Strength Improvement Studies of Concrete Using Ureolytic Bacteria

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***Abstract*— The "Bacterial Concrete" is a concrete which can be made by embedding bacteria in the concrete that are able to constantly precipitate calcite. The bacterial concrete makes use of calcite ($CaCO_3$) precipitation by bacteria. This phenomenon is called microbiologically induced calcite precipitation. As part of metabolism, some bacterial species like Bacillus pasteurii, Bacillue sphaericus ect. produce urease, which catalyzes urea to produce CO_2 and ammonia, resulting in an increase of pH in the surroundings where Ca^{2+} and CO_3^{2-} ions precipitate as calcite. This microbiologically induced calcite precipitation is highly desirable because the calcite precipitation is pollution free and natural and can be used to improve the compressive strength of concrete specimens, repair cracks in concrete. 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. 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.**

Key Words: Concrete, bacteria, urease, precipitation, ureolytic.

I. . INTRODUCTION

Concrete which forms major component in the construction industry as it is cheap, easily available and convenient to cast. 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. Based on the continuous research carried out around the globe, various modifications have been made from time to time to overcome the deficiencies of cement concrete. The ongoing research in the field of concrete technology has led to the development of special concretes considering the speed of construction, the strength of concrete, the durability of concrete and the environmental friendliness with the use of industrial material like fly ash, blast furnace slag, silica fume, metakeolin etc. Recently 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)[1]. In this technique urolytic bacteria are used hence the concrete is called Bacterial concrete[2]. The “Bacterial concrete” can be prepared by adding spore forming bacteria in the concrete that are able to continuously precipitate calcite. 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[3] and crack repair on surfaces of concrete structures[4]. The Calcite precipitation occupies the voids between cement matrixes and therefore leads to denser concrete. The approach does not deplete any natural resources since the bacteria used can be easily reproduced by cultivation process. The use of biological approach in concrete is also considered as a green technology as its production does not involve greenhouse gas emission. Therefore bacterial induced Calcium Carbonate (Calcite) precipitation has been proposed as an alternative and environment friendly way for improvement of strength of building materials[5].

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

Cement:In this experiment 43 grade Ordinary Portland cement is used. The testing of cement is done as per IS 4031-11- 1988 Code the specific gravity of cement found is 3.0.

Fine aggregates: In this experiment the locally available sand is used and the specific gravity of fine aggregate is to be obtained by using the IS 2720 part 3 code. The specific gravity is found 2.65. The fine aggregates used which passes through the 4.75mm sieve.

Coarse aggregates: In this experiment the locally available aggregates are used and the specific gravity of course aggregate is done by using the IS2386 part 3 1963 code. The specific gravity is found 2.82. The coarse aggregates which are used of 20 mm size.

Water:The least expensive but the most important ingredient of concrete is water. The water which was used for mixing concrete was clean and free from harmful impurities such as oil, alkali, acid etc. Portable water was used for making concrete cubes and curing work.

Bacteria: The selected aerobic alkaophilic bacteria *Bacillus subtlis* strain MU12 was used in the present study. The bacteria were grown in B4 broth for about 48 hrs. at 25°C. After growth, the broth was centrifuged at 5000 rpm (rotations per minute) for 20 min. at room temperature. The pellet was suspended in 100 ml distilled water and haemocytometer count was taken. The different concentrations of bacteria were taken to prepare cement concrete made of cement, coarse aggregate, fine aggregate and water (M20-1:1.5:3). 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.

Preparation of specimen for compressive strength test:

The cubes were prepared for concrete mix with and without addition of bacteria. The size of the cubes was taken as 150mm x 150mm x150mm. The water to cement ratio was fixed at 0.42 as per standard methods. Control specimens were prepared for 7, 14 and 28 days compressive strength tests in a standard manner according to Indian specifications. Total numbers of 12 cubes were prepared and tested for different days and concentrations.(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.

Fig1 Casting of cubes

Fig 2 Immersing of cubes in water until tested

Compressive strength Test

Compression test has been conducted confirming to IS 516-1959(5), on the concrete specimens on the universal testing machine in Department of Civil Engineering, PAU Ludhiana on 7^{th}, 14^{th} and 28^{th} days of soaking.(Fig. 2) Test cube, after wiping out the surface moisture, were placed with the cast faces not in contact with the plates of testing machine. Load has been applied at a constant rate of stress equal to 15mpa/min according to the relevant IS code and loading was continued till the dial gauge needle just reverses its direction of motion. The reversal in the direction of motion of the needle indicates that the specimen has failed. The load at which the specimens failed has been recorded. Thus from the results, the compressive strength is obtained.

III. .RESULTS AND DISCUSSION

This study was conducted with the aim of isolating locally urease producing bacteria that could be potentially used in various biocementation processes. Ureolytic bacteria were isolated from various sources like soil (collected from various places Himachal Pradesh, Rajasthan and Punjab), milk, and open air.

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	Morethan 300	1
10	Milk (Unpasteurised)	200	1
11	Milk (Pasteurised)	40	1
12	Air	50	1
13	Garden soil	80	4

Medium used: Urea agar; Temperature 25± 2°C; pH: 7.0; Incubation period, 3-7 days

These samples were serially diluted and one ml of each sample was used to isolate the urease producing bacteria. Colour of the media was observed for urease production as colour change from yellow to pink conformed the urease production(Fig.3). Results from Table 1 shows that a total of 13 samples were used to isolate urease producing bacteria. Approximately, 1500 bacterial colonies were purified from these samples. However, only 17 isolates were urease positive and were able to change the media colour from yellow to pink (Fig. 4).

