

Effect of Biogrouting in Cochin Marine Clay – An Experimental Study

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Abstract—Biogrouting is a new ground improvement method based on Microbial Induced Calcite Precipitation (MICP). MICP involves formation of calcium carbonate precipitate within the soil mass after injecting microbes and cementation reagent including urea and calcium chloride dihydrate. Non-ureolytic bacteria *Bacillus subtilis* is used in this study which accelerates the CaCO_3 formation through addition of nucleation sites. Improvement in shear strength, permeability, compaction and plasticity characteristics of Cochin Marine Clay (CMC) by varying count of bacteria (1×10^7 , 1×10^8 and 1×10^9 cells/ml), concentration of cementation reagent (0.5 M, 1 M and 2 M) and treatment duration (10, 20 and 30 days) was studied and reported. Greater improvement is observed at 10^9 cells/ml, 1 M and at 30 days. Improvements has reduced at higher concentration of cementation reagent. Influence of treatment duration in soil improvement was observed. The calcite precipitation was confirmed by set of test tube experiments and XRD analysis.

IndexTerms—Biogrouting, Cementation reagent, Cochin Marine Clay, MICP.

I. INTRODUCTION

Cochin areas are located along the coastal belt of India. Marine deposits accumulated along the coastal areas have further contributed to the formation of marine soil. Marine clays are sensitive and soft clays having low shear strength and high compressibility. Settlement of foundations are one of the major problem associated with these soil type. The engineering properties needs to be improved for any further construction works to be performed. Considering the economy and environmental impacts of traditional and commonly used soil stabilization techniques, there arise the need of ecofriendly ways. Biogrouting is an in-situ soil improvement technique involving Microbial Induced Calcite Precipitation (MICP). The calcium carbonate precipitate formed during MICP usually bridges the voids within soil mass. The presence of non-ureolytic bacteria increases the calcite precipitation through addition of nucleation sites in form of bacterial cells.

The studies have reported that MICP is more effective in sandy soils than silts. The usefulness of *Bacillus subtilis* in Cochin Marine Clays (CMC) needs to be investigated. The impact of biogrouting in consistency limits, CBR and compaction characteristics have to be found out. This paper aims to find the optimum concentration of *B. subtilis* and cementation reagent including urea and calcium chloride dihydrate in improving engineering properties of CMC. The role of treatment duration is also studied.

II. TEST METHODOLOGY

The chemicals including equimolar urea and calcium chloride dihydrate are mixed with the soil thoroughly. The bacterial solution provided with organic supplement of egg white and yolk diluted into required concentration was then added. Proper mixing was ensured. The procedure was repeated for three concentrations of bacteria – 1×10^7 , 1×10^8 and 1×10^9 cells/ml & 0.5 M, 1 M and 2 M concentration of cementation reagent. Treatment duration of 10, 20 and 30 days were selected.

A MATERIAL COLLECTION

The materials used in this study includes Cochin marine clay, *Bacillus subtilis*, Nutrient, Urea (NH_2CONH_2) and Calcium Chloride Dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$).

1. Cochin Marine Clay (CMC)

Marine clay was collected from Njarakkal at a depth of 2 m. Material properties are shown in table 1.



Fig. 1 CMC

Table 1

Material properties of CMC

Properties	Values
Specific gravity	2.72
Percentage of clay size particles (%)	27
Percentage of silt size particles (%)	73
Optimum moisture content (%)	21
Maximum dry density (g/cc)	1.67
CBR Value	2.74 for 5 mm penetration
Coefficient of permeability (cm/s)	5.443×10^{-4}
UCC (kg/m ²)	0.18
Cohesion (kg/m ²)	0.09
Liquid limit (%)	116
Plastic limit (%)	45
Plasticity Index	71
Soil Classification (Indian Standard Classification System)	Inorganic clay of high plasticity (CH)
Coefficient of consolidation (cm ² /min)	0.073

2. *Bacillus subtilis*

Pure culture of *Bacillus Subtilis* strain JC3 was collected from the department of Agricultural Microbiology, College of Horticulture, Vellanikkara, Thrissur.

3. Chemicals

The cementation reagent consisting of equi-molar contents of chemicals, Urea (NH₂CONH₂) and Calcium Chloride Dihydrate (CaCl₂.2H₂O) were used in the study. Concentration of cementation reagent is an important parameter that affects the urea hydrolysis.

