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Bio-Cementation of Fat Clay Using MICP

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Abstract. The current study emphasizes on analysing the efficacy of Microbially Induced Calcite Precipitation (MICP) which uses the bacterial processes in enhancing the cohesive soil properties. In this study urea hydrolysis was catalyzed by urease enzyme secreted from the Sporosarcina Pasteurii. The soil used in the present study was sourced from Pinnapuram Pumped Storage Project, Kurnool, Andhra Pradesh and was classified as high compressible clay (CH). The effect of urea hydrolysis on the unconfined compressive strength (UCS) variation and its particle size gradation have been studied. The studied parameters include curing period and concentration of urease solution. The UCS value of the soil improved significantly (1.5-2.5 times) following MICP, which increased further with increase in the curing period. The rate of gain in strength is attributed to the formation of $CaCO_3$ precipitate. The particle size distribution upon MICP treatment showed an increase in silt sized particles and a decrease in clay sized particles. The change in gradation is due to the flocculation of particles due to MICP treatment. A successful accomplishment of this MICP technique implies that an innovative and sustainable technique can be developed to effectively stabilize highly plastic clays.

Keywords: Clay; Microbial Induced Calcite Precipitation; Sporosarcina Pasteurii; Unconfined Compressive Strength; Particle Size Gradation.

1 Introduction

Numerous ground improvement techniques have been developed to improve the poor soils and to make them suitable for construction. Mechanical, hydraulic, admixture stabilization, soil reinforcement and confinement methods were broadly introduced to take up the ground improvement. Although these techniques are usually quite effective, they are uneconomical besides high variability in suitability, availability, and constructability which hinders the adoption of these methods on large scale stabilization works. Hence bio-stabilization techniques that have developed in recent years provide a sustainable solution to the chemical grouts that are harmful to the environment. (Mitchell and Santamarina, 2005[21]; Ivanov and Chu, 2008[19]; DeJong et al., 2006[11], 2010[12], 2013[13]; Harkes et al., 2010[14]; Van Paassen 2007[27]). This technique is used to enhance the soil strength and stiffness by utiliz-

ing the by-products that are formed due to metabolism of microorganisms. Bio-stabilization has its potential to modify the physical and mechanical properties of geo-materials through precipitation by using natural and environmental friendly additives to make the unstable and weak soils suitable for construction (Ramakrishnan et al., 2005[24]; Bhaskar et al. 2016, 2017, 2018, 2019[3-6]) and also to alleviate the liquefaction hazards (Burbank et al., 2011[7]). Animesh et al., (2016) [2] studied the impact of MICP on two different plasticity values (CI and CH) soil. The results of the study have proven that with the use of MICP, there was a significant improvement of about 1.5—2.9 times in the unconfined compressive strength in both the soils. The authors found that the effect of MICP on the UCS of CH soil (1.50—2.91) was more when compared to that in CI soil (1.45—2.26).

1.1 Bio-cementation

Microbially Induced Calcite Precipitation (MICP) can be accomplished through many techniques such as De-nitrification, Iron Reduction, Sulfate Reduction, Urea Hydrolysis etc. Among these techniques, calcite precipitation (MICP) formed through Urea hydrolysis is considered highly efficient. The urea hydrolysis which is a very slow process will be catalyzed by urease enzyme secreted by the bacterium. The basic mechanism of MICP through urea hydrolysis involves the decomposition of urea into NH_3 and CO_2 within the cell of bacteria. [Eq. 1]

$$NH_2\text{-}CO\text{-}NH_2 + H_2O \rightarrow 2NH_3 + CO_2 \quad (1)$$

NH_3 reacts with water and converted into NH_4^+ ion carbon dioxide quickly decomposes in the presence of water into bicarbonate ions (HCO_3^-) and further forms carbonate ions (CO_3^{2-}) when it reacts with hydroxyl ions. [Eqs. 2, 3 & 4].

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (2)$$

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+ \quad (3)$$

$$HCO_3^- + H^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2H_2O \quad (4)$$

The carbonate ions, in the presence of supplied calcium source, precipitates calcium carbonate [Eq. 5] which binds the soil particles and alters the engineering properties of soil.

