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Study of Strength Variation of Concrete Using Ureolytic Bacteria

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Abstract— **This paper presents the results of a study carried out to investigate the ability of ureolytic bacteria to enhance the compressive strength of concrete. The urease producing aerobic alkalophilic bacteria *Bacillus subtlis* strain MU12 was used in the present study. Ureolytic bacteria used in the present studies were isolated from various sources like cowshed, poultry farm, milk, soil and pigeon dung. All the isolates were screened for ureolytic activity on the basis of urease test. These isolated cultures were purified on phenol red agar plates. Four different cell concentration (10^4, 10^5,10^6,10^7 cells/ml) of bacteria were used in making the concrete mixes. Tests were performed for compressive strength of concrete cubes at 7 days, 14 days and 28 days. Inclusion of MU12 @ 10^7 cells/ ml in cement concrete enhanced the compressive strength in 7^th^ and 14^th^ days concrete samples.**

Index Terms— **Urease, Calcium Carbonate Precipitation, Characterization, Endospore.**

I. Introduction

Concrete is an absolutely essential component of construction materials used in infrastructure and most buildings. Despite its versatility in construction, it is known to have several limitations. A lot of research has been carried out around the globe to improve properties of concrete. Recently, it has been found that microbial mineral precipitation resulting from metabolic activities of favorable microorganisms in concrete can improve the overall behavior of concrete. In this technique ureolytic bacteria (microorganism) are used hence the concrete is called Bacterial or Microbial concrete. The “Microbial 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) [1]. Microbial calcite precipitation is mainly due to ureolytic activity and carbonate biomineralization of bacteria. Under suitable conditions, most bacteria are capable of inducing carbonate precipitation. In addition, carbonate particles can also be produced by ion exchange through the cell membrane [2]. 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 $CaCO_3$ formation through urea hydrolysis is known as Bacterial Calcite Precipitation [3]. Bacterial Cultures improves the strength of cement sand mortar [4] and crack repair on surfaces of concrete structures [5, 6]. Bacterial Calcite Precipitation (BCP) is highly desirable because it is pollution free and natural. There are several applications of BCP; the most important is strength development. Bio concrete has many advantages over the ordinary concrete; it needs a much shorter time; it is suitable for in-situ process; raw material of bio cement are produced at a lower temperature and can be used as eco-construction material as it consumes less energy and less CO_2 [7]. Bacteria to be incorporated in concrete should be alkali resistant to endure the high pH of concrete. The “Bacterial Concrete” can be made by embedding bacteria in the concrete that are able to constantly precipitate calcite. Several bacteria have the ability to precipitate calcium carbonate. These bacteria can be found in soil, sand, natural minerals. The selection of the bacteria is depend on the survive capability of bacteria in the alkaline environment. Most of the microorganisms die in an environment with pH value of 10 or above [8]. The use of microbial concrete in Civil Engineering has become increasingly popular. From enhancement in durability of cementious materials to improvement in sand properties, from repair of limestone monuments, sealing of concrete cracks to highly durable bricks, microbial concrete has been successful in one and all. This technology also offers the advantage of being novel and eco- friendly. This special kind of concrete has multiple usage.

With recent encouraging reports on compressive strength enhancements achieved in conventional concrete through Microbiologically Induced Calcium Carbonate Precipitation (MICCP). The present study was aimed at isolation and characterization of more efficient urease producing bacterial strains for improving the strength of cement concrete.

II. MATERIALS AND METHODS

2.1 Isolation and purification of urease producing bacterial strain

Ureolytic bacteria used in the present studies were isolated from various sources, like poultry dung compost, cowshed, milk sample, etc. Ureolytic bacteria were isolated by inoculating 1ml of serially diluted soil suspension on modified Urea agar (composition gl^{-1}: Potassium dihydrogen phosphate: 2.0; Glucose: 1.0; Peptone: 0.2; Sodium chloride: 5.0; Urea: 20.0; Phenol red: 0.012; Agar: 15.0; pH: 7) plates and incubating at $25\underline{+}2^oc$ in dark [9]. Colonies that turn the agar red or pink were picked and streaked for isolation onto Urea agar plates.

Further**,** colonies were picked and further streaked for purification. The cultures were maintained at refrigerated temperature on nutrient agar as by repeated sub-culturing fortnightly.

2.2 Cultural characteristics of Bacteria

Cultural characteristics of all the isolates were studied on the basis of Gram’s staining [10] and colony

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morphology. The size, shape, pigmentation, texture and elevation of the colonies were the characteristics used to describe the morphology of a bacterial colony.

2.3 Materials used

The following are the details of the materials used for making concrete cubes:

Cement: Portland cement of 53 grade available in local market was used in the investigation. The cement used was 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.

Sand: Natural river sand well graded passing through 4.75mm sieve having fineness modulus 2.71and specific gravity of 2.64 was used.

Aggregate: Locally available coarse aggregate with equal proportion of size 10 mm and 20 mm conforming to IS: 383-1970 having fineness modulus 6.613, specific gravity of 2.56 and water absorption 0.5% were used as coarse aggregate.