4. Nutrient

Sufficient nutrients should be supplied to bacteria for microbial activity to effectively happen. Egg white and yolk were used as organic supplement after removing fat (1% by weight of soil).

III. TESTING PROGRAM

Calcium carbonate precipitation during MICP was confirmed initially using test tube experiments. A series of Standard Proctor test, Unconfined Compressive strength test, Permeability test, CBR test and Atterberg's limits were conducted on treated CMC. The XRD analysis of the sample showing highest increment in properties were conducted. The effect of biogrout on compaction, shear strength and permeability characteristics of treated CMC were studied and the results are reported below.

1. Calcium Carbonate Precipitation

Visual examination of the calcium carbonate precipitation induced by microbes was checked using set of test tube experiments. The *Bacillus subtilis* concentration was kept constant and equi-molar solutions of cementation reagent was at 0.5 M, 1 M and 2 M was used. The bacterial solution was provided with organic supplement of egg white and yolk. The solution was prepared to obtain 50 mL volume and then mixed. After 10, 20 and 30 days of treatment visual examination was performed.

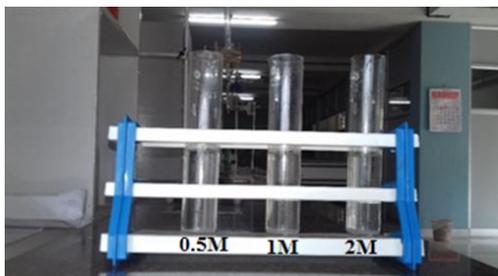


Fig. 2 Test tube experiment

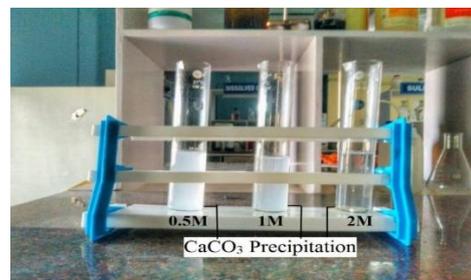


Fig. 3 Test tube experiment after 10 days



Fig. 4 Test tube experiment after 20 days

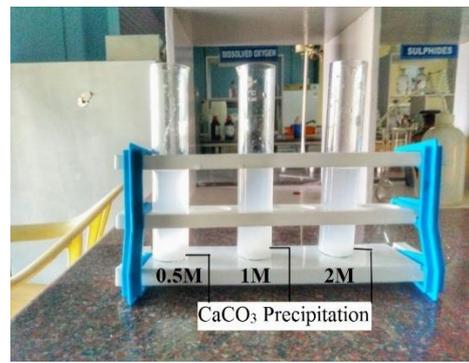


Fig. 5 Test tube experiment after 30 days

Greater precipitation was observed at 1 M concentration of cementation reagent. Thus the efficiency of *Bacillus subtilis* in accelerating calcite precipitation was confirmed. The test results showed that higher concentration of cementation reagent inhibited the reaction and precipitated calcite reduced.

2. *Optimum Moisture Content (OMC)*

The proctor compaction test was done to determine the soil compaction properties with change in moisture content.

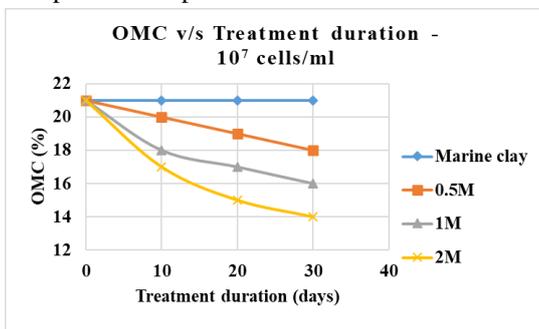


Fig.6 Variation of OMC for 10⁷ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

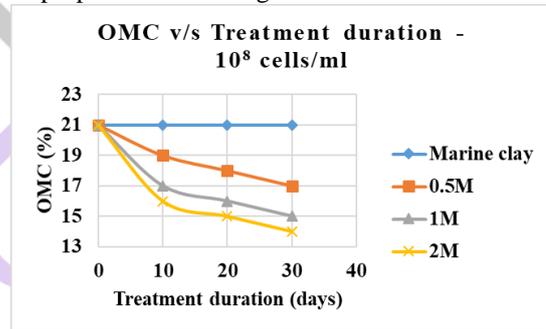


Fig. 7 Variation of OMC for 10⁸ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

Soil porosity has reduced with microbial induced calcite precipitation. Thus the soil became rigid and denser. Hence the OMC value has decreased significantly from the control value of 21%. In clayey soil due to the lower grain size, the chemical reaction was limited. Thus the water requirement reduced with increase in concentration of microbes.

