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Improvement of Strength of Locally Available Soils using Microbial Induced Calcite Precipitation

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Abstract: Engineering properties of soils are improved by adopting various proven methods such as mechanical and chemical. Strength of locally available soil (Black cotton soil and red soil) was enhanced by application of Microbial Induced Calcite Precipitation (MICP) using species of Bacillus pasteurii. Microbial culture improves the unconfined compressive strength and shear strength of locally available soil. Microbial culture developed from Bacillus pasteurii, was used to stimulate and catalyze the process of calcite precipitation triggered by urea hydrolysis which includes reaction between urea and calcium chloride. This paper includes outcomes of effectiveness of MICP on locally available soil, on three parameters measure of the cementation reagent, measure of Bacillus pasteurii and duration of treatment process. The results elaborated that with the application of MICP, unconfined compressive strength of black cotton soil increased 1.6 to 2.3 times and red soil from 1.8 to 3 times. This gives optimum quantity of microbes and concentration of Cementation reagent as additive to improve strength of black cotton soil and red soil.

Index Terms: Calcite Precipitation, Bacillus pasteurii, unconfined compressive strength, Black cotton soil, Red soil

I. INTRODUCTION

For civil construction on a particular site, geotechnical engineers are always concern about ability of soil to resist load. Weak soil which lacks adequate stiffness and strength to resist upcoming load of superstructures, needs soil improvement. Engineering properties such as compressive strength, permeability, erosion, compressibility etc are improved by using conventional soil stabilization technique such as mechanical or chemical aids. A study on use of Geo-polymer as a one of the chemical method to improve compressive strength of soil was carried out[1].Neuru Fuzzy Inference System-ANFIS model based study was carried out to evaluate effect of geopolymerization on unconfined compressive strength of soil [2].

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Bioremediation as another soil improvement technique. Microbiological degradation of contaminants present in soil, play vital role in remediation [3]. Importance of unconfined compressive strength of soil-cement specimens and % weight loss due to freeze and thaw durability test defined [4]. MICP is a recent, eco-friendly and sustainable technology, which involve uses micro-organisms along with chemicals and torestore soil properties.

Effect of MICP to get decrease in permeability, compressibility, volumetric response and increase in compressive, shear strength and stiffness is possible [5, 6]. Present study is to ascertain various parameters and its possible implication of MICP on locally available fine grained soils. Previous study shows that bio-mineralization is the process in which chemical alteration of environmental take place due to existence of microbial activity which leads to precipitation of CaCO3 [7,8].The success of MICP on sandy soil was studied [9,10]. Formation of calcium carbonate due to presence of microbial cells and their bio-chemical activities was noticed [11]. Mechanism of calcium carbonate precipitation by photosynthesis [12, 13], by urea hydrolysis [7, 11, 14], by sulfate reduction [15, 16] was studied. Amongst all above methods; urea hydrolysis is the common and most widely used method of calcium carbonate precipitation [5, 11, 17]. The rate of CaCO3 precipitation depends on cell growth and is higher than chemical precipitation [7]. Amount of CaCO3 precipitation depends on various factor viz type of bacteria and its cell concentration, pH, temperature, duration of treatment etc [18]. It is observed that urea activity is essential to occur microbiologically-induced calcite precipitation [19]. Microorganisms/bacteria are of size 0.5μm-3μm and have capability to change mechanical properties of soil and accelerate geo-chemical reactions. However inherent pore size of soil and size of bacteria limits post sedimentation/precipitation of clayey soil having more than 12% clays [20].This laboratory based experiment will confirm feasibility of application of MICP on locally available soils in Maharashtra, India. In this study variable parameter to cause carbonate precipitation includes concentration of bacterial cells (colony forming unit, cfu/ml), concentration of cementing reagent and duration of treatment. Conclusions were drawn based on experimental results obtained.

. 1.1. MECHANISM OF MICP

Now a day's, MICP is emerging field of soil stabilization and has potential in various geotechnical application because of its clean, sustainable and economical solution.

