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Performance Study on Stabilization of Fine Grained Clay Soils Using Calcium Source Producing Microbes

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ABSTRACT

In recent years, the method to produce bio-cementation in sand using bacterial calcium carbonate precipitation (BCCP) process has become more popular. The major objective of this research paper is to study the capability of BCCP to enhance the unconfined compressive strength of clayey soils. Two types of bacteria were used to generate calcium carbonate precipitation. The experimental design variables adopted in this study are bacteria types (*L. fusiformis* and *S. pasteurii*), soil types (low compressible clay and intermediate compressible clay), types of externally supplied calcium solution (calcium chloride and eggshell solution), molarities of cementing solution (0.25, 0.50, 0.75 and 1.00 M) and curing period (1, 3 and 7 days). The experimental test results showed that the BCCP process significantly improves the unconfined compressive strength (UCS) of both soils. The improvement however varied with bacterial types, soil types, types of externally supplied calcium solution, molarities of cementing solution and curing period. In BCCP treatment, *S. pasteurii* treated soils give more strength than *L. fusiformis* because of high urease activity of *S. pasteurii* in the order 450 U/ml. The maximum improvement ratio was achieved in CL soil (2.51) compared to CI soil (2.26) due to particle sizes. The optimum externally supplied calcium solution and molarity of cementing medium were established as an eggshell solution and 0.50 M, respectively. The images from scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) analysis confirmed the experimental findings.

1. Introduction

Geotechnical engineers declare clay soil as problematic soil due to its poor strength and excess settlement characteristics (Soon et al., 2013). By improving the engineering behavior of these soils, it can further be used for geotechnical purposes mainly for highway construction. This can be accomplished by adopting any one of the various ground improvement and soil stabilization techniques that enhance the engineering properties of clay soils. A variety of ground improvement techniques are advised viz. Preloading, Compaction, Column techniques, Soil replacement, Soil nailing, grouting, etc. Among the above methods, chemical grouting is found to be the habitually used ground improvement technique in many civil and geotechnical engineering applications (van Paassen et al., 2010).

In the chemical grouting method, the chemical grout made of various chemicals like Silicate, Cement, Arcylamides, Aminoplasts, Aluminium sulphate, Ligno-sulfonates, Polyurethane etc. are used (Cheng et al., 2014). These are either injected into the soil mass with high pressure or mechanically mixed with soil mass which in turn increases the strength capacity and reduces the settlement characteristics of soft soils (Chu et al., 2011). The main adversity in using this method is that most of the grouts stated above are toxic to the environment and living beings (van Paassen et al., 2010).

In recent times, the entire humankind looks forward to a green and sustainable technology in all fields and aspects. Bacterial calcium carbonate precipitation (BCCP) is an efficient alternative for chemical methods that gains higher attention for its sustainability and low cost (Canakci et al., 2015). BCCP is an interdisciplinary

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process that combines the fields of microbiology, geotechnical engineering, and chemistry (Mortensen et al., 2011). It is a natural process performed in the soil which can be exaggerated by introducing a higher amount of bacteria with urease enzyme and cementing material within the soil mass. This in turn raises the production of calcium carbonate end product and improves the mechanical behaviour of the weak soils (Soon et al., 2013). In BCCP process, the calcite precipitation is formed with help of bacteria. BCCP will be executed through two mechanisms. They are bio-cementation and bio-clogging (Chu et al., 2013). Bio-cementation is defined as a biological process to attract the soils through bio-cement generated by bacterial activities in the soil, the engineering behavior of soil might be enhanced. As a result of bio-cementation, bio-clogging takes place.

In most of the previous research works *Sporosarcina pasteurii (S. pasteurii)* was used as a urease producing microorganism for stabilizing coarse-grained soils (Li et al., 2018). The several urease producing bacteria generally available are *Pararhodobacter*, *Bacillus, Sporosarcina*, *Clostridium* and *Desulfotomaculum.* Some of the researchers have compared the improvement made by *S. pasteurii* with any one of the above-mentioned species.

Shreedhar et al. (2018) studied the impact of biological loading on standard sand and aeolian sand by using *Proteus Vulgaris* bacteria. The experimental results showed that the improvement is not up to the level when compared to with *S. pasteurii*. All the previous research concluded that *S. pasteurii* gives higher strength or effective bacterial soil stabilization. In this present study, two different clay soils were stabilized by using *L. fusiformis* and *S. pasteurii* bacteria (Ivanov et al., 2015). Both *L.fusiformis* and *S. pasteurii* bacteria were urease positive microorganisms and increase the strength of the clay soils (Keykha et al., 2014). The increase in strength will be determined by conducting unconfined compression test in the laboratory.