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \downarrow \quad (5)$$

The calcite once formed, will dissolve very slowly at a geological time scale. Hence when sufficient quantity of calcite is precipitated, a durable stabilisation technique is achieved (Van Paassen et al., 2007 & 2009[26, 27]). MICP technique proves to be very effective in granular soils. To date, very little work was undergone on fine grained soils. On the other hand, Enzyme Induced Calcite Precipitation (EICP) has been successfully used to immobolize heavy metal ions on soils exhibiting different plasticity values (Moghal et al. [22, 23]). Enzyme Induced Calcite Precipitation (EICP) has also been successfully used to improve the Shear Strength of different

type of Soils (Chandra A and Ravi K [9, 10]). MICP was not earlier applied to clayey soils owing to distribution of micropores in these soils upon compaction. Latest studies by Bhaskar et al. [3-6] have revealed that, even fat clays when compacted to their respective OMC and MDD values have sufficient micropores in them which will allow the bacteria to grow and precipate calcite. In the current study, an attempt is made to stabilize high compressible clay (black cotton soil) through urea hydrolysis by utilizing the Ureolytic bacterium (sporosarcina pasteurii).The objective of this research is to develop an innovative ground improvement alternative that uses the MICP technique for shallow depths.

2 Materials and Methods

2.1 Soil

The soil used for the current study was collected from 2.5m depth from a location at Pinnapuram, Kurnool, India. Experiments were conducted on the collected soil, to identify the basic index properties according to IS: 2720 (Part-5) [17]. The properties obtained are mentioned in table 1 and the particle size distribution curve is shown in figure 7 as per IS: 2720 (Part-4) [16]. The soil was classified as Highly Compressible (CH) clay. Highly plastic clays are also termed "fat clays". Accordingly, in the present study the term has been used.

Table 1. Geotechnical properties of studied soil

Soil Property	Value
Specific gravity (G)	2.27
% Gravel	0
% Sand	10
% Silt	24
% Clay	66
Liquid limit (LL)	64
Plastic limit (PL)	22
Plasticity index (PI)	42
Soil classification	CH
Maximum Dry Density (g/cc)	1.8
Optimum Moisture Content (%)	18
Cohesion (C) kg/cm^2	0.24
Angle of internal friction (Φ^0)	14.2
UCS(kg/cm^2)	1.73

2.2 Bacterial culture and ambient conditions

The Sporosarcina Pasteurii bacteria usually exists in most of the natural soils. In this study, rather than extracting it from the soil and culturing the cells, the freeze-dried cells were procured from Microbial Type Culture Collection (MTCC). The microbe is

gram-positive i.e., its properties will not be affected by the pressure and temperature and it is rod shaped and size ranges between 0.5-3 μm in length (Bhaduri (2016) [8]). The optimum conditions for the growth of bacterial cells are 37^0c, proper nutrient media and aerobic environment. The nutrient media used in this study was Nutrient Broth (NB) media rich in NaCl, peptone and beef/meat extract.

2.3 Bacterial cell solution

NB-Urea solution was mixed into the soil sample after inoculation with the bacterial cells. The solution comprised of 25g of Luria – Bertani (LB) media , 20g of urea, 2.12g of sodium bicarbonate and 10g of ammonium chloride per L(litter) of double-distilled (DD) water (Al-Qabany et al., 2011[1]). The solution is adjusted for its pH value to 8.5 using 4M HCl before autoclaving.

In order to prepare the bacterial cell solution, a seed culture was prepared by inoculation of one colony (taken from Petri plate) or about 20μl into 5mLmedia and allowed to grow for about 8 – 10 hours in the shaker incubator at a prescribed temperature. The inoculum was transferred into the main culture at a dilution ratio of 1mL for every 100mL main culture and the cells were allowed to grow for about 32-34 hours at the same temperature in shaker incubator by the end of which the cells reached an equilibrium growth. The main culture is then allowed to cool down and stored at 4^{o}C before its use. The cells were harvested at a temperature of 4^{o}C in a centrifuge operated at 7000rpm for about ten minutes. The harvested cells were then washed twice with the 0.1M Sodium Phosphate Buffer solution (pH = 7) to clear away the metabolic waste that was produced during the growth of the bacteria which otherwise would have retarded the further growth of cells. The centrifuged pellet was then re-suspended in a small quantity of bacterial cell solution autoclaved earlier and was transferred into the actual bacterial cell solution (Fig. 1).