Use of MICP has application in the fields like improvement of soil strength, oil recovery, bio-clogging, slope stabilization, sand impermeability, improvement of strength and durability of concrete, cement mortar, bricks, remediation and preservation of historical monuments and sculpture.Effect of MICP on sandy and residual soil for shear strength and reducing hydraulic conductivity was studied [10]. The results reveal that there is effective increase in shear strength (1.41to 2.64 times).Under optimum conditions for improving engineering properties of residual soil using MICP [21, 22], 69.1% and 90.4% improvements were noted for un-drained shear strength and hydraulic conductivity respectively. As compared to aerobic oxidation, de-nitrification, sulphate reduction etc methods of MICP, urea hydrolysis possesses has the highest calcite conversion rate as studied by [23], over other methods studied by [24, 25].

Urea hydrolysis is the mechanism of chemical reaction where decomposition of urea- $CO(NH_2)_2$ is done by Urease enzyme or by microorganisms. Urease enzyme could be added externally [26, 27], or Urea hydrolysis byurease positive type bacteria, i.e. genera *Bacillus*, *Sporosarcina* [9, 25, 28]. Effectiveness of MICP depends on availability of nucleation sites in soil matrix, appropriate PH of soil, bacterial concentration and its ionic strength, and availability of air space and nutrients.

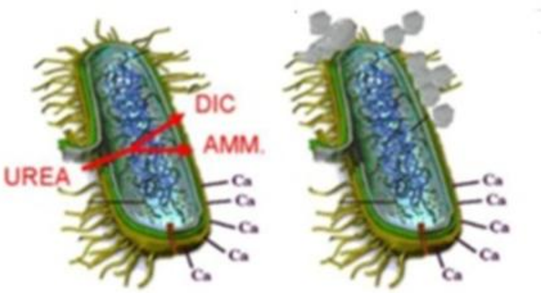
During urea hydrolysis, 1 mole of urea is hydrolyzed (decomposed) into 2 moles of ammonium in the presence of urease positive type bacteria as per following reaction

$$CO(NH_2)_2 + 2H_2O \longrightarrow 2NH4^{+} + CO_3^{2-}$$

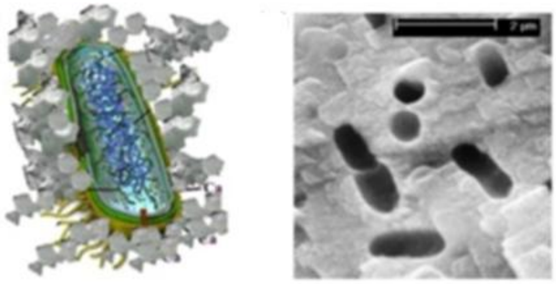
Increase in pH of soil matrix is due to ammonium ($NH4^{+}$) which is released in the process of urea hydrolysis. This Increase in pHtriggers the precipitation of calcium carbonate (calcite). The large number of negative ions present on the wall of microorganisms helps adsorption of calcium ions. These calcium ions react with carbonate ions ($CO3^{2-}$) from the urea hydrolysis leading to formation of calcite.

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3$$

The sticky/gelatinous nature of CaCO3 precipitates helps bonding between the soil particle and thereby responsible for improving the engineering properties of soil. The process of calcite precipitation by gram positive type bacteria is explained in sequences by Fig.1 [29].



a)calcium ion attracted to cell wall b) calcite precipitated near cell wall



c) Calcite increased in quantity d) imprint of cell that takes and encapsulate cell part in calcite precipitation (De Muynck et al. 2010a).

Figure 1. The process of calcite precipitation

II. MATERIALS AND METHODS

For this study, the materials used were locally available soil samples and Microorganisms Bacillus *(Sporosarcinapasteurii)* (NCIM-2477) obtained from National Chemical Laboratory (NCL), Pune.Nutrient broth was used for growth of the culture. Cementation reagent was made from Urea, Calcium Chloride.

2. 1. SOIL SAMPLES

In India, black cotton soil and red soil are available in 20-25% each. Black cotton soil is predominant in states of Maharashtra, Karnataka, Madhya Pradesh Andhra Pradesh, while red soil in konkan region of Maharashtra covering Deccan Trap basalt rock. Both soils are fertile and useful to farmers; on the contrary it's unfavorable for most of the civil engineering construction. Location of sample collection from site is as mentioned in Table 1.