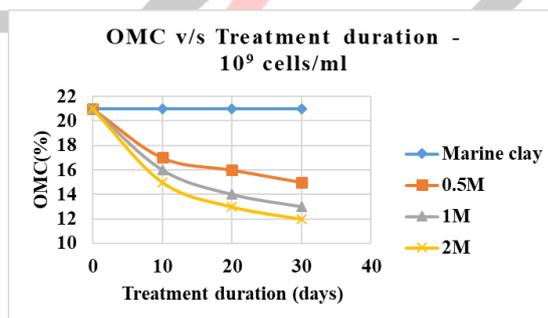


Fig. 8. Variation of OMC for 10⁹ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

With increase in molarity of cementation reagent, the reaction gets inhibited and the reduction in OMC value was reported. The thickness of diffusible double layer reduced due to calcite precipitation in between the clay particles. Particles moved closely in lower water contents due to reduction in interparticle repulsion and thus OMC value reduced. With increase in treatment duration, produced calcite gets linked together resulting in reduced water demand.

3. *Maximum Dry Density*

MDD is an important property that affects the cementation behavior of fine grained soils. The MDD is observed when there are no voids in the soil. The precipitated calcite increased the degree of crystallinity and thus dry density improved with increase in concentration of microbes.

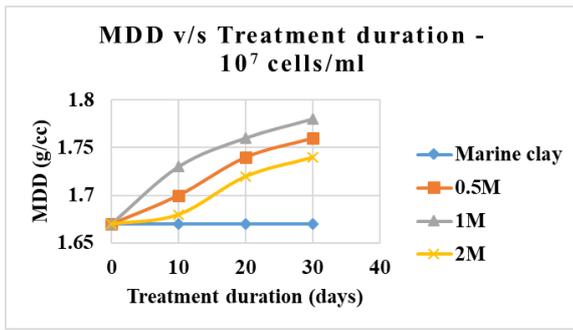


Fig. 9 Variation of MDD for 10^7 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

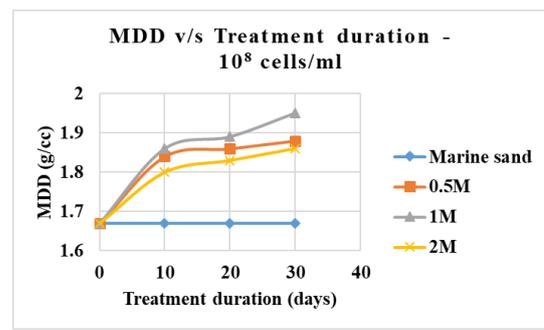


Fig. 10 Variation of MDD for 10^8 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

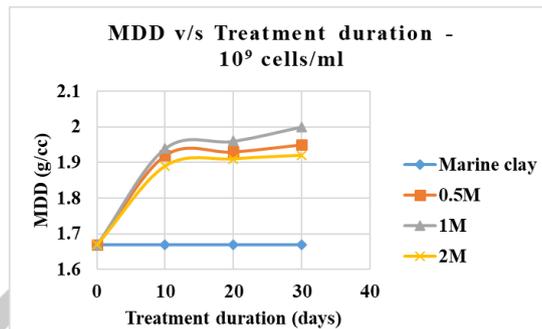


Fig. 11 Variation of MDD for 10^9 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

Bacterial action gets decreased with increase in concentration of cementation reagent. Thus MICP has significantly increased with increase in concentration of cementation reagent up to 1 M and then reduced considerably. Increased treatment duration has caused even distribution of the precipitated calcite and MDD value increased.

4. Coefficient of permeability

The permeability of fine grained soil is an important parameter to be addressed.

