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Effect of microorganism on engineering properties of cohesive soils

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Abstract. This paper presents the study of the effect of microorganism *Bacillus pasteurii* on the properties such as *Atterbergs*' limit and unconfined compressive strength of cohesive soils. The results of this study reveal that the liquid limit and plasticity index for all clay soils decreased and the unconfined compressive strength increased. Decrease in plasticity index is very high for Kuttanad clay followed by bentonite and laterite. The unconfined compressive strength increased for all the soils. The increase was high for Kuttanad soil and low for laterite soil. After 24 h of treatment the improvement in the soil properties is comparatively less. *Besides* the specific bacteria selected *Bacillus pasteurii*, other microorganisms may also be taking part in calcite precipitation thereby causing soil cementation. But the naturally present microorganisms alone cannot work on the calcite precipitation.

Keywords: atterberg's limit; unconfined compressive strength; microorganism; *Bacillus pasteuri*; stabilization; cohesive soil.

1. Introduction

The enhanced engineering properties and performance of cemented soils over uncemented soils led to the development of artificial cementation methods which can effectively cement large volume of soil. All of the current methods to improve the engineering properties of soil have benefits as well as drawbacks and there continues to be a need to explore new possibilities of soil improvement, particularly as suitable land for development becomes scarcer. An innovative alternative approach to effectively improve engineering properties of soils lies with the combined use of microorganisms, nutrients and biological process naturally present in subsurface soil. The success of biological treatments have been demonstrated in other fields such as stabilization of metals (Etemadi *et al.* 2003), environmental stabilization of contaminated soils (Khachatoorian *et al.* 2003), microbially enhanced oil recovery (DeJong *et al.* 2006), encapsulation of hazardous and other contaminants in natural soils and acid mine tailings etc (DeJong *et al.* 2007). The paper presents the effect of biological treatment on the engineering properties such as index properties, unconfined compressive strength (UCS), influence of microbial cementation on the particle size and the effect of treatment

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time on the cementation of soil, using the bacteria *Bacillus pasteurii.*

2. Background

In the recent years a new method for improving the soil properties has been introduced, which is based on the use of microorganisms and the related biological process. There are 10^9 to 10^{12} organisms in a kilogram (DeJong *et al.* 2006) of soil near the ground surface. Single cell microorganisms include all types of bacteria, archea and eukarya. Bacteria and archea have a simple cell structure with no membrane-enclosed nucleus, and are distinguished by their chemical composition rather than by their structures. Some bacteria can make spores to endure adverse environmental changes. Bacteria vary in shape and may nearly round to rod-like or spiral. The cell diameter usually in the range of 0.5 to 3 μm and spores can be as small as 0.2 μm. The size of bacteria may decrease under stressed condition. They can survive *pH* ranging from 2 to 10 and in salinities much greater than that of sea water. The growth of microorganisms is exponential. Most bacterial cells have a negative surface charge for a ground water *pH* between 5 and 7, which is typical for near surface soils. The negative surface charge decreases with increasing concentration and volume of ion in the pore fluid. Surface charge also decreases with decreasing *pH*. Thus there are some similarities between bacteria cells and charged clay particle. Other environmental factors which affects the bacterial growth in soil involves *pH*, redox potential, temperature, the presence of predatory microorganisms which may limit bacterial population and space limitations. Although microorganisms are free to move in the pore space between large soil grains and aggregations, narrow pore throats formed by small soil grains prevent their entry. Therefore bacteria are not expected to enter through pore throats smaller than ~0.4 μm (Mitchell and Santamarina 2005). Microorganisms are important in the formation of fine grained particles and clay minerals. A continuous bacterial monolayer can form a mineral surface within minutes. The presence of microfossils in some fine grained soils as soil particle can have a profound effect on the behaviour of soil mass, conferring unusual geotechnical properties that deviate from general parameter correlations including high porosity, high liquid limit, unusual compressibility and uniquely high friction angle.

2.1 Bacterial soil cementation

There is growing evidence that microbial activity plays an important role in calcite precipitation. The microbial calcium carbonate formation has been found in the studies of Braissant *et al.* (2003) and Baskar *et al.* (2006). *Bacillus pasteurii* is a common alkalophilic soil bacterium with a highly active urease enzyme (Ferris *et al*. 1996). *Bacillus pasteurii* uses urea as an energy source and produces ammonia, which increases *pH* in the proximal environment, causing Ca^{2+} and CO_3^{2-} to precipitate as $CaCO_3$ (Kroll 1990). DeJong *et al.* (2006) reported that the local rise in *pH* often causes the microbes themselves to serve as nucleation sites for crystallization. In calcite precipitation, the overall equilibrium reaction is

$$Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3 \downarrow$$

Microbiologically induced calcite precipitation occurs according to the reactions

$$Ca^{2+} + HCO_3^- + OH^- \rightarrow CaCO_3 \downarrow + H_2O$$

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 \downarrow + CO_2 + H_2O$$

The high *pH* environment is provided by the decomposition of urea according to the reaction