2. Materials and Methods

2.1 Soil Samples

The soil samples used in this present study were of two types namely soil sample A and soil sample B. The soil sample A was collected from Vellode site, Erode district, Tamil Nadu while the soil sample B was taken from a site in Agricultural University, Coimbatore district, Tamil Nadu. The index properties of soil samples were tabulated in Table 1. All the index properties of the soil samples were determined with the help of Indian Standard soil testing procedures. Based on the test results of index properties, the soil sample A was classified as low compressible clay (CL) and the soil sample B was classified as intermediate compressible clay (CI) under IS soil classification system.

2.2 Microorganisms Used

Two different microbes used in this study were *L. fusiformis* and *S. pasteurii*. The mother cultures of *L. fusiformis* and *S. pasteurii* were purchased from MTCC 1286 and MTCC 1761, Chandigarh, respectively. The American type culture collection (ATCC) number of *L. fusiformis* is ATCC 7055 and the ATCC number of *B. pasteurii* is ATCC 11859. *L. fusiformis* is a urease gram-positive, rod-shaped, non-motile microbe. This microbe has a limited length between 2.5 to 3 µm and a limited width between 0.5 to 0.9 µm. The optimum temperature for growth of this microorganism is 30ºC and pH is 9 (http://en.wikipedia.org/wiki/Lysinibacillus_fusiformis). *S. pasteurii* is a gram-positive, rod-shaped and non-motile microbe positive for urease. This microbe has a limited length between 2.7 to 5 µm and a limited width between 0.7 to 1.2 µm. The optimum temperature for growth of this microorganism is 35ºC and pH is 9.5. Under risky environmental conditions, both these microbes can form inactive spherical endospores which are opposing heavier loads, high temperatures, harmful chemicals, and ultraviolet rays (Bhaduri et al., 2016).

Table 1. Index Properties of Soil Samples

Description	Symbol	Soil sample A	Soil sample B
Specific Gravity	G_S	2.65	2.57
Gravel (%)	G	0.7	0.4
Sand (%)	S	28.2	25.5
Silt (%)	M	30.8	31.2
Clay (%)	C	40.3	42.9
Liquid limit (%)	W_L	34	39
Plastic limit (%)	W_P	22	24
Shrinkage limit (%)	W_S	11	10.5
Plasticity index (%)	I_P	12	15
pH of the sample	pH	7.95	7.81
Optimum moisture content (%)	OMC	12	14
Maximum dry unit weight (kN/m^3)	$\gamma_{d,\,max}$	17.5	16.9
Unconfined compressive strength (kN/m^2)	q_u	199	94
Soil classification	IS	CL	CI

The role of *S. pasteurii* and *L. fusiformis* is to produce enzyme urease through its metabolic activity under proper cultivation process (Soon et al., 2013). This urease enzyme hydrolyzes urea into ammonium and bicarbonates. Then the external input calcium solution is applied to form the $CaCO_3$ end product which is responsible for the strength enhancement of clay soils. Eq. (1) indicates the conversion reaction. 1 mole of urea transforms into 2 moles of ammonia and bicarbonates (Kim and Park, 2013).

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

The generation of ammonia gas (NH_4^+) increases the pH and forms a perfect platform for BCCP with the help of calcium ion Ca^{2+} from the externally provided calcium chloride and eggshell solution as given in Eq. (2) (Yasuhara et al., 2012).

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

The generation of calcium source ($CaCO_3$) as the end product is

authoritative for cementing the soil samples. This calcium carbonate end product combines the soil particles through the bio-cementation process and increases the cohesion and hence improves the strength of soils (Cheng et al., 2013). The expedited calcium carbonate firmly combines with soil samples which results in more particle to particle interaction that leads to denser packing of soil samples.

2.3 Cementing Materials

The cementing materials play a crucial role to generate calcite precipitation in the BCCP biological process. The different ingredients used in the cementing technique are summarized in the Table 2. All the ingredients used are of analytical reagent grade. Two different external input calcium solutions (calcium chloride and eggshell solution) are used in different molarities 0.25, 0.5, 0.75 and 1.0 M.