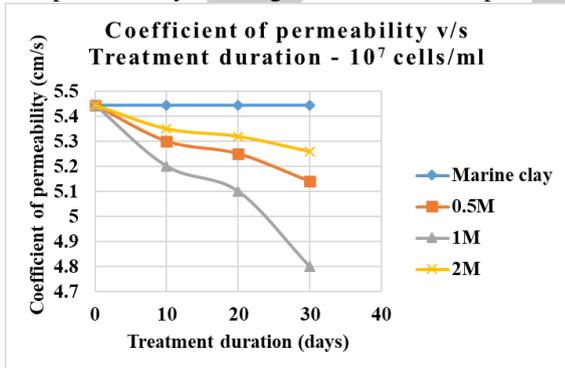


Fig. 12 Variation of k value for 10^7 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

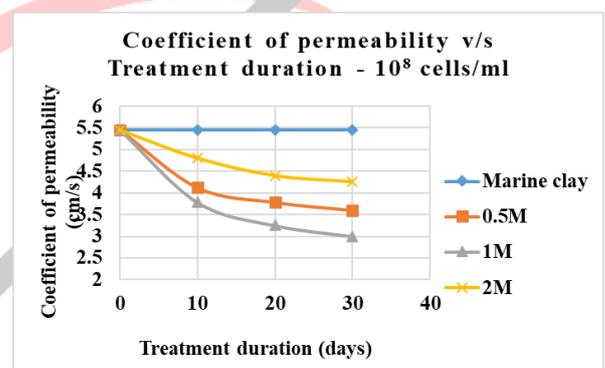


Fig. 13 Variation of k value for 10^8 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

Due to calcite precipitation, unit weight of the soil got increased and the soil became denser. Greatest reduction in permeability was observed for 10^9 cells/ml bacterial concentration, 1 M concentrated cementation reagent at 30 days. The bacterial action was negatively affected at higher salty environment created by higher concentration of cementation reagent after 1 M. Thus higher concentration of urea- CaCl_2 produced lesser reductions in permeability coefficient.

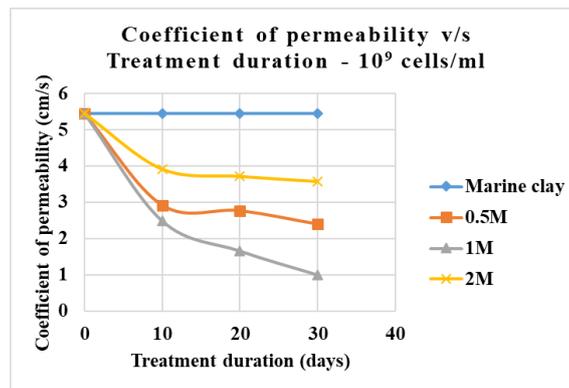


Fig. 14 Variation of k value for 10⁹ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

The treatment duration played an important role in MICP, such that the permeability has reduced considerably. The growing bacterial cells increased the rate of calcite precipitated with increase in treatment duration. Relatively small amount of calcite was produced in CMC due to the small size of soil particles. Clayey soil exhibited fewer pore space for the movement of bacteria and thus no larger diminution in permeability was achieved. However, the produced calcite reduced permeability of CMC.

5. Unconfined Compressive Strength

Unconfined compression tests (UCC) were performed on treated CMC and the results were analyzed. Increment in UCC values were observed with increase in concentration of bacterial cells. The highest increment was observed for 10⁹ cells/ml at 1 M cementation reagent after 30 days. The obtained value is 0.28 kg/cm² which comes under the category of soft consistency soil. Clogging of the voids through calcite precipitation led to the densification of treated soil. But the calcite bonds with soil particles were little weaker due to the presence of fines content. Hence the increment in UCC value was observed to be lower. UCC value has increased with increment of cementation reagent concentration of 1 M and then reduced beyond that. At higher concentration of cementation reagent, bacterial action got reduced and hence UCC value reduced at 2 M concentration of urea-CaCl₂.

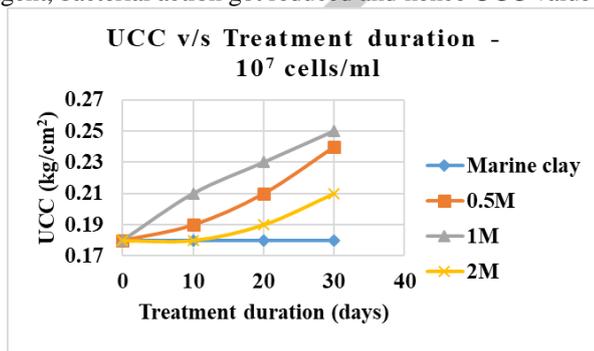


Fig. 15 Variation of UCC value for 10⁷ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