Water: Ordinary drinking water available in the construction laboratory was used for casting all specimens of this investigation.

2.3 Preparation of specimen for compressive strength test

Cement concrete cubes of 150 mm X 150 mm X 150 mm were made using different concentration of bacterial isolate *Bacillus subtilis* strain MU12 for M20 concrete design mix. The cell suspension was mixed with B4 broth in different concentrations so as to make final concentrations of 10^4, 10^5, 10^6 and 10^7 cells/ml for biodeposition experiment. The cubes were prepared for concrete mix with and without addition of bacteria. The water to cement ratio was fixed at 0.42 as per standard methods. Total numbers of 12 cubes were prepared and tested for different days and concentrations. The mixing of concrete constituents was carried out with materials being laid in uniform layers, one on the other in the order–coarse aggregate, fine aggregate and cementitious material. Dry mixing was done to obtain a uniform colour. Required amount of bacterial suspension was added along with the water. Cubes were cast and compacted with a vibration machine and left in the molds for 24 hrs (Fig.1). The cubes were remoulded after 24 hours and subsequently cured in a water bath for 7, 14 and 28 days of compressive strength testing (Fig. 2). The results were interpreted with the help of graph obtained where stress in terms of Mega Pascal (MPa) and Position (mm) were plotted by the computer software. Cubes were tested for compressive strength using universal testing machine in Department of Civil Engineering, PAU Ludhiana on 7th, 14th and 28th days of soaking. The formula used for calculation of compressive strength is

Fig.(1): Remoulding of cubes

Fig.(2): Immersing of cubes in water until tested

Compressive Strength= Total Failure Load / Area of the Cube

III. RESULTS AND DISCUSSION

The objective of this experimental study was to highlight the effect of urease producing microorganism on the strength enhancement in cement concrete. Total of 13 samples were used to isolate urease producing bacteria (Table 1). These samples were taken from different locations of Punjab, Himachal Pradesh and Ludhiana. Approximately, 1500 bacterial colonies were purified from these samples. Urea agar was used for the selection of urease producing microorganisms, producing a red-pink color due to the presence of phenol red, a pH indicator (Fig.3). Only 17 isolates were urease positive and were able to change the media color from yellow to pink (Fig. 4). Maximum colonies

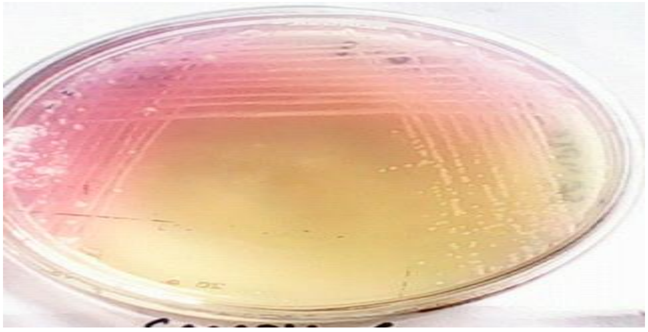

Fig.(3): Pink coloured isolate producing pink

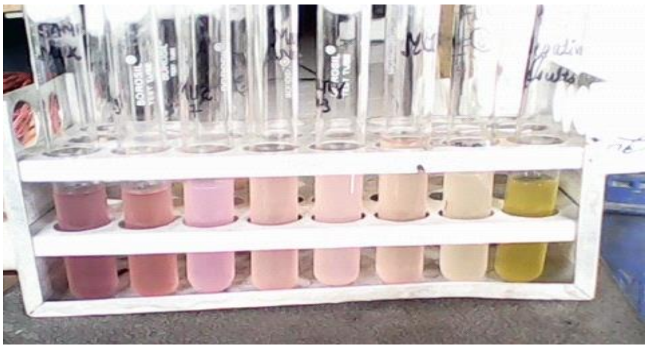

Fig. (4): Urease test for screening of isolates were observed from Pigeon dung sample followed by soil sample from Poultry droppings (GADVASU).

Six soil samples were used for isolating ureolytic bacteria. The samples included: poorly graded tidal sand, an organic soil, mined alluvial poorly gradedsand, quartz poorly graded sand, and Paloues loess soil [11].

Table (1): Isolation of Urease producing bacteria

S. No	Sample Source	No. of Colonies/Plate	No. of Pink Colonies
1	Soil from Cowshed (Rajasthan)	50	Nil
2	Soil from Cowshed (Himachal Pradesh)	100	2
3	Soil from Cowshed (Ludhiana)	60	1
4	Soil (lentil farm, PAU Ludhiana)	40	1
5	Soil (construction site, PAU Ludhiana)	150	1
6	Soil sample (COAET)	60	1
7	Soil from Poultry farm (GADVASU Ludhiana)	150	2
8	Poultry droppings (GADVASU)	90	1
9	Pigeon dung	More than 300	1
10	Milk (Unpasteurised)	200	1
11	Milk (Pasteurised)	40	1
12	Air	50	1
13	Garden soil	80	4

*Average of triplicate data

The compressive strength results of concrete cube test specimens for control concrete and four different cell concentrations are presented in Table 2 and the comparison between the results is presented in form of bar chart (Fig.5). All the values are the average of the three trials in each case in the testing program of this study.