$$NH_2 - CO - NH_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2$$

More details of the above reaction are available from DeJong *et al.* (2006). Calcite precipitation studies conducted by Ercole *et al.* (2007) proposed a new method for the restoration of limestones in historic buildings and monuments. The paper describes the formation of calcite crystals by extracellular polymeric substances. They isolated bacterial outer structures (glycocalix and parietal polymers), such as exopolysaccharides (*EPS*) and capsular polysaccharides (*CPS*) and checked for their influence on calcite precipitation. *CPS* and *EPS* extracted from both *Bacillus firmus* and *Bacillus sphaericus* were able to mediate $CaCO_3$ precipitation *in vitro*. X-ray microanalysis showed that in all cases the formed crystals were calcite. Scanning electron microscopy showed that the shape of the crystals depend on the fractions utilized. These results suggest the possibility that biochemical composition of *CPS* or *EPS* influences the resulting morphology of $CaCO_3$. Laboratory experiments involving calcium carbonate precipitation by bacteria isolated from stalactites sampled from three caves in Sahastradhara, Dehradun, India were conducted by Baskar *et al.* (2006) to determine whether geomicrobiological processes might be involved in stalactite formation. The culture experiments demonstrate that *B. thuringiensis* and *B. pumilis* mediate the precipitation of calcite under well-defined conditions. The optimum temperature for calcite precipitation was 25°C. Galinat *et al.* (2001) and Day *et al.* (2003) report the results of the scanning electron micrograph (*SEM*) analysis of concrete crack remediation by polyurethane immobilized *Bacillus pasteurii* whole cells. The results of this study reveal that the compressive strength of concrete cubes increased and cracks were remediated with the cells. A common soil bacterium, *Bacillus pasteurii*, was used to induce $CaCO_3$ precipitation.

2.2 Strength studies

There are limited numbers of studies on the effect of bioremediated reactions on the strength and stiffness of the soil. An investigation conducted by Villarraga and coworkers as reported by Mitchell and Santamarina (2005) showed an increase in undrained strength, undrained stiffness, drained strength of 20-100%, 50-100%, 10-50 kPa respectively and a decrease in permeability of one to two orders of magnitude. They concluded that microbial processes influence rock weathering, mineralization, soil formation and fabric and soil grain surface properties. Inherent pore size restrictions in relation to the size of microorganisms limit the post sedimentation bioengineering of clays and clayey soils. Therefore good candidate soils for biomodification include *GW*, *GP*, *SW*, *SP*, *ML* and organic soils. The successful development of a treatment procedure to beneficially alter the behavior of uncemented cohesionless soil using natural microbial processes has been reported by DeJong *et al.* (2006). Factors determined critical to the success of the microbial treatment include *pH*, oxygen supply, metabolic status, concentrations of microbes, ionic calcium in the biological and nutrient treatment flushes as well as the timed sequence of injections. Specimens cemented with gypsum and microbially induced calcite both exhibited similar specimens. Both gypsum and microbially induced calcite precipitation (*MICP*) cementation were observed on the sand-particle surfaces as well as at particle contacts. The gypsum cement was characterized by well-formed needle-shaped crystals while the microbially induced calcite cement exhibited a more grainy texture

with little structure at the investigation magnification. The results presented have established that substantial cementation in loose sand structures can be engineered through harnessing and controlling natural biological processes. From the literature presented above, it is evident that the effect of biological treatment on the engineering properties such as index properties and unconfined compressive strength (*UCS*), influence of microbial cementation on the particle size and the effect of treatment time on the cementation of cohesive soils using bacteria *Bacillus pasteurii* has not been investigated. Hence this research is taken up and the results are presented in this paper.

3. Materials used and experimental procedure

3.1 Cohesive soils

Kuttanad comprising of about a total area of 1100 km^2, in coastal region of central Kerala, India is in many respects a unique land. Particularly the whole area is about 0.6 to 2.2 m below mean sea level. The major portion of the area remains submerged under water for more than a month of every year. The clays in this region exhibit swelling properties and are rich with the nutrient elements needed for the microbial growth. The Kuttanad clay was collected from a place Kuttanad in Kerala, India from a depth of 2 m and 3 m from ground surface from five different bore holes for study. Laterite soil was collected locally in Trivandrum, India. Commercially available bentonite was procured from the local market for the study. Kuttanad and laterite clay was collected in sealed polythene bags and directly transported to the laboratory. The sealed polythene bags were stored in water filled containers to preserve the natural condition. Index and engineering properties of soils were found in the laboratory as per respective Bureau of Indian Standards. The properties of the Kuttanad clay, bentonite and laterite soil are presented in the Table 1. The particle size distribution curve for these soils is shown in Fig. 1. The major nutrient levels for the microbial growth in