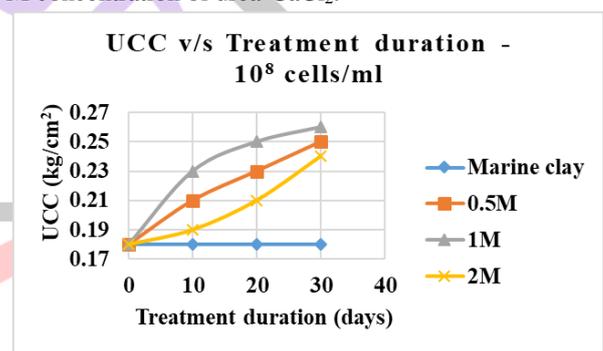


Fig. 16 Variation of UCC value for 10⁸ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

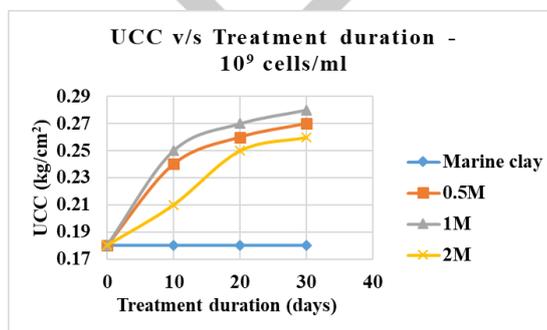


Fig. 17 Variation of UCC value for 10⁹ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

The shear strength increased with increase in treatment duration as the chance of distribution of produced calcite got increased with time.

6. California Bearing Ratio

CBR tests were carried out on treated soil samples and the values are graphically represented in following figures.

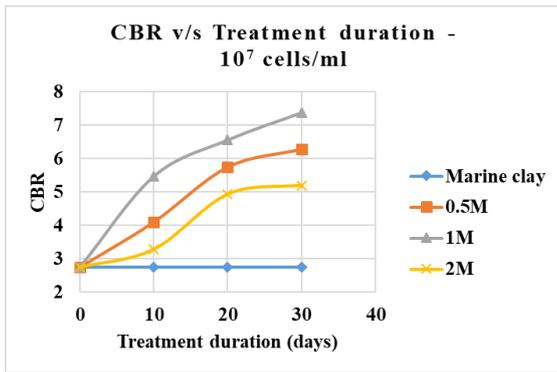


Fig. 18 Variation of CBR value for 10^7 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

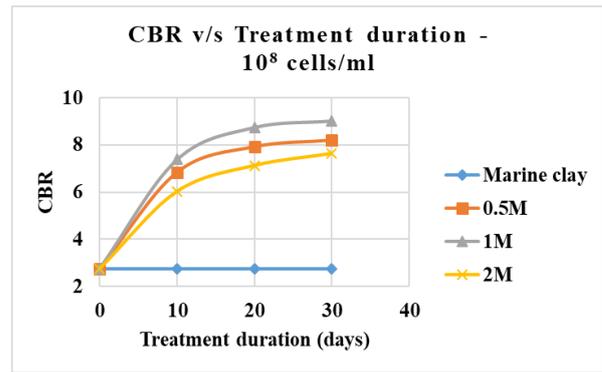


Fig. 19 Variation of CBR value for 10^8 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

Highest increase in CBR value was observed at 10^9 cells/ml concentration of bacteria, 1 M concentration of cementation reagent and at 30 days of treatment. CBR value of 12.86 comes under the range of 10-30, hence the CMC can be categorized as strong soil.

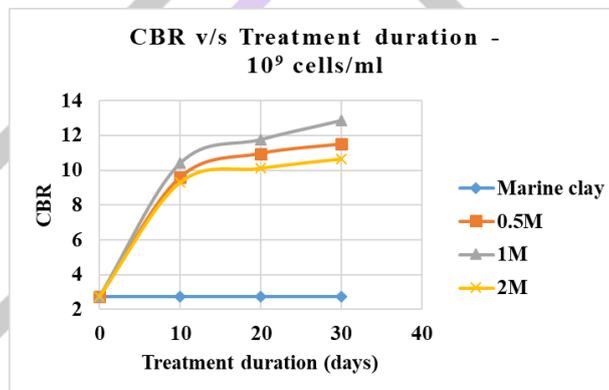


Fig. 20 Variation of CBR value for 10^9 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

Studies have reported that CBR value is affected by cell types, concentration of cementation reagent and bacteria and degree of compaction. The laboratory tests on treated CMC showed that CBR value has increased with increase in bacterial concentration. *B. subtilis* used in the study was growing cell type and thus the calcite precipitate was formed and thereby CBR value increased. Also the bacteria used can resist mechanical impact and can withstand in extreme environmental conditions. Higher cementation reagent concentration can inhibit the role of microbes and thus the CBR value has reduced at 2 M cementation reagent. The treatment duration helped the bacterial cells to attach firmly into the soil and hence the CBR has increased with number of treatment days.