Fig. 1. Bacterial cell solution

2.4 Cementation solution

Cementation solution is the solution with a calcium source ($CaCl_2$). The solution consists of equimolar Urea-$CaCl_2$. For the survival of microorganisms, diluted nutrient media was also added to the cementation solution. Cementation solution was prepared freshly before mixing into the soil, to avoid precipitation. In this study the concentration of solution was varied as 0.25M, 0.5M and 1M.

2.5 Sample preparation

In the present study soil samples are prepared by mixing the soil and bacterial cell solution (OD_{600} of about 1.5) followed by cementation solution (0.25 M, 0.5 M, 1 M). The quantity of bacterial cell solution and cementation solution was taken as equal to one pore volume to ensure the maximum calcite precipitation (Inagaki et al., 2011[15]). All the samples are prepared at constant density (maximum dry density) to make sure that change in strength is only due to MICP but not density.

2.6 Curing

Curing is one of the important factors affecting the strength of bio-stabilised soil as it accounts for 45.97% increase in strength (Sotoudehfar et al., 2016[25]). In this study, wet burlap curing method was adopted the samples were cured for 3, 7, 14 and 28 days respectively. Cementation solution was sprayed at regular intervals for maintaining ambient moisture at 25^0C-35^0C.

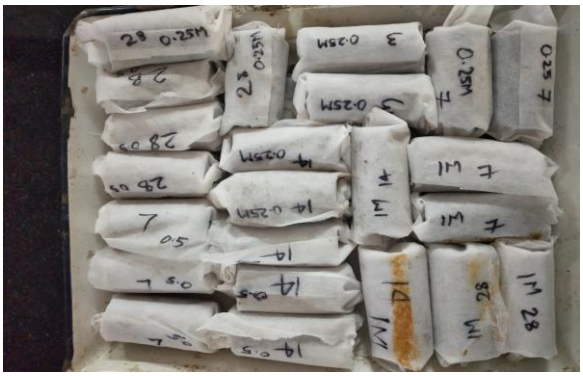

Fig. 2. Curing of specimens

3 Results and Discussions

Unconfined compressive strength (UCS)

The UCS values of the samples at respective maximum dry density (MDD) and optimum moisture content (OMC) values at varying curing periods (3 days, 7days, 14 days and 28 days) and varying cementation solution concentrations (0.25, 0.5 and 1M) are determined by plotting stress-strain curves [Fig 3, 4, 5 & 6] as per IS: 2720 (Part-10)[18]. From these stress-strain curves, the peak stress values are identified and the corresponding UCS values are obtained and provided in Table 2. The strength

increases with increase in the concentration from 0.25M to 0.5M. However, the strength decreases when the concentration increases from 0.5M to 1M as the water soluble components are increased which largely disable the activity of bacterial cells. Similar observations have been reported by Inagaki et al., (2011) [15]. Hence the optimum concentration of cementation solution is considered as 0.5M.

