Application of Microbial-Induced Carbonate Precipitation for Soil Improvement via Ureolysis

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Abstract The challenges to develop or strengthen the weak soil always prompted the need for further research investigation to develop a new, eco-friendly, and sustainable method of ground improvement. The MICP (microbially induced carbonate precipitation) technique is one such method in which metabolic pathways of microorganism are utilized to form calcite precipitation inside the soil matrix leading to improve the engineering properties of soil. Ureolysis or urea hydrolysis is the most efficient process among all MICP methods of carbonate generating reaction, as it has the potential to produce large amount of calcite ($CaCO_3$) within a short period of time. This study aims to investigate the effectiveness of MICP technique on fine grained soil as clayey sandy silt or loam in improving its shear strength. In this study, three species of urease positive, alkaliphelic aerobic bacteria, namely *Sporosarcina pasteurii*, *Bacillus megatarium,* and *Morganella morgani* were used for ureolysis and microbially induced calcite precipitation. Quantitative analysis of calcite precipitation in the soil samples was done by Piper method. The target soil was mixed with each microorganism individually before it was compacted into the mould. In the experimental programm, four different treatment conditions were considered for each types of microorganism such as (1) untreated, (2) treated with cementation reagent (mixture of 0.5 M $CaCl_2$ and 0.5 M urea), (3) treated with bacteria only and (4) treated with both bacteria and cementation reagent. These experiments revealed that all these three types of microorganism can induce sufficient amount of calcite precipitation that can result in measurable improvement of the strength of soil.

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Calcite · Urease positive · Shear strength

1 Introduction

Due to rapid infrastructural demand for the ever-growing population in the country like India, development activities has to be carried out on the weak and problematic soil due to the shortage of competent land space. Grouting is one of the commonly used methods which are widely adopted for soil stabilization technique. Traditional grouting method for ground improvement employs particulate (cement/bentonite) or chemical grout. These methods are effective only near vicinity of grouting equipment and are not useful to treat large volumes of soil. On the other hand most of the chemical grouting methods are harmful to the environment. Synthetic chemical grouting techniques are even more toxic or hazardous except sodium silicate (Karol 2003). Hence, this technology does not provide effective and economical solutions considering issues of environmental pollution of air and underground water.

Therefore, this recent issues of environmental degradation has prompted the development of new, eco-friendly, and sustainable technology for ground improvement. Bio-mediated soil improvement technique is one of such new and innovative research field within geotechnical engineering which can be applied as an alternative approach of ground improvement technique taking care of the concerning of environmental issues. In this technique, calcium carbonate precipitation has been induced inside the soil matrix by microorganism through their metabolic process to improve the engineering properties of soil. Hence, this technique is also called as microbial induced carbonate precipitation or MICP.

Though the application of this technology in ground improvement is relatively young, many studies has been reported including DeJong et al. (2006), Whiffin et al. (2007), Harkes et al. (2010), Kim et al. (2012) etc. Dejong et al. (2006) found higher initial stiffness and shear capacity at failure for treated sand specimen than untreated loose specimen. Whiffin et al. (2007) have been successful to consolidate 5 m long sand column by applying MICP treatment. Harkes et al. (2010) investigated on the methodology to distribute and fix *Sporosarcina pasteurii* homogeneously in sand bed and found that two phase injection procedure (injection of bacteria followed by fixation fluid) can be applied to achieve homogeneous distribution of bacteria. Most of the researchers use *S. pasteurii* as microorganism and sand as soil material for MICP treatment. It is reported that the presently available method of MICP treatment is not favorable for fine grained soil due to small pore throat size. Therefore the aim of this study is to investigate the effectiveness of MICP technique by using different urease positive microorganism on fine grained soil as clayey sandy silt or loam in improving its undrained shear strength.

2 Theoretical Background

Several metabolic pathways of microorganism are identified in nature which can produce cementatious compound such as calcite. Among all, urea hydrolysis or ureolysis is the most efficient process through which large amount of calcite can be precipitated within a short period of time. In ureolysis, urea is decomposed via hydrolysis to ammonium and carbonate ions by urease enzyme produced by urease positive bacteria under aerobic condition. This mechanism yields higher pH along with high concentration of carbonate ions and create an environment favorable for the precipitation of calcium carbonate or calcite. Calcite is precipitated through the reaction between carbonate ions (CO_3^{2-}) from the urea hydrolysis and calcium ions (Ca^{2+}) from the supplied calcium chloride. Following equations describe the entire pathway of carbonate generation through urea hydrolysis (Castanier et al. 1999; Hammes and Verstraete 2002; Whiffin 2004).