7. Liquid Limit

Liquid limit is a very important property of cohesive soils.

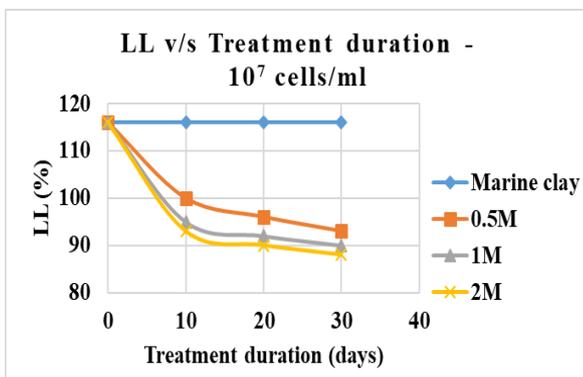


Fig. 21 Variation of LL for 10^7 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

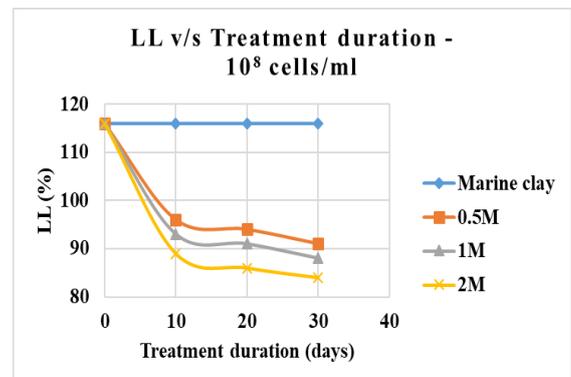


Fig. 22 Variation of LL for 10^8 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

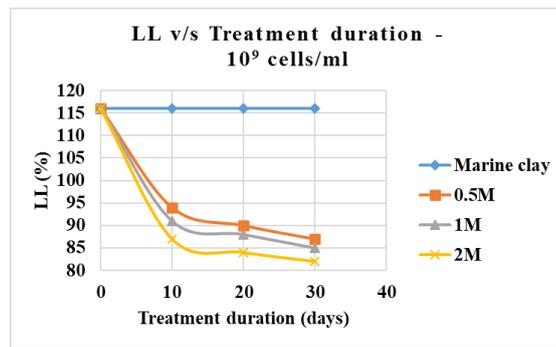


Fig. 23 Variation of LL for 10⁹ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

The results revealed that the LL has reduced considerably with increase in concentration of cementation reagent, bacteria and treatment duration. The reduction in LL was attributed due the presence of calcite precipitation in clayey soil. The calcite gets bonded in between the clay particles and thus the thickness of diffusible double layer reduced. Hence the LL reduced due to reduction in interparticle repulsion due to which the particles moved closely in lower water contents. With increased concentration of bacterial cells, the calcite precipitated increased and hence the specimen became more stable. With increased treatment duration, the clay became denser and thus the LL reduced. Liquid limit of soil can be used to predict the consolidation properties of soil. Thus the structures constructed above treated CMC can have reduced settlement of foundations. Also the bearing capacity will be improved further.

8. Plastic Limit

The plastic limit is defined as the water content below which the soil stops behaving as plastic material. At this water content, the soil loses its plasticity and it passes to the semi-solid state. The greater reduction in PL value was observed at 10⁹ cells/ml bacterial concentration, 2 M concentration of cementation reagent and at 30 days of treatment.

