the surroundings where ions Ca2+ and CO32- precipitate as CaCO3. Possible biochemical reactions in medium to precipitate CaCO3 at the cell surface that provides a nucleation site.

**CONCLUSION:**

Based on laboratory investigations, the following findings are accessible:

1. Lower water permeability is attained by bacteria built-in concrete compared to controlled concrete

2. Due to high impermeable nature, Bacterial concrete is a potential self-healing remediation technique for cracks in concrete.

3. In bacterial concrete interconnectivity of pores is disturbed due to plugging of pores with calcite crystals. Since interconnected pores are significant for permeability, the water permeability is decreased in bacteria treated specimens. Thus concrete permeability depends on its pore Structure. Even the best of concrete is not gas-tight or watertight unless the pores are closed.

4. Microbiologically induced calcium carbonate crystals, in the cracks and pores of concrete using built-in bacteria B.subtilus JC3, seals the cracks and pores in hardened concrete thereby making the concrete impermeable. This process of self-healing using microorganisms is an innovative method for remediation of cracks in old and new concrete structures.

Thus we conclude that concrete-immobilized spores of such bacteria may be able to seal cracks by biomineral formation after being revived by water and growth nutrients entering freshly formed cracks, hence the application of bacteria will improve the strength and durability of cement concrete therefore it appears promising field in near future.