TABLE 1. Location of soil Sample Collection

Sample	Location	Latitude	Departure
Black Cotton soil	Askarwadi, Pune-Bopdev-Saswad Road Tq. SaswadDist Pune	18.394	73.915
Red soil	Waravatne,Maihsala-Khargaon- Divyaagar road, DistRaigad	18.141	73.085

Usually to make the construction economic, it is a practice to use locally available materials.Withthis objective, how to make use of such abundance locally available quantity of material for civil engineering construction which is challengeable. The widely spread location and types of soil in the state of Maharashtra was the basis of selection of sample. Table 2 shows, engineering properties of soil sample under consideration

TABLE 2. Basic Properties of Virgin Soil Sample

Test	**Symbol**	**Black cotton soil**	**Red soil**
Gravel	G	0.12	0.24
Sand	S	28	32
Silt		39	30

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clay		32	38
Soil Classification		Clayee (CH)	MH Well graded
Optimum moisture content	OMC	31%	25%
Maximum dry density	$\gamma_{d\ max}$	1.33 gm/cc	1.62 gm/cc
Dry unit weight		13.048 KN/m^3	16.05 KN/m^3
Liquid limit	W_L	76.62%	56.61%
Plastic limit	W_P	36.02%	39.57%
Shrinkage limit		11.61%	9.31%
Unconfined compressive strength	q_u	59.63Kpa	83.72Kp

Fig. 2 shows test result of grain size distribution of soil sample collected. Figure.3 shows experimental setup for unconfined compressive test of virgin soil samples. The samples were tested in triplicate.
The effect of MICP treatment with variable parameters is assessed by comparing the unconfined compressive strength soils before and after treatment.

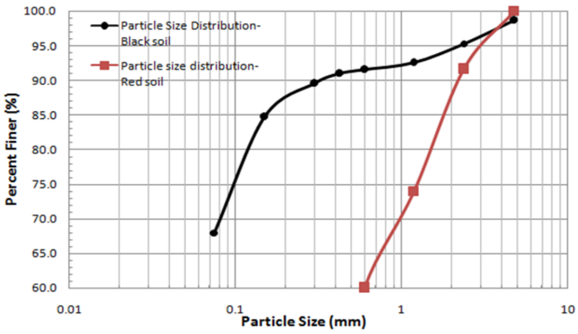


Figure 2. Grain Size Distribution

Figure 3. Experimental Set-up

2.2. MICRO-ORGANISM AND CEMENTATION REAGENTS

Calcite Precipitation was carried out with the helpSporosarcinapasteurii. Prepared solutions of cementation reagent and microbes were mixed directly with the soil in this experimental work; Microorganisms Bacillus (*Sporosarcinapasteurii*) (NCIM-2477) was obtained from National Chemical Laboratory (NCL), Pune, Maharashtra, India. Pure bacterial culture was isolated. Nutrient broth was used for growth of the culture. Concentration of Bacillus pasteurii was varied as $1x10^5$, $1x10^6$, $1x10^7$cfu/ml [10] as one of the parameter. Cementation reagent as next governing parameter was made from Urea, Calcium Chloride, and Nutrient broth. It is important ingredient for enhancing precipitation. From the studies of [10, 30], the optimum amount of nutrient broth 3 gm/lit, is required to be added in to the solution for optimum growth of bacteria. For MICP process, different calcium sources such as calcium nitrate, calcium oxide, calcium chloride, calcium acetate can be used. Calcite precipitation was obtained using sword bean extract as a urea catalyst and two calcium source namely calcium chloride and calcium hydroxide in place of micr-organisms like *Sporosarcinapasteurii* [31]. Out of this calcium chloride is the better calcium source as it yields higher urease activity and more calcite production [32].Equal moles of Urea and calcium chloride (Ca^{2+}+ $CO_3^{2-}\rightarrow$ $CaCO_3$) were used to prepare cementation reagent solution. This solution is prepared in different concentration as 0.25 M, 0.5 M, 0.75 M and 1.0 M.

2. 3. MIXING AND CURING

To start with, bacteria were added directly to the soil and it is ensured that they are mixed properly, followed by addition of cementation reagent. As per the study by[10], the addition of amount of bacteria and cementation reagent was decided. Properfixation and distribution of bacteria in soil was ensured [30]. The soil samples were compacted so as to get 90% maximum dry density.UCS.test was carried out as per IS 2720 (Part 10) 1991.To evaluate effect of treatment duration/curing, particular soil samples of given bacterial and molar concentrations were allowed for curing for the period of 1, 3 and 7 days. During the curing period chemical reacts and precipitation of CaCO3 take place. Temperature of 20^0C to 28^0C was maintained by way of moist gunny bags [27].