Table 1 Properties of cohesive soils

Property	Laterite soil	Bentonite soil	Kuttanad soil				
			BH 1	BH 2	BH 3	BH 4	BH 5
Gravel (%)	11.2	0	0	0	0	0	0
Sand (%)	49.7	0	25	27	24	22	28
Silt (%)	38	16	28	24	29	28	26
Clay (%)	1.1	84	47	49	47	50	46
Specific Gravity	2.65	2.55	2.5	2.5	2.5	2.4	2.62
Liquid limit (%)	46	237	120	98	115	116	76
Plastic limit (%)	28	50	85	61	74	61	44
Shrinkage limit (%)	20		20	26	31	14	26
Plasticity index (%)	18	187	35	37	41	55	32
Unconfined compressive strength (*UCS*) (kN/m^2)	9.6	13.62	5.3	4	5.6	4.63	4.2
Free Swell	3	15	2.8	2.7	2.8	2.8	2.6
pH	7.5	8	7.5	7.5	8	7.5	7.5

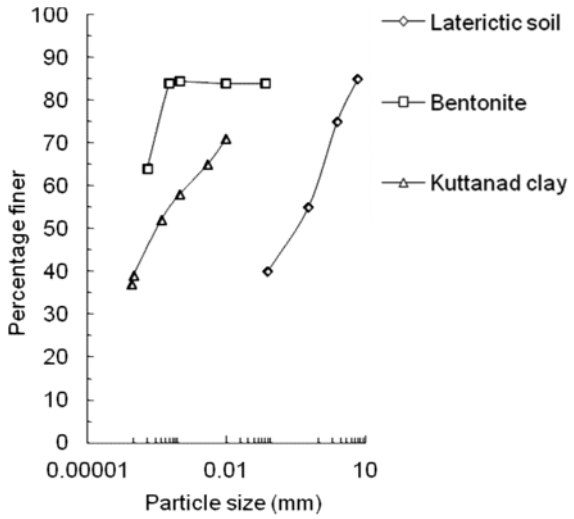

Fig. 1 Particle size distribution curves for cohesive soils

Table 2 Major chemical constituents in cohesive soils for microbial growth

Element	Range of value (kg/ha)			Laterite soil		Bentonite soil		Kuttanad clay	
	Low	Medium	high	Quantity (kg/ha)	Remark	Quantity (kg/ha)	Remarks	Quantity (kg/ha)	Remark
Organic carbon	< 0.5	0.5%-0.75%	> 0.75%	0.37%	Low	0	Low	3.834%	High
Nitrogen	< 280	280-560	> 560	58.17	Low	89.38	Low	107.96	Low
Phosphorous	< 10	10-25	> 25	5.92	Low	6.27	Low	7.735	Low
Potassium	< 220	220-520	> 520	1787.52	High	497.28	High	417.76	Medium
Calcium			> 600	1337.50	High	13330.8	High	919.97	High

Kuttanad clay, bentonite and laterite soil determined by the chemical analysis are presented in Table 2. For mineralization to take place in soils by the microbes the major constituents to be present in the soil is nitrogen. Hence nitrogen was added to the soils in the form of urea.

3.2 Selection of microbes and procedure of microbial treatment

DeJong *et al.* (2006) reported that the bacteria type microorganism *Bacillus pasteurii* is effective in the microbially induced calcite cementation. The same bacterium was selected for microbial treatment in the present study. For microbially induced calcite precipitation to be effective, a microorganism must be capable of CO_2 production paralleled by a *pH* rise in the surrounding environment to an alkaline level that induces precipitation of calcium carbonate. Aerobic microorganisms capable of consuming urea as an energy source are particularly good candidates because they provide two sources of supply of CO_2, respiration by the cell and decomposition of urea. In addition, cells of *Bacillus pasteurii* do not aggregate; this ensures a high cell surface to volume ratio, a condition that is essential for efficient cementation initiation. The specific microbes needed for the study was bought from *Institute of Microbial Technology*, *Chandigarh*, India. The microbes

Table 3 Constituents of treatment solution (modified after DeJong *et al.* 2006)

Solution	Constituent	Quantity
Urea medium	Bacto nutrient broth	3 g/per liter of distilled water
	Urea (NH_2CONH_2)	20 g/ per liter of distilled water
	NH_4Cl	10 g/ per liter of distilled water
	$NaHCO_3$	2.12 g/ per liter of distilled water
Microbial treatment solution	Bascillus pasteurii containing urea medium	20 ml (after 24 h culture)
	Urea medium	400 ml
	$CaCl_2$ stock solution	8 ml solution (solution contains140 g/per liter of water)

were obtained in lyophilized culture form. The microbial cells were initially grown on nutrient broth medium and then stored in nutrient agar medium for future use. The cells required for a treatment were transferred to fresh liquid medium. After 19 h at 37ºC under agitation, the cells were spun down in a centrifuge at 1000 g and 4ºC for 10 minutes. At the conclusion of the centrifuging, the supernatant was removed. The cells were resuspended in 20 ml fresh urea growth medium. The urea medium prepared was sterilized and cooled. The sterilization was done using autoclave. The microbial injection solution and precooled urea growth medium were prepared simultaneously. The constituents needed for the urea growth medium were used as reported by DeJong *et al.* (2006) and is listed in the Table 3. The *pH* of the medium was adjusted with 5 N HCl. The soil specimens were air dried in shadow to avoid changes in chemical properties which otherwise has major effect on microbial growth. Thereafter, the soil specimens were powdered and filled in the specially fabricated container made of acrylic material of size 15 × 15 × 15 cm^3. Microbial treatment solution