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-} \quad (1)$$

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

3 Material and Methodology

3.1 Selection of Bacteria and Batch Cultivation

Considering the potentiality of calcite precipitation, three non-pathogenic species of urease positive, alkalifilic, aerobic bacteria have been chosen in this study. These are **(1) *Sporosarcina pasteurii (MTCC-1761)*, (2) *Bacillus megatarium (MTCC-428)* and (3) *Morganella morgani (MTCC-662)***. All the microbes were procured from Microbial Type Culture Collection Centre and Gene Bank housed at IMTECH, Chandigarh, Govt. of India.

All these three microorganism were cultivated in nutrient broth, growth medium no. 3(MTCC) [Composition: Beef extract 1.0 gm/L, Yeast extract 2.0 gm/L, Peptone 5.0 gm/L, NaCl 5.0 gm/L, Agar 15.0 gm/L]. Incubation temperature for *S. pasteurii (MTCC-1761)* and *B. megatarium (MTCC-428) was* 30 °C, whereas for *M. morgani (MTCC-662),* it was 37 °C. For all bacteria the incubation period was 24 h.

3.2 Soil Sample

Soil sample was collected from the surface layer of sediments on the banks of river Hooghly during low tide time. The physical and engineering properties of the soil

Table 1 Engineering properties of soil sample

Properties	Values
MDD	16.97 kN/m^3
OMC	17%
Liquid limit (LL)	37%
Plastic limit (PL)	21%
Grain size	10% Sand, 70% Silt, 20% Clay (D_{50} = 0.02 mm)
IS classification	MI

specimens were determine as per Indian Standard (IS 2720) and tabulated in Table 1. Standard proctor test was performed to establish moisture-density relationship of the test soil.

3.3 Cementation Reagent

For MICP process, cementation reagent serves as the essential raw materials. In this study, cementation reagent comprises of the mixture of the solutions of 0.5 M urea ($CO(NH_2)_2$) and 0.5 M Calcium Chloride ($CaCl_2$). All the chemicals used in this research were analytical grade to ensure the consistency of the test results.

3.4 Preparation of Soil Specimen

Prior to prepare the specimen, the test soil samples were air dried for several days. To maintain the reasonable pore space for smooth percolation of reagent solution through the soil specimen, the density of the test sample was kept lower than the MDD. In this study the soil specimens were compacted in the mould (38 mm dia) by applying the equivalent energy as per the Standard Proctor Test at a density of 15.79 kN/m^3 (93% of MDD). The water content was taken from the Proctor density curve corresponding to the desired density. To inoculate the soil specimen, soil samples were mixed with each cultivated bacteria directly at a time and compacted into mould. In that case quantity of water to be mixed with the soil was replaced by the growth medium with the bacteria. Length to diameter ratio for all the test specimens was maintained approximately as 2:1.

3.5 Quantitative Analysis of Calcite Precipitation

Quantitative determination of precipitated calcium chloride was done following modified Piper's method or acid neutralization method (Piper 1966). In this method sodium hydroxide is used to titrate the excess hydrochloric acid applied to dissolve the precipitated calcium carbonate

$$\% \text{ of } CaCo_3 = \frac{5x\,(B - S) \times N \times \text{mcf}}{\text{Samplewt}\,(\text{gm})}, \tag{3}$$

where, B = Vol in ml NaOH used for blank, S = Vol in ml of NaOH used for sample, N = Normality of NaOH and mcf = moisture correction factor

3.6 Experimental Variables

In this investigation four different treatment conditions were considered for each type of microorganism. (1) Untreated in which the specimen was prepared at desired density and made saturated before testing for comparison purpose. (2) Specimen prepared at desired density and treated with cementation reagent. (3) Specimen prepared with bacterial solution and made saturated before testing to observe the effect of bacterial cell on the strength of soil. (4) Specimen inoculated with bacterial solutions during preparation and then treated with cementation reagent.

3.7 Experimental Procedure

MICP treatment was done by injecting the reagent solution (0.5 M urea/$CaCl_2$) into the soil specimen through a pressurized tank containing reagent solution. Figure 1 shows the laboratory test setup for MICP treatment of soil specimen. Total volume of reagent solution injected into the soil specimen was 4 L in 72 h. The pressure was maintained at 100 kPa throughout the treatment for all the specimens. After completion of treatment the soil specimens were extruded from the mould for strength determination by unconfined compression test.