III. RESULTS AND DISCUSSION

The Unconfined Compressive test result for virgin Black cotton soil was 59.65 kPa and that for Red soil was 83.75 kPa. After treatment with MICP, and allowing for curing, re-tests were conducted on both soils. Tabular presentation of test result is as shown in Table 3.Results imply thatUCS values do have effect of treatment duration. About 50-60% increase in strength is achieved in a first 24 hrs followed by 20-30 % in next 72 hrs. At the last, 10-15 % in next 168 hrs.[22]. For Black cotton soil, proportion of 1 $\times10^7$cfu/mlbacterial concentration and0.5M molar concentration of cementation reagent yields highest increment. Similar observations as that of black cotton soil were noticed in case of Red soil, for the combination for of 1 $\times10^6$cfu/ml bacterial concentration and 0.5M of cementation reagent.
Usually the size of microbes ranges from 0.5µm -3µm [30]. Distribution and travel of microbes depends on pore size of soil composites. Both the soil, offered considerable percentages of the microbial movement; however UCS values of Red soil shows relatively higher increment ratio. This can be attributed to coarser to finer particle size of red soil which provided a closely packed soil particles and offers extra particle-to-particle contact, more specific surface area for the calcite precipitation. These parameters improve cohesion of soil.

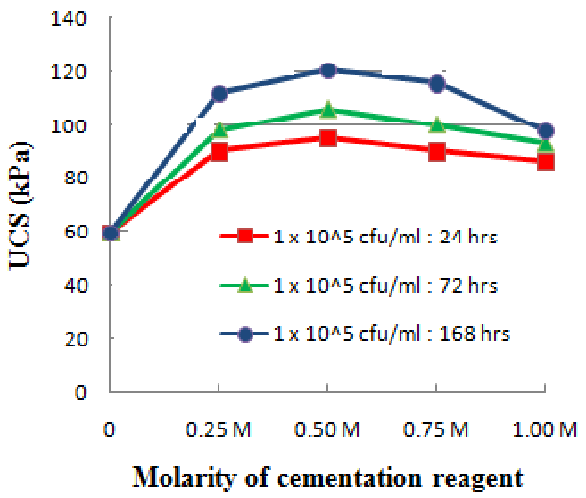
The increase in cohesion ultimately enhances shear strength and compressive strength of soil.

TABLE 3.UCs Test result after MICP treatment for Black cotton and Red soil

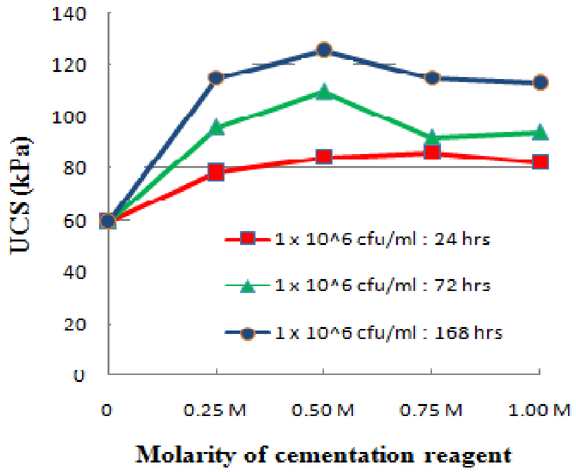
Cementation Reagent	Virgin Value of UCS (kPa): Black Cotton Soil 59.65 kPa, and for Red Soil 83.75 kPa					
	24hr curing period		72 hr curing period		168 hr curing period	
	B.C Soil	Red Soil	B.C Soil	Red Soil	B.C Soil	Red Soil
Count of *BacillusPastuerii1* 1x10⁵cfu/ml						
0.25 M	90.2	125	98.1	129.4	111	176.5
0.50 M	95.1	131	105	168.7	120	229.5
0.75 M	90.2	124	100	159.8	115	221.6
1.00 M	86.3	120	93.1	153	98.1	206.9
Count of *BacillusPastuerii1*1 x10⁶ cfu/ml						
0.25 M	78.4	121	96.1	129.4	114	198.1
0.50 M	84.3	154	109	205.9	125	289.3
0.75 M	86.3	139	92.2	170.6	114	225.5
1.00 M	82.4	131	94.1	161.8	112	221.6
Count of *BacillusPastuerii1*1 x10⁷cfu/ml						
0.25 M	76.5	125	86.3	158.8	96.1	217.7
0.50 M	96.1	137	111	187.3	137	263.8
0.75 M	91.2	125	107	156.9	127	219.6
1.00 M	87.3	117	98.1	141.2	123	191.2