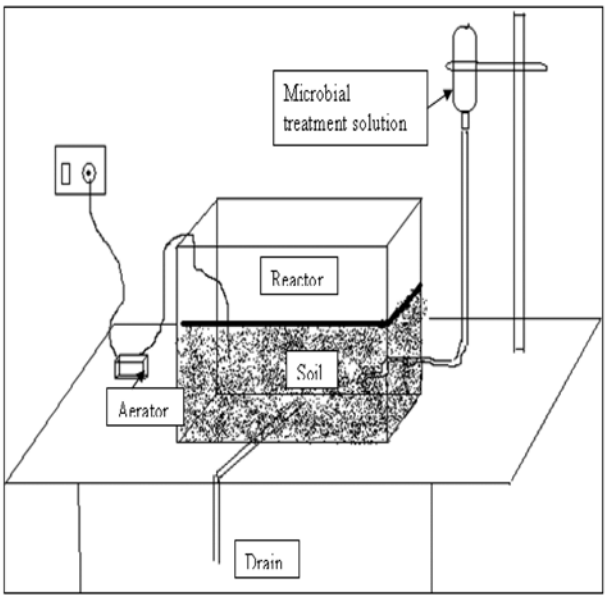

Fig. 2 Test set up

Bacteria cells in Nutrient agar Bacteria cells in Nutrient broth

Fig 3 Nutrient agar and nutrient broth medium

was added through a tube from the base at a rate of 4 ml/min. The schematic diagram of test set-up is shown in Fig. 2 and the photographs nutrient agar medium and nutrient broth is shown in Fig. 3. The microbial treatment solution was aerated using electronic aerators throughout the treatment period for proper mixing and to adjust *pH*. The soil was aerated and solution was kept in the soil for the required time and drained out. In order to study the changes in soil properties due to microbial treatment, soil specimens were initially tested for various properties for 24 h as the time for metabolism of bacteria is less than 24 h. To confirm that the microbial activity is the reason for the improved behavior of the soil, the soil samples were treated with the treatment solution without microbes (solution consisting of calcium chloride and urea medium). To ensure that the specific organism *Bacillus pasteurii* and not the naturally present microbes, is contributing to the calcite precipitation and then to cementation, treatment on sterilized soil samples were also carried out. Sterlization was done using autoclave. Treatments were done by varying the concentration of microbial treatment solution to find the effect of concentration on the change in properties of soil. To confirm that the microbial calcite precipitation is irreversible and to find the optimum treatment period, soil samples were treated and tested up to 5 days.

3.3 Soil testing

Microbially treated soil samples were tested in the laboratory as per the Bureau of Indian Standards. The liquid limit, plastic limit and shrinkage limit were determined as per IS: 2720 part 5 and 6. Unconfined compressive strength as in IS: 2720 part 10. The variation of clay and silt after the treatment were found out by conducting Hydrometer analysis and free swell as per ASTM standards.

4. Results

Microbial treatments using the bacterial type *Bacillus pasteurii* were cunducted on Kuttanad clay, bentonite and laterite soil. After microbial treatment the samples were dried in oven at 105° ±5°C and the results are presented in this section.

4.1 Kuttanad clay

The liquid limit, plastic limit and plasticity index values of the soil are a measure of amount of clay present in the soil. The soils with high organic content generally have low plasticity index values. Atterberg limits and unconfined compressive strength (*UCS*) before and after microbial treatment of soil samples collected from 5 boreholes (BH1, BH2, BH3, BH4 and BH5) are presented in Table 4. A study of Table 4 reveals that the liquid limit values of the soil samples from all the five boreholes before treatment varied from 76% to 120%. These values of liquid limit varied from 55% to 97% after the microbial treatment. The average decrease in the liquid limit due to microbial treatment was 36%. Further study of Table 4 reveals that the average decrease in plastic limit due to the microbial treatment was 12.4%. The plasticity index of the soil samples before treatment varied from 32% to 55%. The average decrease in plasticity index was 24%. The

Table 4 Atterberg limits and unconfined compressive strength test results of Kuttanad clay before and after treatment with microbial treatment solution

