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An Eco-sustainable approach to increase compressive strength of cement mortar using ureolytic bacteria

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Abstract— **Sustainability is an important theme in all fields at this moment in order to maintain an equilibrium in the use of natural resources and to minimise environmental damages. In the case of construction sector, the technique of microbial induced carbonate precipitation is gaining importance as a sustainable technology to improve the mechanical properties of cement mortar cubes and for self-healing of concrete. *Comarca Lagunera*, a region comprising Coahuila and Durango states of Mexico is rich in fauna and flora. Bacterial strains showing urease activity were isolated from these soils and one strain ACRN 1 was tested for its potential to increase the compressive strength of mortar cubes. Cement mortar cubes were prepared in the ratio of 1:3 (cement and sand) with the water cement ratio of 0.4. ACRN 1 was added to water at different concentrations, from 10^4 to 10^8 cells per ml of water and was compared with control (without bacterial strains). It was observed that the concentration of 10^5 cells per ml of water was the optimum concentration and increased the compressive strength of the cement mortar cube by 18.57% at 28 days of curing. Statistical analysis also showed that the results obtained on compressive strength of mortar cubes by addition of 10^5 cells per ml of water was significantly ($p<0.05$) different than control and other bacterial concentrations tested.**

Index Terms—**Bacteria, Carbonate precipitation, Cement mortar cubes, Urease.**

I. INTRODUCTION

Cracks and fracturing are perennial problems in concretes and various commercial products, *viz*., structural epoxy, resins and epoxy mortar are available for quick remedy [16]. Prevention of crack formation has not been achieved till date and considerable expenses are incurred in maintenance work at regular intervals to safeguard the structures. A novel strategy to restore corroded structures and repair concrete cracks is microbiologically induced calcite precipitation (MICP) [1], [2], [19], [20].

Sustainability involves the use of environment friendly green technology, and in most cases employs the use of an agent of biological origin. In the case of civil engineering and construction field, there is a need to develop alternative sustainable technologies since the production and use of conventional Portland cement is a significant contributor to emission of greenhouse gases and the resultant global warming. Less dependence on fossil energy and the use of innovative materials are global challenges. The term "bio mimicry" was defined by Janine Benyus as innovation inspired by nature; it is looking to the natural world for developing sustainable technologies [3].

Existing biological principles and advances in knowledge on microbial induced carbonate precipitation (MICP) offers the opportunities to use natural stable systems to meet these challenges. Microbial mineral precipitation involves various types of microorganisms and their metabolic pathways. Carbonate precipitation is mainly carried out by ureolytic bacteria by the production of urease enzyme. This enzyme catalyzes the hydrolysis of urea to CO_2 and ammonia, resulting in an increase of pH, resulting in carbonate precipitation in the bacterial environment [1], [5]. Recent studies reveal that the addition of bacteria like *Bacillus pasteurii* promoted self-healing of the cracks in concrete since they are capable of carbonate precipitation [20]. Moreover, it is reported that the durability of the concrete increased with the increase in the concentration of bacteria. Application of bacteria as an integrated healing agent to the concrete mixture would aid to mitigate environmental problems and cost of reparation [6], [7], [14], [22].

The Importance of microbial mineral precipitation has been widely recognized in Petroleum, Geological and Civil Engineering based on the reports on the remediation of cracks in rock formations, especially in oil reservoirs, sand consolidation, ornamental stone repair, etc. [9], [19], [21], [23]. After analyzing the behavior of microorganisms in plugging the pores of rock by adhering to the available surfaces through extracellular organic compounds, the research has been initiated in remediation of cracks in man-made structures such as concrete. Various research groups from United States of America (USA), Spain, Belgium, India, United Kingdom (UK) and Netherlands are working to solve this macro problem by use of microbial technology [2], [7], [13], [15], [21], [23].

This study is aimed at isolation and selection of urease producing bacterial strains from the soils of *Comarca Lagunera* of North-East Mexico. Of the twenty-four strains isolated, six were selected based on their urease activity. In this paper, the behavior of one bacterial strain ACRN 1 and its potential in increasing the compressive strength of cement mortar under different cell concentrations was evaluated.

II. MATERIALS USED & METHODS

A. Cement

Ordinary Portland cement available in local market was used in this study. The cement used has been tested for various properties as per ASTM C187 - 98 and C191-08.

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B. Fine aggregate

Sand available in the local market was used in this work. The sand was graded to meet the requirements ASTM C 778 and ASTM 136 – 06 specifications.