3. 1. GRAPHICAL REPRESENTATION OF UCS TEST RESULTS AFTER MICP TREATMENT

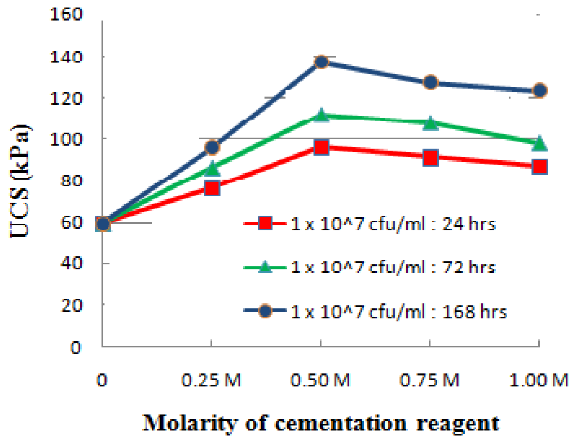
The graph in Figure.4 (a) to (c) shows comparative results of UCS test with varying concentration of cementation reagent, after MICP treatment on Black cotton soil.



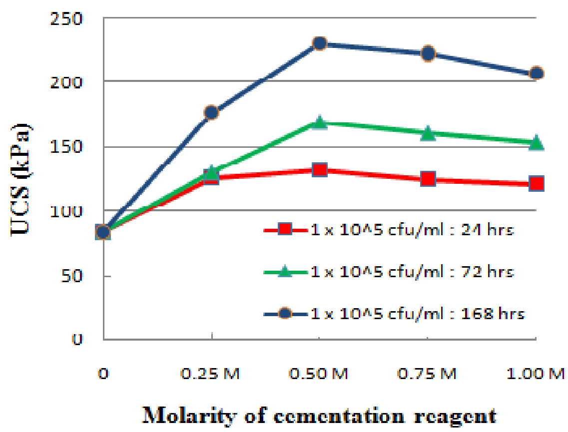
a)1 x 10^5cfu/ml



b) 1 x 10^6cfu/ml



c)1 x 10^7cfu/ml for Black Cotton soil



d) 1 x 10^5cfu/ml

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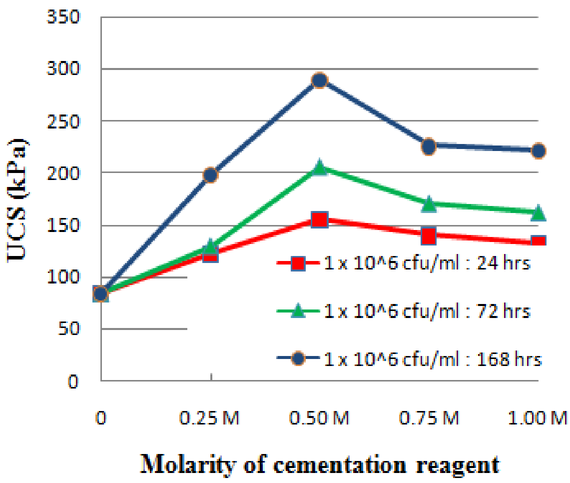
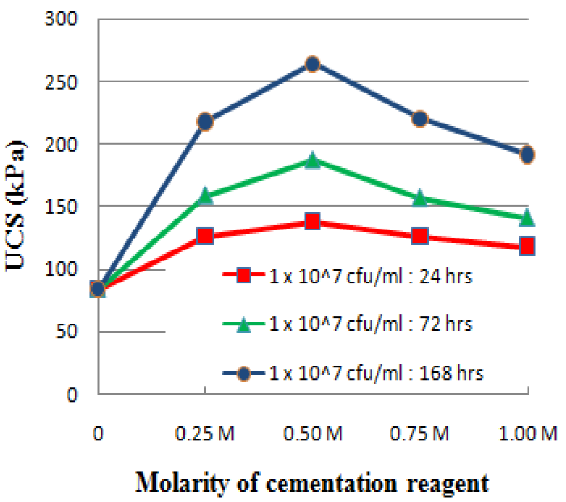