Property	Sample No.	Before treatment	After treatment	% decrease
Liquid limit (%)	BH 1	120	97	23
	BH 2	98	76	22
	BH 3	115	55	60
	BH 4	116	58	58
	BH 5	76	59	17
Plastic limit (%)	BH 1	85	74	11
	BH 2	61	54	7
	BH 3	74	50	24
	BH 4	61	41	20
	BH 5	44	44	0
Plasticity index (%)	BH 1	35	23	12
	BH 2	37	22	15
	BH 3	41	5	36
	BH 4	55	17	38
	BH 5	32	14	18
Shrinkage limit (%)	BH 1	20	18	2
	BH 2	26	22	4
	BH 3	31	26	5
	BH 4	26	23	3
	BH 5	26	25	1
				% increase
UCS (kN/m^2)	BH 1	5.3	7.3	38
	BH 2	4	9	125
	BH 3	5.6	9	61
	BH 4	4.6	9.6	109
	BH 5	4.2	7	67

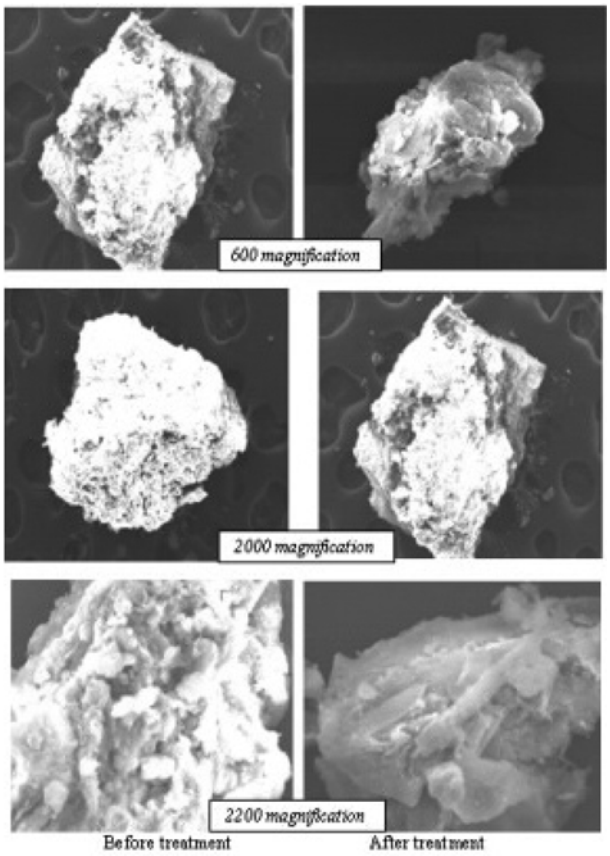

Fig. 4 Scanning Electron Microscopy images for the untreated and treated samples

shrinkage limit of samples varied from 14% to 31% and the percentage reductions after the microbial treatment were comparatively less and varied from 1% to 5%. There was an increase in the *UCS* after the microbial treatment. The *UCS* of the soil samples was ranging from 4 kN/m^2 to 5.6 kN/m^2 before the treatment. The percentage increase in *UCS* varied from 38 to 125% after the treatment and the average increase was 80%. Scanning Electron Microscopy (*SEM*) images are shown in Fig. 4 for the untreated and treated samples of the soil. These images clearly indicate that the pores in the soil are disappearing after the microbial treatment. Further it has been observed that there are some similarities between the microbially induced stabilization and chemical stabilization, especially lime stabilization. The lime treatment enlarges the size of the clay particles by coagulation to silt size, there by changing the soil structure leading to stabilization. Lime reduces the plasticity index of soil thereby decreasing the swelling potential of the soil.

The literature reports that the air dried Kuttanad clay have lower Atterberg limits and higher unconfined compressive strength. Therefore, the properties of air dried as well as oven dried (105° ± 5°C) sample were compared with the microbially treated soil samples. The results of comparison are presented in Table 5. A study of this table reveals that there was an increase of 41% in the unconfined compressive strength after the microbial treatment as compared to air dried soil samples.

To check that the specific microbe selected *Bacillus pasteurii* was the reason for the improved

Table 5 Comparison of properties of air dried and oven dried sample with treated Kuttanad clay sample

Property (Sample BH3)	Before treatment		After 24 h treatment	Increase/decrease from natural condition	Increase/decrease from dried condition
	As such from Field	After air drying			
Liquid limit (%)	115	64	55	60	+9*
Plastic limit (%)	74	44	42	30	+5
Shrinkage limit (%)	31	21	28	3	−7**
Plasticity index (%)	41	20	13	28	7
UCS (kN/m^2)	5.6	6.4	9	61	41

* + for increase and ** − for decrease

Table 6 Properties of untreated sample, sterilised treated sample and unsterilised treated Kuttanad clay sample

Property (Sample BH 3)	Untreated soil (air dried)	Sterilised & treated soil	Unsterilised treated soil
Liquid limit (%)	115	107	55
Plastic limit (%)	74	73	42
Shrinkage limit (%)	31	24	13
Plasticity index (%)	41	34	28
UCS (kN/m^2)	5.6	6.4	9

behavior of soil and not the other bacteria naturally present in the soil, microbial treatment were done on sterilized soil. The properties were found out after the microbial treatment and are presented in Table 6. Study of Table 6 reveals that the soil treated after sterilisation also indicates the