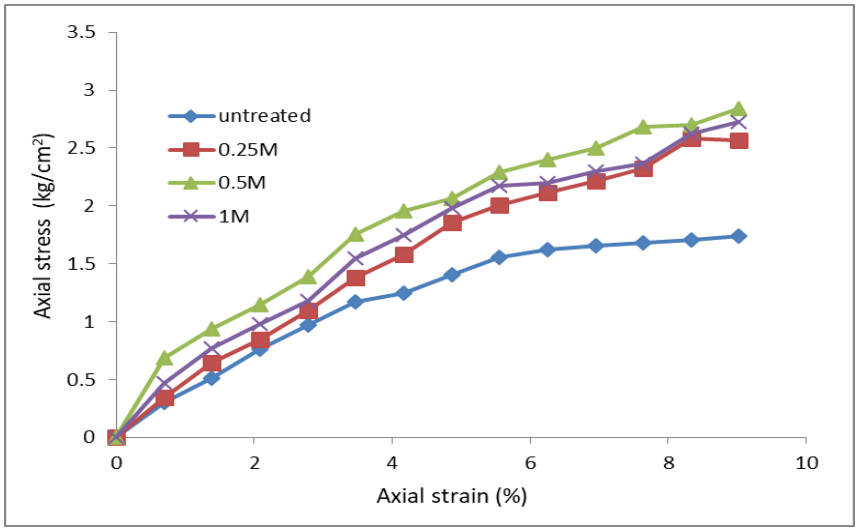

Fig. 3. Stress- Strain plot of samples tested after 3 days of curing

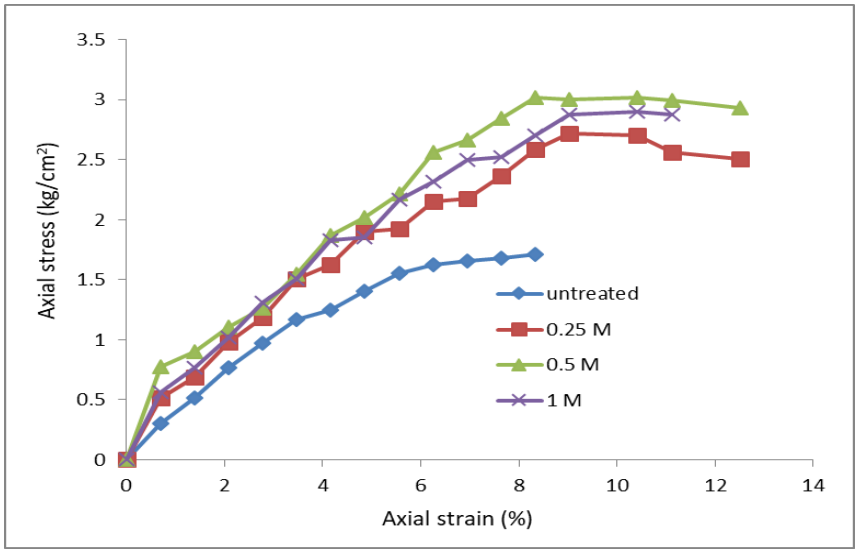

Fig. 4. Stress- Strain plot of samples tested after 7 days of curing

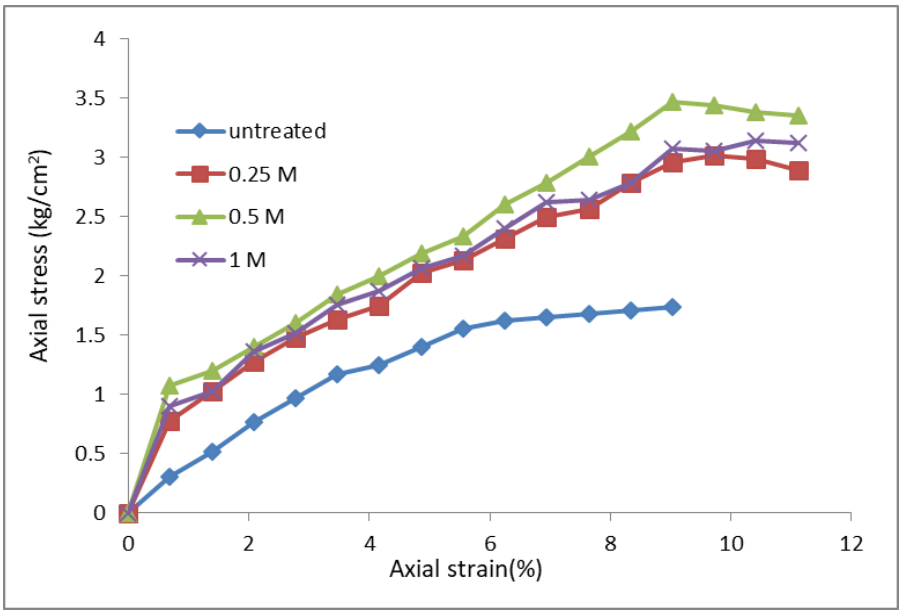

Fig.5. Stress- Strain plot of samples tested after 14 days of curing

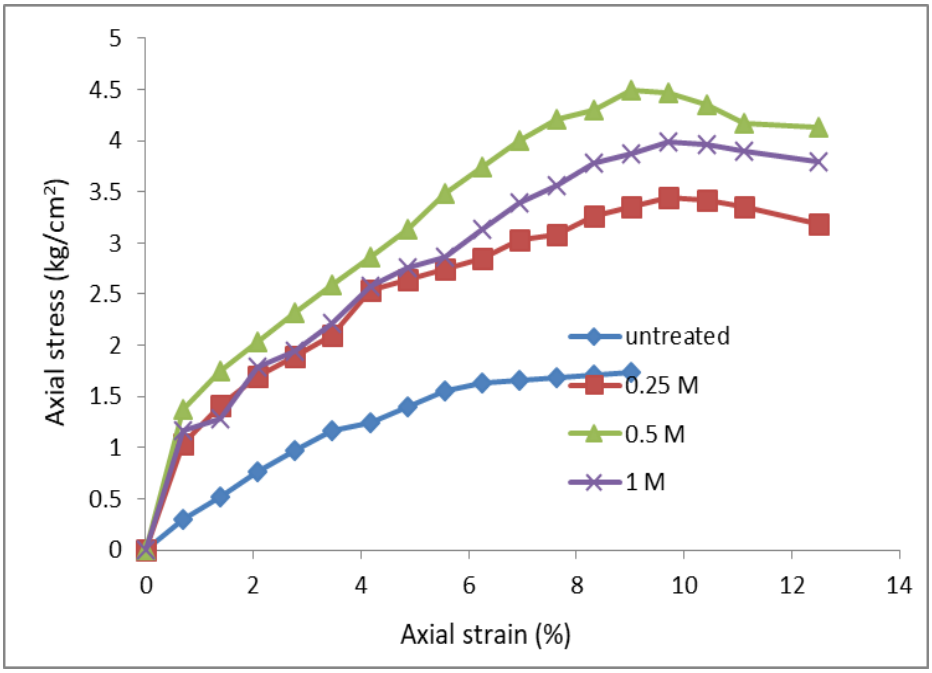

Fig.6. Stress- Strain plot of samples tested after 28 days of curing

Table 2. UCS values of treated specimens

Curing period (Days)	3			7			14			28		
Concentration of solution (M)	0.25	0.5	1	0.25	0.5	1	0.25	0.5	1	0.25	0.5	1
UCS value (kg/cm^2=0.1MPa)	2.58	2.84	2.72	2.72	3.01	2.89	3.01	3.47	3.14	3.44	4.49	3.99

Grain size analysis

The particle size distribution of the sample treated with 0.5M cementation solution after 28 days of curing period was carried out. Fig 7 shows the gradation curve of the soil before and after bio-treatment. It is evident that MICP affects the grain size distribution of the soil due to flocculation of particles. Similar observations have been reported by Yuze Wang et al., (2019) [28].