Table (2): Compressive strength (in MPa) of bacterial concrete using different cell concentrations

S.No		Compressive strength (MPa)		
	Inoculum conc. (cells/ml)	7 Days	14 Days	28 Days
I.	Control Specimen	4.66	8.63	26.2
II.	10^4	1.18	10.45	14.63
III.	10^5	7.99	15.87	20.30
IV.	10^6	8.08	16.4	20.93
V.	10^7	13.00	17.43	21.42
C.D. at 5%	0.88	2.81	2.92	

As observed on 7th day, cell concentration of 10^4 cells/ ml showed sudden decrease in the compressive strength. But as cell concentration was increased to 10^5 cells/ ml compressive strength increases from 4.66 MPa (control) to 7.99 MPa. Cell concentration of 10^6 and 10^7 cells/ ml showed further increase in compressive strength to 8.08 MPa and 13 MPa respectively. As cell concentration was increased compressive strength was also increased. Similar trend was observed during 14th day of soaking.

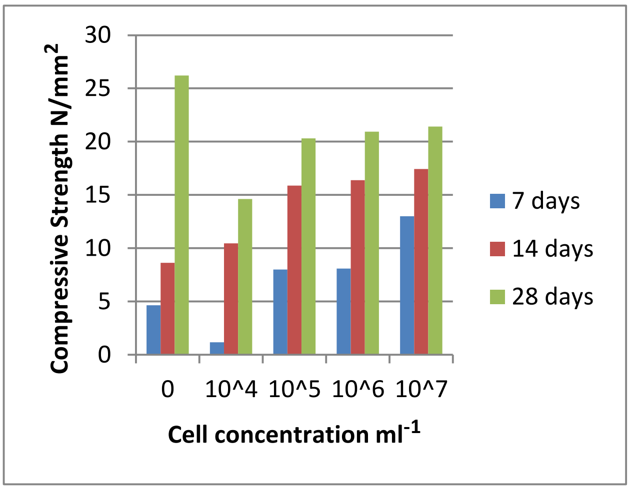

Fig (5). Compressive strength (in MPa) of bacterial concrete with different cell concentrations

Different concentrations of bacterial cells ranging from 0-10^7 cells/ml were with cement and sand mortar/ concrete during casting and it was reported that concrete showed an increase in compressive strength with increase in cell concentrations and maximum strength was found at 10^5 ml. The percentage increment of compressive strength was found to 22.6% in concrete [11].

Table (3): Percent change in compressive strength of cement concrete using *Bacillus subtilis* strain MU12

		Percent change in compressive strength		
S. no.	Inoculum conc. (cells/ml)	7 Days	14 Days	28 Days
1	10^4	74.6	21.09	-44.16
2	10^5	71.46	83.89	-22.5
3	10^6	73.39	90	-20.11
4	10^7	>100	>100	-18.24

IV. DISCUSSION

Results from Table 3 indicates that when a concentration of 10^4 cells/ ml was added to the concrete, the compressive strength decreased as compared to control and showed a decrease in compressive strength. As cell concentration was increased from 10^4-10^5, there was a considerable increase in the compressive strength of cement concrete. Cubes with 10^5 cells/ ml showed 71.46% increase in compressive strength. Similarly, as cell concentration was increased to 10^6 and 10^7 cells/ ml, compressive strength was increased and percent increase observed was 73% and >100% respectively on 7th day of testing. Similar trend was observed in case of cement cubes of 14th day. Concentration of 10^4 cells/ ml results in small increase in compressive strength but 10^5, 10^6 and 10^7 cells/ ml showed 83.89%, 90% and >100% increase in compressive strength respectively. However, a decreasing trend in compressive strength was observed during 28th day as compressive strength of cement cubes was decreased as compared to control cubes. The increase in compressive strengths is mainly due to filling of the pores inside the cement mortar cubes with microbiologically induced calcium carbonate precipitation initially. The reduction in the compressive strength after 28 days of curing might be due to reason that survival of microorganisms are greatly influenced by the pH of an environment. The *Bacillus subtilis* strain MU12 did not survived the high pH of concrete and there was a significant loss of compressive strength.

V. CONCLUSIONS

Out of all isolated cultures developed and tested, it was observed that *Bacillus subtlis* strain MU12 has offered to precipitate calcium carbonate under laboratory conditions. Compressive strength studies have been carried out on concrete cubes by incorporating *Bacillus subtilis* strain MU12 with various concentrations along with control mix. From the strength studies, it was observed that the strength improvement is significantly higher for all concentrations after 7 and 14 days for all concentrations but the strength drastically reduced after 28 days testing as compared with the control concrete. Further studies can be carried out to create an environment for survival of *Bacillus subtlis* strain MU12 and also for higher grades of concrete.

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