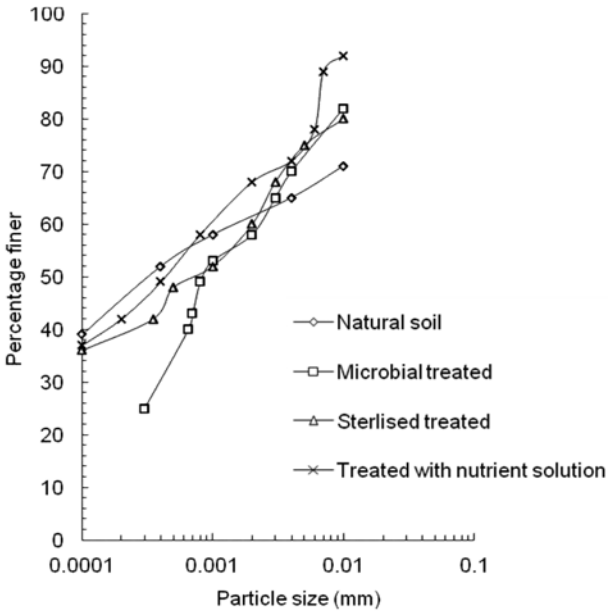

Fig. 5 Particle size distributions of soil samples treated in different condition

improvement in the properties but the improvement was less than the unsterilised soil samples. Keeping the above in view, it can be concluded that the naturally present microbes may also be taking part in calcite precipitation thereby causing the cementation of the soil samples. Particle size distribution curve of natural soil and microbially treated soil are shown in Fig. 5. A study of this figure reveals that the percentage of clay in soil was reduced from 47% to 30% and the percentage of silt increased from 29% to 48% after the microbial treatment. Fig. 5 also contains the particle size distribution curve of sterilized treated soil and natural soil. Further study of this figure reveal that the results were similar to unsterilised treated soil, but the reduction in the clay content was comparatively less in comparison to the unsterilised soil samples.

In order to find the effect of nutrient medium without microbes on the cementation process of soil, treatment was done with nutrient solution alone. The treatment was done for 24 h and the treated soil was tested for engineering properties. The results of these tests are presented in Table 7 and Fig. 5. Study of Table 7 and Fig. 5 reveals that there were comparatively very small changes in the properties of the soil with the addition of nutrient solution alone. Thus it can be concluded that the impact of nutrient solution was less on the cementation process. The small improvement noticed was entirely due to the presence of already existing microbes in the soil samples. Keeping the above in view, it can be concluded that the specific calcite precipitating natural soil microorganism *Bacillus pasteurii* was the main reason for the improvement of the soil behavior after the treatment. It is pertinent to mention here that the naturally present microorganisms may also contribute to the

Table 7 Properties of untreated soil, microbially treated soil and soil sample treated with nutrient solution of Kuttanad clay

Sample No.	Property	Untreated soil	Treated with Nutrients (without microbes)	Microbial Treated soil
BH 1	Liquid limit (%)	120	120	97
	Plastic limit (%)	85	78	74
	Plasticity index (%)	35	42	23
	UCS (kN/m^2)	5.3	3.2	7.3
BH 4	Liquid limit (%)	116	80	58
	Plastic limit (%)	61	53	41
	Plasticity index (%)	55	27	17
	UCS (kN/m^2)	4.6	5.6	9.6

Table 8 Properties of soil samples treated at different concentration of microbial treatment solution of Kuttanad clay

Sample No.	Concentration (gm/ml)	% increase in *UCS*	% decrease in Liquid limit
BH 1	1.6	38	3
BH 2	1.4	125	22
BH 3	1.0	61	60
BH 4	1.25	109	58
BH 5	1.1	67	17

Table 9 Variation of unconfined compressive strength values with treatment time of Kuttanad clay

Sample No.	*UCS* value (kN/m^2)		
	Untreated sample	Treated for 24 h	Treated for 48 h
BH1	5.3	7.3	8.1
BH2	4	9	10
BH4	4.6	9.6	15

Table 10 Atterberg limits, unconfined compressive strength and free-swell of soil samples (mixture of BH1 and BH3 sample) treated for different time intervals of Kuttanad clay

Property	No. of days treated					
	Untreated	1 day	2 days	3 days	4 days	5 days
Liquid limit (%)	108	85	88	83	80	88
Plastic limit (%)	67	59	59	52	60	57
Shrinkage limit (%)	33	31	31	31	22	25
Plasticity index (%)	41	26	29	31	20	31
Unconfined compressive strength (kN/m^2)	8.5	10.7	10.93	10.66	12.78	17.6
Free Swell (ml/2gm)	2.8	2.4	2.3	2.5	2.5	2.4

improvement of the soil behaviour.

The soil samples were treated with different concentration of microbial treatment solution. The change in properties of soil treated is presented in the Table 8. Study of this table reveals that the percentage increase in *UCS* increases up to a concentration of 1.4 gm/ml and after that it decreases.