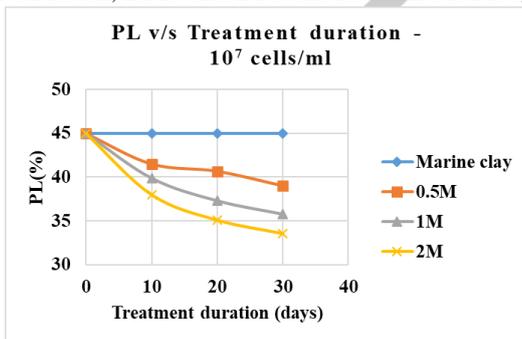


Fig. 24 Variation of PL for 10⁷ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

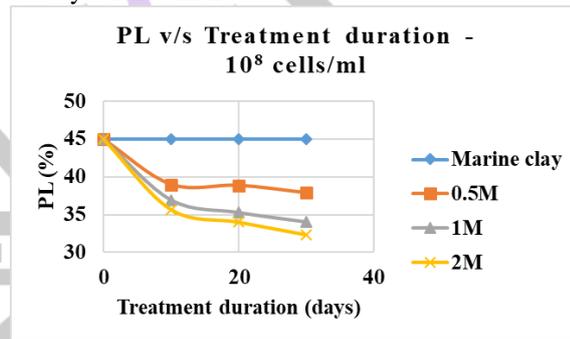


Fig. 25 Variation of PL for 10⁸ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

The plastic limit has reduced with increase in treatment duration, concentration of cementation reagent and bacterial cells. Due to calcite precipitated, the soil became brittle and thus plasticity has reduced.

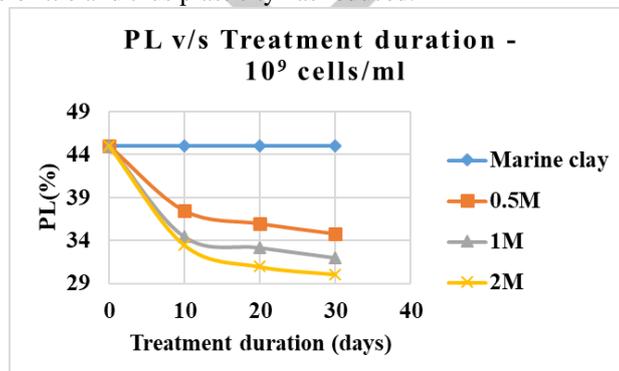


Fig. 26 Variation of PL for 10⁹ cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

The increased treatment duration has contributed to the growth of bacterial cells and thus improvement in precipitated calcite deposition was observed.

9. Plasticity Index

The variation in plasticity index values are graphically represented in following figures.

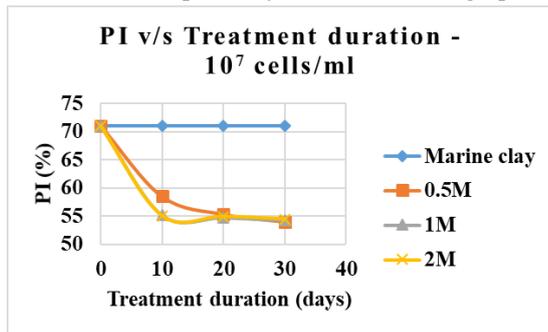


Fig. 27 Variation of PI for 10^7 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

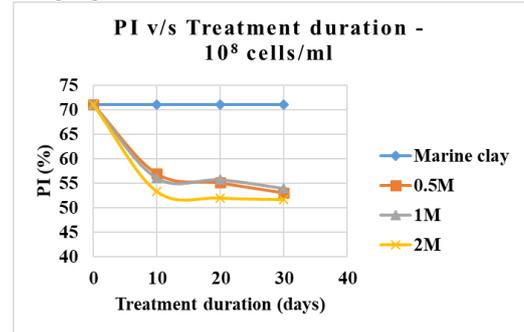


Fig. 28 Variation of PI for 10^8 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

The term Plasticity Index (PI) is defined as the range of water content over which the soil remains in the plastic state. The greater reduction in PI values was observed at 10^9 cells/ml bacterial concentration, 2 M concentration of cementation reagent and at 30 days of treatment duration. From the values of liquid limit and plastic limit, the plasticity index value was calculated further. The liquid limit and plastic limit has reduced and thus the plasticity index reduced considerably.