C. Water

Locally available potable water confirming to ACI 318 - 2008 was used.

D. Isolation of urease producing bacteria & assay of enzyme activity

Bacterial strain ACRN 1 was isolated from the soils of *Comarca Lagunera* by using a selective medium containing (g/l) $NaHCO_3$, 2.12; urea, 20; peptone, 0.5; meat extract, 1.5; NH_4Cl, 2.12; $CaCl_2 \cdot 2H_2O$, 30 mM; agar, 20. Urease activity of the strain was determined and one unit of urease activity is defined as the release of one µmol of ammonia per min at 37° C [17]. Later, ACRN 1 was grown in a urea broth, harvested after 48 h, cell concentrations were adjusted and used to prepare mortar cubes.

E. Cement Mortar cube preparation and resistance test

Cement and sand were mixed properly at the ratio of 1:3 and a water cement ratio of 0.4 was used. A total of 16 mortar cubes of dimensions 50x50x50 mm were prepared by adding bacteria at different cell concentrations (10^4, 10^5, 10^6, 10^7 y 10^8 per ml of water). Control samples were prepared with only water. Mortar cubes were cast and the molds were placed in water in the moist curing cabinet. After 7, 14, 21 and 28 days, the cubes were removed; wiped clear of water and the compressive strength were determined by following the protocols mentioned in ASTM C109/C109M-93 Standard Test Method for Compressive Strength of Hydraulic Cement Mortars.

III. Results & Discussion

A. Results of Compressive strength of mortar cubes

Results on the compressive strength of mortar cubes prepared with different concentrations of ACRN 1 bacteria, *viz*., 10^4, 10^5, 10^6, 10^7 y 10^8 per ml of water of bacterial strain ACRN 1 and at 7, 14, 21 and 28 days of curing is presented in Table 1 and Fig. 1. Compared to control, the mortar cubes prepared with 10^5 cells per ml of water showed higher compressive strength of 22.81 and 18.57% after 14 and 28 days of curing respectively.

Table 1. Average Compressive strength of the specimens with various concentrations of bacteria ACRN 1

Concentrations of ACRN 1 per ml of water	Average Compressive Strength of the Specimens (kg/cm2)							
	7 days		14 days		21 days		28 days	
		Increase in %		Increase in %		Increase in %		Increase in %
Control	174	-	225	-	261	-	253.3	-
1×10^8	177	1.72	193.33	-14.08	183.67	-29.63	234.67	-7.35
1×10^7	188.07	8.08	227	0.89	238.67	-8.56	293.2	15.75
1×10^6	213.67	22.8	219.67	-2.37	257.67	-1.28	254.33	0.4
1×10^5	200.33	15.13	276.33	22.81	244.33	-6.39	300.33	18.57
1×10^4	180.33	3.64	257	14.22	230.67	-11.62	238	-6.04

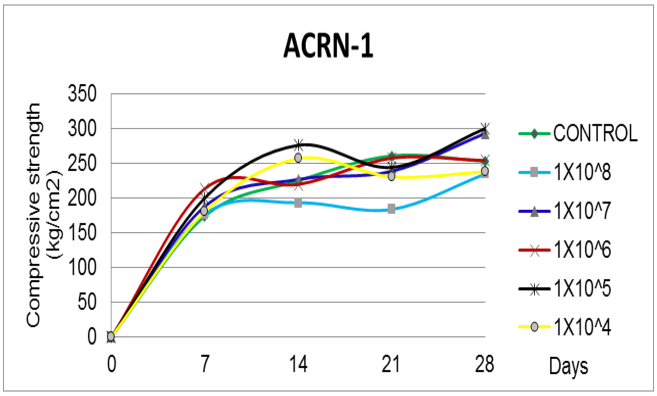

Fig.1. Compressive strength of mortar cubes in relation to bacterial population ACRN 1

A. Statistical Analysis on Compressive Strength results of the Mortar cubes with Bacterial strain ACRN 1

Univariate and Multi variant ANOVA analysis were performed to compare the mean values of the compressive

strength of mortar cubes to determine the influence of ACRN 1 bacterial strain at different cell concentrations and at different curing periods.

From the Statistical analysis, it can be concluded that the compressive strength of mortar cubes prepared at a concentration of 10^5 ACRN 1 bacterial cells per ml of water was significantly higher (18.57% increase) at 28 days of curing than other treatments (Tables 2 & 3, Fig. 2 & 3).