The soil samples were treated for 24 h and 48 h to check the influence of treatment time on the soil cementation. The treated soils were tested for *UCS*. The test results are shown in Table 9. Study of Table 9 reveals that the *UCS* increased with the increase in treatment time up to 48 h. Keeping this in view, it was decided to study whether the treatment time increases the soil improvement. A representative soil sample was treated for 1 to 5 days. The properties of samples treated for different time were found out and the results are shown in the Table 10. Certain clay soils containing montmorillonite mineral swell considerably upon imbibing water from outside. These soils also shrink upon removal of water. Kuttanad clay is rich in montmorillonite mineral. Also as swelling clays they have a plasticity index of above 25%. Generally soils of high plasticity index are swelling clays, so free swell test on the soil sample have been performed. Free swell of the treated and untreated soil samples were found out as per *ASTM* standards and presented in Table 10. Study of Table 10 reveals that there was a reduction in the free swell values, but the variation with time of treatment is not much. Table 10 further indicates that as the treatment time increases, the *UCS* of the soil increases. A close examination of Table 10 reveals that the Atterberg limit decreases after microbial treatment, but the reduction seen in initial 24 h, after that the reduction is not much with the increase in treatment time. Particle size distribution of the soil samples treated for different time interval is plotted and is shown in Fig. 6. Study of Fig. 6 reveal that the average particle size increases after the treatment but there is not much effect of treatment time on the particle size.

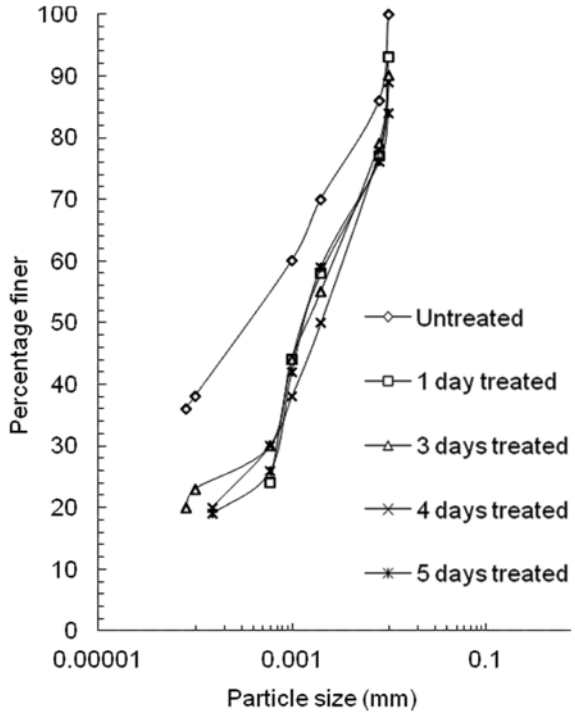

Fig. 6 Comparison of Particle size distribution curve of soil samples treated for different time intervals

4.2 Bentonite soil

The results of bentonite specimens tested for Atterberg's limits and *UCS* before and after microbial treatment are presented in Table 11. A study of this table reveals that for natural soil the liquid limit and plastic limit values due to microbial treatment is varied. The decrease in liquid limit, plastic limit and plasticity index due to microbial treatment is 21.25%, 37.2% and 17% respectively. The percentage increase in *UCS* after treatment is 16.7%. There is marginal improvement for the sterilized soil treated with nutrient solution alone but with microbes and nutrient solution the sterilized as well as unsterilized samples has some improvement. Therefore it can be concluded that the naturally present microbes may also be taking part in calcite precipitation leading to cementation

Table 11 Atterberg limits and unconfined compressive strength test results for different treatment of bentonite

Conditions	*UCS* (kN/m^2)	Free swell	*LL*	*PL*	*PI*
Natural soil	13.62	16	237	50	187
Natural soil treated with nutrient and microbes	15.89	18	189	31	158
Unsterilized treated bentonite	15.89	17	189	31	158
Sterilized and treated bentonite	14.12	17	198	33	165
Microbially Treated	15.89	17	189	31	158
Sterilized soil treated with nutrient only	12	17	240	60	180

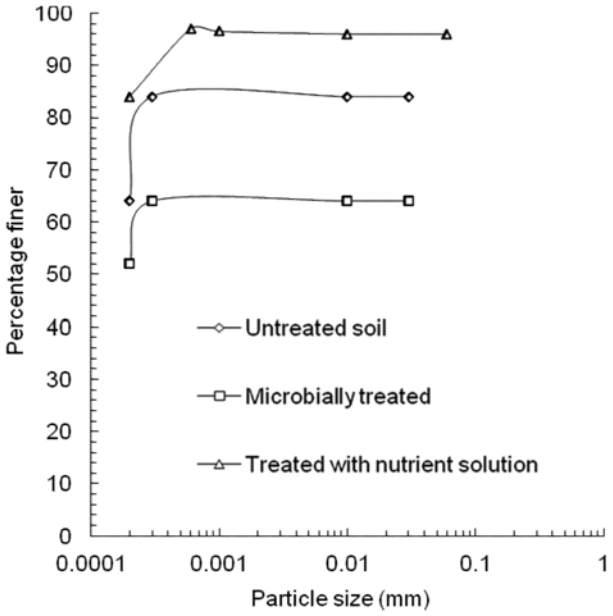

Fig. 7 Comparison of particle size distribution curve of untreated soil sample, sample treated with nutrient solution without microbes and microbially treated bentonite soil