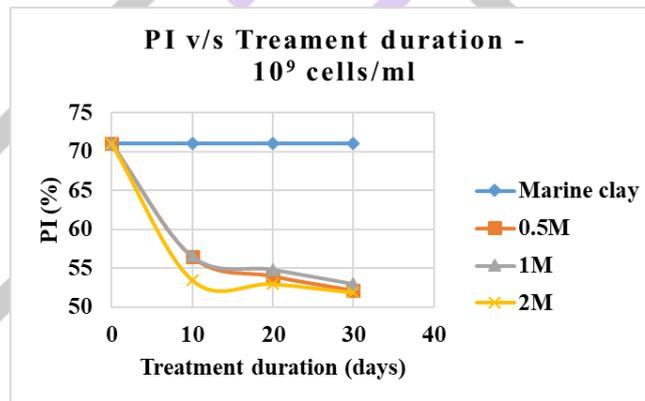


Fig. 29 Variation of PI for 10^9 cells/ml with increase in treatment days for 0.5 M, 1 M and 2 M cementation reagent

The plasticity index reduced with increase in treatment duration. The calcite bond formation induced by microbes in clayey soil contributed to reduction in plasticity properties of treated CMC.

10. XRD Analysis

CMC at bacterial concentration of 10^9 cells/ml, cementation reagent concentration of 1 M and at treatment duration of 30 days offered highest increment in results. XRD analysis was done on treated CMC sample which showed best results after testing. The XRD analysis determines the orientation of crystals within the specimen. The XRD test results on treated CMC is represented below.

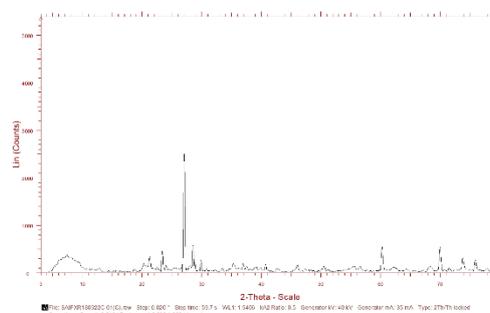


Fig. 30 XRD test results on treated CMC

XRD analysis showed that lesser peaks in treated CMC. This may be due to comparatively lesser transport of bacteria in fine grained soils and thereby reduction in MICP to happen.

IV. EFFECT OF MICP ON CMC

From the tests conducted on CMC, it can be concluded that MICP improves soil shear strength and reduces plasticity and permeability characteristics of marine clay. The calcium carbonate precipitate filled up the voids of soil samples and bind soil particles together. A higher increase in soil properties was not observed, since the performance of microbes are lower in fine grained particles due to reduced porosity. Greater improvement was analyzed at 10^9 cells/ml bacterial concentration, 1 M cementation reagent concentration and at 30 days of treatment. The use of a low urea-calcium chloride solution resulted in stronger samples. Considering economic point of view and percentage improvement with increase in concentration of microbes, the tests were limited up to 10^9 cells/ml microbial concentration.

Applications of MICP on CMC

- Improved strength and load bearing capacity of structure constructed above biogrooted soil due to increased density.
- Settlement of existing foundation can be reduced due to increased density.
- Minimized seepage due to improved permeability properties.
- The treated CMC can be used for pavement subgrade applications due to the increased CBR value

V. CONCLUSION

Biogrouting is an effective and environmental friendly technique of soil improvement. From the test results on treated CMC, the following conclusions are made.

- The OMC of treated CMC has reduced from 21% to 13% with percentage reduction of 38.09%. MDD value has increased from 1.67 to 2 g/cc. Percentage improvement in MDD value for CMC was 19.76%.
- The coefficient of permeability value has reduced from 5.44×10^{-4} to 0.991×10^{-4} in clayey soils. Percentage reduction in permeability value for treated clayey soils was 81.78%.
- UCC value showed improvement from 0.18 to 0.28 kg/m². Percentage improvement in shear strength for treated CMC was obtained as 55.56%. Lower improvement in UCC may be due to the uneven distribution of the produced calcite.
- CBR value of clayey soil has increased from 2.74 to 12.86 respectively. CBR value showed percentage improvement of 369.34% for clayey soil.
- The value of PI has reduced from 71% to 53% with percentage reduction of 25.35% at 10^9 cells/ml bacterial concentration, 1 M concentration of cementation reagent and at 30 days. The calcite precipitate within the soil particles has caused reduction in diffusible double layer thickness and thereby reduced adsorption of water. Thus the plasticity properties reduced.
- The influence of treatment duration in biogrouting is established. The properties get increased with duration as there is a chance of distribution of calcite produced.
- Proper organic supplement should be provided for microbial cells for metabolic activities to effectively happen.
- Lower concentration of cementation reagent is recommended for MICP applications.

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