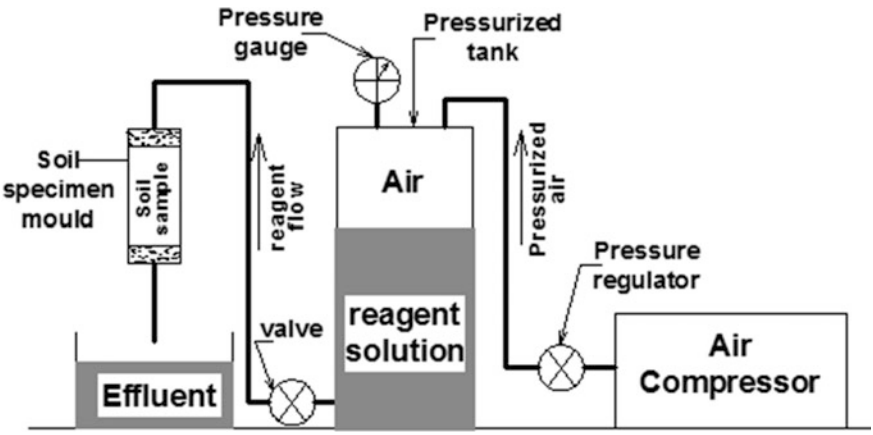

Fig. 1 Test setup for MICP treatment

4 Results and Discussion

The stress strain curves of all the soil specimens tested in the unconfined compression testing machine are shown in Fig. 2. Unconfined compressive strength or UCS (q_u) is defined as the peak stress or the stress corresponding to 20% axial strain whichever is lower. Undrained cohesion (C_u) is taken as half of the UCS value, i.e., ½ q_u. It was observed that unconfined compression strength was improved for all MICP treated soil specimen as compared to the untreated samples.

Carbonate content in the soil specimen was determined by averaging the calcite content value of three different positions (top, middle, and bottom) of the soil specimen. Percentage calcium carbonate content of different soil samples (untreated and MICP-treated) are tabulated in Table 2. It was also found that some carbonate (0.698%) was present in the untreated soil sample. The carbonate content is slightly increased (0.86%) in the samples treated with cementation reagent only. Therefore the actual amount of carbonate precipitation for MICP treated soil specimens with MTCC662, MTCC1761, and MTCC428 were 0.495, 1.042, and 1.752% respectively.

Comparison of strength improvement ratio of different test specimens with respect to untreated specimen is given in Fig. 3. The improvement ratio is maximum (1.49) for the sample treated with MTCC428 (*B. megatarium*) among all the bacteria used in this investigation. The specimen treated with cementation reagent only also exhibited a slight (15%) improvement in shear strength. This implies that the existing bacteria inhabited in the soil samples may have favoured the MICP process though it was not appreciable. Furthermore, no measurable improvement was observed in UCS strength for the soil specimen treated with microorganism only. Therefore, those results were not included in Figs. 2 and 3.