of the soil. The particle size distribution curve was plotted for soil samples before and after microbial treatment is shown in Fig. 7. Study of this figure reveal that the percentage finer decreased for particles of smaller size. There was comparatively less variation in percentage finer for particles of large size as evident from Fig. 7. Therefore, it can be inferred that microbial treatment has higher cementing effect on soils of smaller size.

The soil samples treated for 1, 2, 3, 4, 5 and 15 days to check the influence of treatment time on the soil cementation presented in Table 12. Study of Table 12 reveals that there are no appreciable changes after 2 days of microbial treatment.

The results of soil samples treated with different concentration of microbial treatment solution are presented in the Table 13. Table 13 reveal that as the concentration of solution increases, *UCS* increases initially, and becomes constant. Further study of Table 13 reveals that Atterberg limit

Table 12 Atterberg limits and unconfined compressive strength of soil samples treated for different time interval of bentonite

No. of days	*LL* (%)	*PL* (%)	*PI* (%)	Free Swell (ml/2g)	*UCS* (kN/m^2)
1 day	189	29	158	18	16.2
2 days	182	30	152	17	16.37
3 days	174	29	145	16	17.29
4 days	172	28	144	16	18.24
5 days	171	27	142	16	18.38
15 days	171	26	141	16	18.40

Table 13 Atterberg limits and unconfined compressive strength of soil samples treated for different concentration of bentonite

Property	Natural soil	Treated 1 ml/g	Treated 2 ml/g	Treated 3 ml/g
Liquid limit	237	189	187	186
Plastic limit	50	31.29	28.71	27
Plasticity index	187	157.71	158.29	159
UCS (kN/m^2)	13.62	16.37	17.29	17.98

Table 14 Atterberg limits and unconfined compressive strength results before and after microbial treatment for laterite soil

Conditions	*LL*	*PL*	*SL*	*PI*	*UCS* (kN/m^2)
Natural Soil	46	28	20	18	9.6
Sterilized Soil	40	24	16	16	14.16
Natural Soil + Urea Medium	50	24	20	26	15.26
Natural Soil + Urea Medium + Microbes	48	26.4	20.73	21.6	12.87
Sterilized Soil + Urea Medium	45.4	26.42	25.4	18.98	16.66
Sterilized Soil + Urea Medium + Microbes	52.6	32.78	21.17	19.82	15.3

decreases initially and becomes constant.

4.3 Laterite soil

The results of laterite soil specimens tested for Atterberg limits and *UCS* before and after microbial treatment are presented in Table 14. Study of Table 14 reveals that there is no large variation in the properties of soil due to microbial treatment compared to Kuttanad clay and bentonite. A close examination of Table 14 reveals that nutrient solution without microbes made the soil more plastic. Further study of Table 14 reveals that the *UCS* of the soil samples before treatment was 9.6 kN/m^2 which increased to 12.87 kN/m^2 after the treatment. The *UCS* increased after the microbial treatment as well as with nutrient solution. This is attributed to the chemical reaction between the nutrient solution and the calcium which was present in the soil.

5. Conclusions

Microorganism *Bacillus pasteuri* was used to study the effect on Atterberg's limit and unconfined compressive strength (*UCS*) of Kuttanad clay, bentonite and laterite soil. On the basis of the results presented in this paper, the following conclusions were drawn.

(1) The liquid limit and plasticity index for all clay soils decreased and the unconfined compressive strength increased. Decrease in plasticity index was very high for Kuttanad clay followed by bentonite and laterite.
(2) The average decrease in plasticity index was 24% and percentage increase in unconfined compressive strength was 80% for Kuttanad clay.
(3) Microbial treatment has higher cementing effect on soils of smaller size. Due to microbial treat-

ment the percentage of clay decreased from 47% to 30% and the percentage silt increased from 29% to 48% for the Kuttanad clay.

(4) The soil treated after sterilisation shows an improvement in the properties but that was less than the unsterilised soil.

(5) Naturally present microbes may also be taking part in calcite precipitation leading to cementation of the soil.

(6) Nutrient solution has very less impact on cementation process of Kuttanad clay and bentonite. But for laterite soil the nutrient solution without microbes made the soil more plastic.

(7) The soil samples treated with different concentration of treatment solution shows that the unconfined compressive strength increases up to a concentration of 1.4 gm/ml for Kuttanad clay, and then it decreases. There was no appreciable change after 2 days of microbial treatment.

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