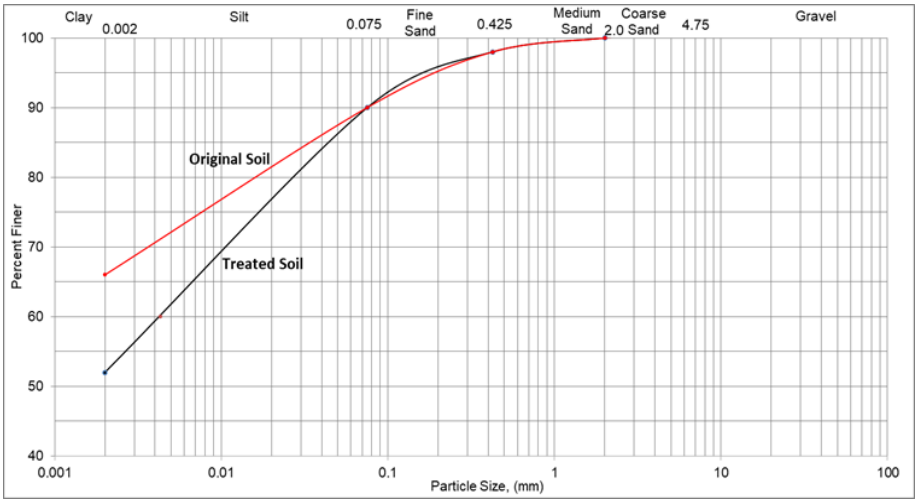

Fig. 7. Grain size distribution curve

Plasticity characteristics

The plasticity characteristics of sample treated with 0.5M cementation solution after 28 days of curing were tested. The results from Table 3 reveal that there is an increase in the liquid limit by about 6% and plastic limit increased by 41% upon treatment. This increase in Atterberg's limits is attributed to formation of an organic polymer called exopolysaccharides (Bhaskar et al. 2016[3]; Bhaskar et al., 2017[4]). However, this increase in Atterberg limits was found to have insignificant effect on swell and shrink behaviour of soil (Bhaskar et al., 2019[5]).

Differential Free Swell Index

DFSI test was conducted on the sample treated with 0.5M cementation solution after 28 days of curing and results are reported in Table 3. It is observed that, there is a significant reduction (almost 60%) in the DFSI values upon MICP treatment.

Table 3. Geotechnical properties of untreated and treated soil

Property of soil	Untreated sample	Treated sample
Liquid limit (%)	64	68
Plastic limit (%)	22	31
Plasticity index (%)	42	37
Differential Free Swell Index (%)	50	20

SEM analysis

Scanning Electron Microscopy (SEM) has been used in this study to identify calcite mineral precipitation on the surface of the soil particles. The MICP treated soil is examined at various magnifications and the SEM images are shown for both bio-treated and the untreated soil in Figure 7. It is observed that the surface of the clay particles is enriched with calcite precipitates. This study ascertains the fact that, MICP can be effectively used to stabilize shallow depths with considerable degree of success. Similar observations have been reported by Bhaskar et al. (2019) [5].

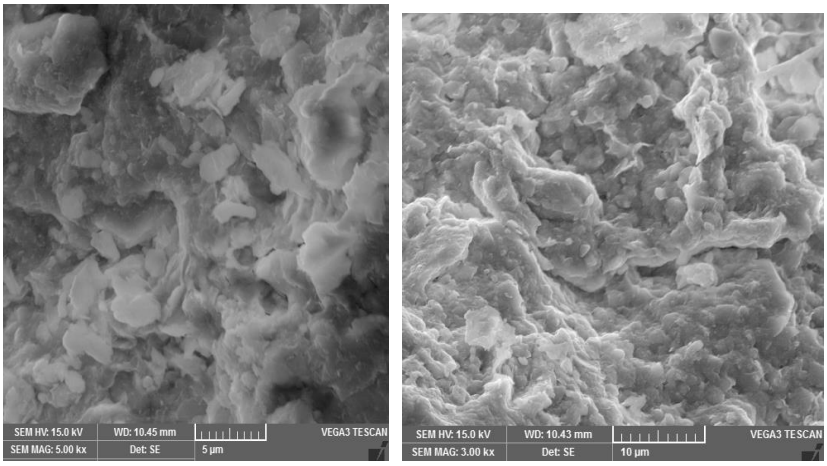

Fig. 8. SEM images a) Untreated b) Treated

4 Conclusions

This study aims at understanding the effect of MICP treatment on fat clay. Sporosarcina pasteurii bacteria was used to induce MICP and the following conclusions are drawn:

1. The UCS values of the soil increased significantly upon MICP treatment. The maximum strength improvement was achieved for 0.5M cementation solution at the end of 28 days curing period.
2. The grain size analysis confirmed that there is an 54% increase in the silt sized particles followed by 21% reduction in the clay sized particles due to flocculation of individual grains followed by MICP treatment.
3. Differential free swell index values decreased by 60% upon MICP treatment.
4. The microstructure analysis confirmed calcite precipitation responsible for the improvement in strength and stiffness of MICP treated soil.

The results of the study have revealed that MICP can significantly enhance the strength and other geotechnical properties of fat clay used in the study. This sustainable and innovative technique can replace existing conventional chemical stabilization techniques.

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