Table 2. Multiple Range Tests of Compressive Strength for Concentration of Bacteria (Method: 95.0 percentage LSD) (ACRN 1)

Concentration of Bacteria	*Cases*	*Mean LS*	*Sigma LS*	*Homogeneous Groups*
8	15	157.733	4.71073	X
4	15	181.2	4.71073	X
0	15	182.667	4.71073	X
6	15	189.067	4.71073	X
7	15	189.387	4.71073	X
5	15	204.267	4.71073	X

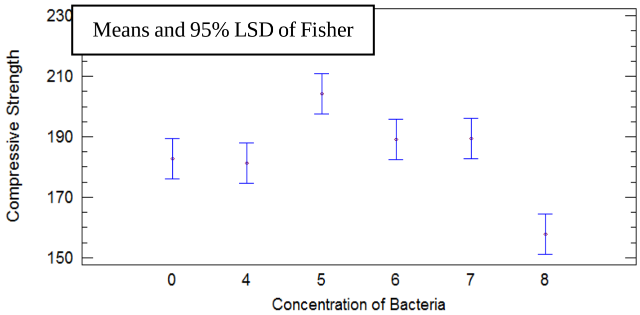

Fig.2. Compressive Strength vs. Concentration of Bacteria ACRN 1

Table 3. Multiple Range Tests of Compressive Strength for Curing Period (Method: 95.0 percentage LSD (ACRN 1))

Curing Period	*Cases*	*Mean LS*	*Sigma LS*	*Homogeneous Groups*
0	18	0.0	4.30029	X
7	18	188.9	4.30029	X
14	18	233.056	4.30029	X
21	18	236.0	4.30029	X
28	18	262.311	4.30029	X

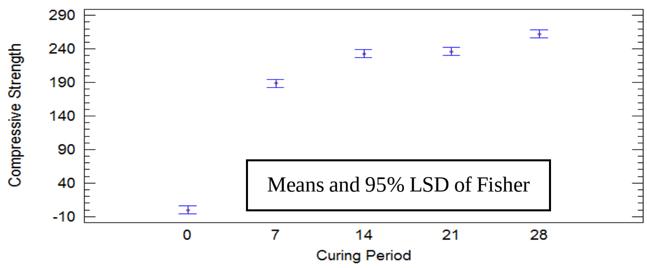

Fig.3. Compressive Strength vs. Curing Period (ACRN 1)

B. Discussion

The main objective of this research was to study the effect of addition of the ureolytic bacterial strain isolated from the soils of *Comarca Lagunera* on the compressive strength of cement mortar cubes.

Comparative analyses of the compressive strength of mortar cubes prepared with ACRN 1 at its optimum concentration in relation to mortar cubes prepared with other bacterial strains reported in the literature is presented in Table 4. It can be observed that the performance of ACRN 1 strain was similar to those reported earlier. Recently, the benefits of addition of bacteria along with other substitutes such as flyash [8] and to cement concrete [9] has been reported.

The increase in compressive strength could be attributed to the precipitation of calcium carbonate particles due to the urease activity of the strain.

Table 4. Increase in compressive strength of different bacterial strains with the control ones

Type of Bacteria	Optimum Concentration of bacteria per ml of water	Increase in Compressive Strength of the Cement Mortar cubes with the control ones (%)		
		7 days	14 days	28 days
ACRN 1	(1 x 10^5)	15.13	22.81	18.57
PU encapsulated *B. pasteurii* [1]	(5 x 10^9)	12	-	2.67
Shewanella sp. [10]	(1 x 10^5)	16.67	21.87	25.29
Bacterium BKH1 [4]	(1 x 10^5)	-	-	25.23
Bacillus subtilis [17]	(1 x 10^5)	13.47	-	16.15

IV. Conclusions

The addition of bacterial strain ACRN 1 showed an increase in the compressive strength of the mortar cubes. The results of the compressive strengths of the mortar cubes prepared adding the ACRN 1 at different concentrations of 10^4, 10^5, 10^6, 10^7 y 10^8 were compared and found that the concentration of 10^5 cells per ml of water is the optimum concentration and the observed compressive strength at 28 days of curing was significantly higher than the control and other treatments.

From the results, it can be concluded that the ureolytic bacteria can play a key role in microbiologically – induced calcite precipitation and to develop eco-sustainable approach for self-healing of concrete.

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