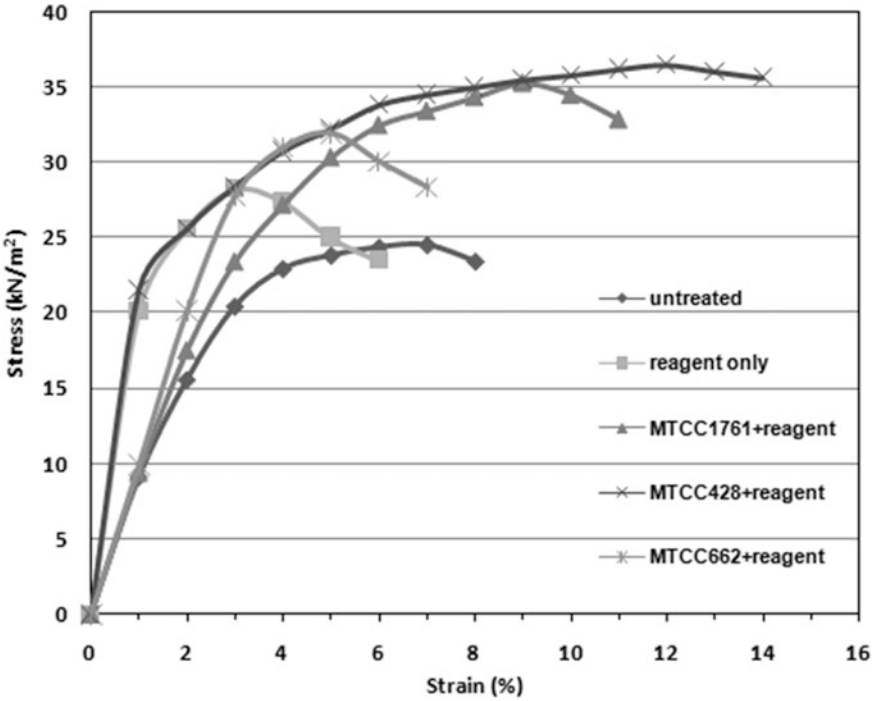

Fig. 2 Stress–strain curves in UCS test

From the test result, it was also observed that the unconfined compression strength is increased with higher percentage of carbonate content. This phenomenon can be attributed to the fact that the more amount of calcite precipitation reduces the pore volume and improve the inter-particle bonding between soil grains and hence the greater shear strength value observed.

5 Conclusions

From this preliminary investigation, the following conclusions can be drawn:

1. All the three microorganism (*S. pasteurii*, *B. megatarium,* and *M. morgani)* can be used for soil improvement by MICP technique. In this particular soil as clayey sandy silt or loam, *B. megatarium* proved to be the most effective microorganism for MICP treatment.

Table 2 Percentage carbonate content in the soil specimens

	Untreated	Treated with cementation reagent only	Treated with MTCC662 + cementation reagent	Treated with MTCC1761 + cementation reagent	Treated with MTCCC428 + cementation reagent
Calcium cobonate content (%)	0.698	0.860	1.193	1.740	2.450

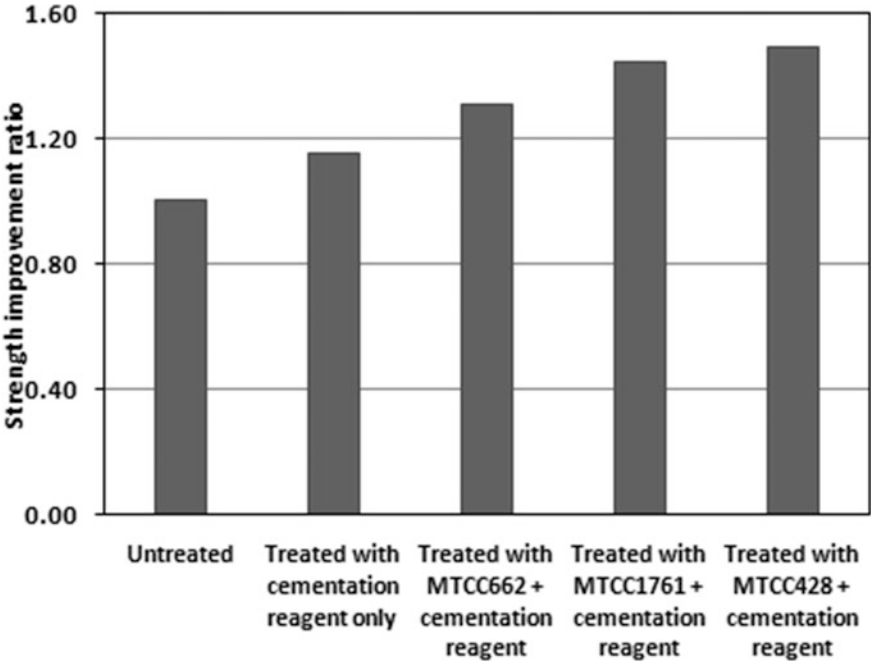

Fig. 3 Shear strength improvement of soil specimen

2. The MICP-treated soil specimen exhibited moderate improvement in shear strength, i.e., 49%. This improvement is estimated for a specified density of soil specimen. To know the effect of density on strength improvement by MICP application more investigations are required.
3. The amount of calcite precipitation in the treated soil specimen ranged from 0.495 to 1.752%. The maximum amount was observed for the soil specimen treated with the microorganism *B. megatarium* (MTCC428)
4. The soil specimen treated with cementation reagents only exhibited slight improvement in shear strength. This result indicated the fact that some calcite forming microorganism was present in the original soil.
5. The presence of biomass only in the soil specimen didn't have any significant effect in improving the strength of the soil.

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