e) 1 x 10^6cfu/ml



(f) 1 x 10^7cfu/ml for Red soil

Figure 4 (a to f) Comparative result of UCs test after MICP treatment

Also graph in Figure.4. (d) to (f) shows comparative results of Red soil. It is inferred from Figure 5, that MICP process has improved unconfined compressive strength to the extent of 1.6 to 2.3 times for black cotton soil and 1.8 to 3 times for Red soil with the 7 days of duration of treatment.

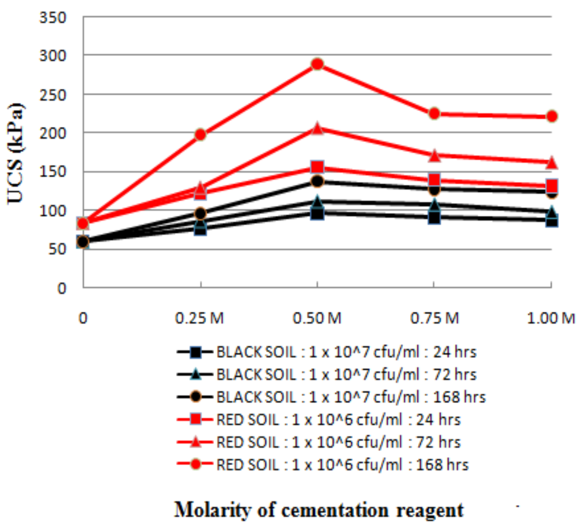


Figure 5. UCs comparison: Black cotton Vs. Red soil

For Black cotton soil, with bacterial concentration of 1 × 10^7cfu/ml and 0.5 M molarity of cementation reagent has given increase in UCS strength from 59.65 kPa to263.80 kPa after 7 days curing. For Red soil, with bacterial concentration of 1 × 10^6cfu/ml and 0.5 M morality of cementation reagent has given increase in UCS strength from 83.75 kPa to 289.30 kPa after 7 days curing. Injection of BacillusPasteuri enhanced urease enzyme which triggered more calcite precipitation and thereby enhancement in UCS ultimately reflects in increased shear strength. The results shows that the UCS of red soil with fine grain size comparatively improved due to the predominant role of inter-particle contacts very well as supported by [25].From Table 3 it is observed that effectiveness of MICP, slightly better performance is exhibited by Red soil as compared to black cotton soil. It can be noted that, attribution towards increase in UCs strength for both soils is, due to Calcite precipitation in presence of Urease positive bacteria which is responsible for reducing voids and cementing of soil particles. Also % variation in achieving incremental strength of soil depends on treatment period.

IV. CONCLUSIONS

In general, MICP, a biological process was found suitable for both soil sample to increase the unconfined compressive strength and there by shear strength. Black cotton soil (1.6 to 2.3 times) in comparison with Red soil (1.8 to 3 times) has small Effect on UCS. For Black cotton soil and Red soil Bacterial concentration of 1 × 10^7cfu/ml and 1 × 10^6cfu/mlrespectively have shown commendable results. It was also found that morality of cementation liquid and curing duration are the influencing factor for increasing the strength. It was observed that the microbial movement for both the soils exist, however red soil has shown the higher influence, because of dense arrangement of particles and higher particle to particle interface in red soil which was on lower side for black cotton soil. This shows relatively more specific surface area for bond and calcite formation was available in red soil as compared to black cotton soil.

Author is of opinion, it is fact that, physical and chemical variation in properties of soil occurs across the globe, Therefor it is desired to check applicability of MICP to the locally available soils in Maharashtra, India.This approach of MICP will reduce cost of civil construction/ engineering project, is new area of research.

.***Abbreviations and Acronyms***

MICP- Microbial Induced Calcite Precipitation

G- Gravel

OMC- Optimum moisture content

γd max Maximum dry density

S- Sand

W_L Liquid limit

q_u Unconfined Compressive Strength(UCS)

KPa Kilo Pascal

Cfu/ml colony-forming units per milliliter

W_PPlastic limit

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