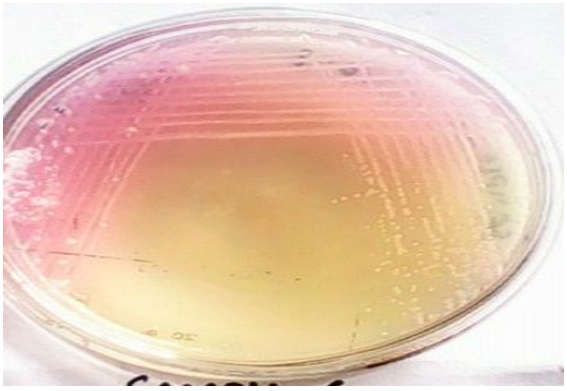

Fig.3: Pink coloured isolate producing

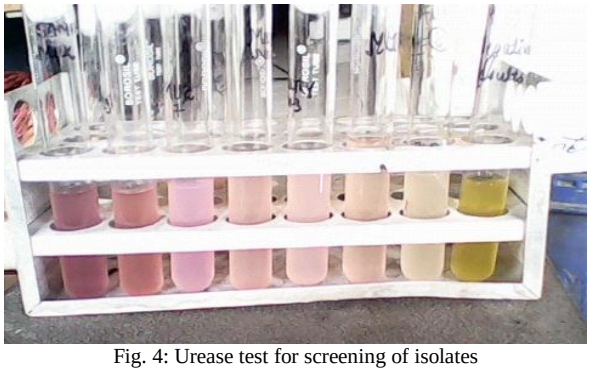

Fig. 4: Urease test for screening of isolates pink colour on urea agar

Comparative Strength

The compressive strength results obtained from the experimental investigations are shown in table 2 and the comparison between the results is presented in form of bar chart. All the values are the average of the three trails in each case in the testing program of this study.

Table 2: Compressive strength of bacterial concrete using Bacillus subtilis

Compressive strength (MPa)				
S.No	Inoculum conc. (cells ml^{-1})	Period of soaking		
		7 Days	14 Days	28 Days
1	Control (without inoculum)	4.66	8.63	26.2
2	10^4	1.18	10.45	14.63
3	10^5	7.99	15.87	20.30
4	10^6	8.08	16.4	20.93
5	10^7	13.00	17.43	21.42
C.D. at 5%		0.88	2.81	2.92

Results from Table 2 indicates that on 7^{th} 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 14^{th} day of soaking. However, a decreasing trend in compressive strength was observed during 28^{th} 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[6]. 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[7]. The *Bacillus subtilis* strain MU12 did not survived the high pH of concrete and there was a significant loss of compressive strength.

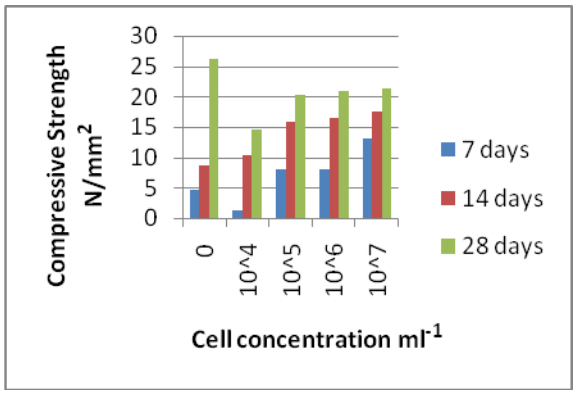

Fig 5. Compressive strength (in MPa) of bacterial concrete with different cell concentrations

IV. . 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.

REFERENCES

[1] Meldrum F.C. [2003] "Calcium carbonate in biominerilisation", *Biomimetic chemistry*, 48,187-224.
[2] Dick J., De Windt W., De Graef B., Saveyn H., Vander Meeren P., De Belie N., Verstraete W. [2006]. "Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species", *Biodegradation*, 17 (4), 354-367.
[3] Ghosh P., Mandal S., Chattopadhyay B.D., Pal S. [2005]. "Use of microorganism to improve the strength of cement mortar" *Cement and Concrete Research* 35(10), 1980-1983.
[4] Ramachandran S.K., Ramakrishanan V., Bang S.S. [2001]. "Remediation of concrete using microorganisms ACI *Material Journal*, 98, 3-9.
[5] Srinivasa Reddy V, Sreenivasa Rao D, Seshagiri Rao M V, Sasikala Ch. Permeation "Properties of Bacterial Concrete" *Journal of Mechanical and Civil Engineering* (IOSR-JMCE) e-ISSN: 2278-1684 Volume 5, Issue 6 (Mar. - Apr. 2013), PP 08-12
[6] Ghosh P, Mandal (2006) development of bioconcrete material using an enrichment culture of novel thermophilic anaerobic bacteria. *Ind J Exp Biol* **44:** 336- 39.
[7] De Muynck W, De Belie N and Verstraete W (2009) Microbial carbonate recipitation in construction materials: A review *Ecol Eng* doi:10.1016/j.ecoleng.2009.02.006.