In all the previous studies, $CaCl_2$ was used as an external calcium source to precipitate $CaCO_3$ end product. Excess usage of $CaCl_2$ in soil may be harmful since it results in strength reduction of soils (Choi et al., 2016). Hence in this study, calcium solutions produced from eggshells are used as a cementing medium and compared with the effect of $CaCl_2$ solution. The $CaCl_2$ was bought from Modern scientific company, Coimbatore. The eggshell solution was prepared as per the following steps. The waste eggshells have 94% of calcium salts and it can be dissolved using white vinegar of 5% acidity. First, the eggshells were washed with distilled water and the washed eggshells were put in the oven for one day at a temperature of 105°. After one day the eggshells were removed from oven and kept in the room temperature for one hour. Finally, the eggshells were crushed into powder (Choi et al., 2016). Then the powdered waste eggshell was mixed with white vinegar in the optimum mixing ratio of 1:8 by weight and this solution was placed in a mechanical shaker for one week. The size of the waste eggshell powder used in this study was less than 0.6 mm, and the specific gravity of waste eggshell was found to be 1.20. The different composition present in the eggshell powder was Calcium 35%, Magnesium 0.3%, Potassium 0.03%, Sodium 0.05% and Phosphorous 0.04%. The molarity of the eggshell after dissolution was prepared by the expression M = (molecular weight of eggshells) × (required molarity say 0.25, 0.50, 0.75 and 1.0) × (100) / 1000.

2.4 Microorganism Cultures

In the current study, *L. fusiformis* and *S. pasteurii* were cultivated in nutrient broth at 130 rpm under an incubation temperature of 37°C. 13 g of Nutrient Broth powder was softened in one litre of deionized water which includes 5 g of Peptone (Himedia Laboratory, India), 5 g of Sodium Chloride (Himedia Laboratory, India), 2 g of Yeast Extract (Himedia Laboratory, India) and 1 g of Meat Extract (Niae Chemicals, India). These chemicals were kept in an autoclave at 12°C for 15 minutes under 15 psi pressure. Then the media was cooled down to room temperature and kept for sterility check at 37°C for 24 hours of incubation. After 24 hours' sterile media was taken and about 1% of inoculums were inoculated and kept in an orbital shaker at 120 rpm in 37°C for about 24 hours. Then the culture was harvested and saved at 4°C before to use. The cell concentration of *L. fusiformis* and *S. pasteurii* was calculated by spread plate techniques. Serial dilution was carried out on an incubated culture medium and the diluted culture was spread on the facial surface of the agar plate and the colonies were computed after 17 hours of incubation. In this study, the cell concentration was fixed at a constant rate of 1×10^7cfu/mL for both the bacteria.

Table 2. Ingredients of Cementing Technique

Constituent	Cementation solution	Sterilization
Urea	0.25 M	Syringe filter
Calcium chloride	0.25, 0.5, 0.75 and 1.0 M	Autoclave
Egg shell solution	0.25, 0.5, 0.75 and 1.0 M	Autoclave
Nutrient broth	3 g	Autoclave
Ammonium chloride	1.86 M	Autoclave
Sodium bicarbonate	2.12 g	Autoclave + dry
Adjusted pH	8.0	-

2.5 Preparation of Soil Samples

Before the BCCP stabilization, the soil samples were air-cured in the soil mechanics laboratory at room temperature for 7 days. Then the UCS soil specimen treated with bacteria was prepared as per the following steps. Initially, the bacteria culture solution was mixed to the soil properly and carefully followed by the inclusion of cementation materials. The soil sample was prepared by, to introduce the bacteria and cementation solution as 50 – 50 ratio of optimum moisture content of soils. For soil sample A, the optimum moisture content is 12%, so the inclusion of bacteria and cementation solution was in a combination of 6% bacteria and 6% cementation solution. For soil sample B, the optimum moisture content is 14%, and hence the inclusion of bacteria and cementation solution was in a combination of 7% bacteria and 7% cementation solution. The UCS samples were prepared by making BCCP on the dry side of optimum to attain 95% of maximum dry density. The required amount of dry soil was taken (for soil sample A 151 g and soil sample B 146 g). Then bacteria solution was directly added to soil and hand mixed the soils with bacteria solution. The thoroughly mixed soil sample with bacteria solution was kept undisturbed for about 2 hours. Finally the cementing solution either eggshell or calcium chloride was directly added to the soils and hand mixed thoroughly to get uniform mix of the soils.

Then the UCS soil samples were allowed to cure for a period of 1, 3 and 7 days with the help of saturated sand and damp gunny bags at a temperature of 20°C to 25°C. The UCS test was conducted for the samples according to IS 2720-Part X. The prepared UCS samples had a diameter of 38 mm and a length of 76 mm with a diameter to length ratio of 2. The vertical load was applied at a constant rate of 1.25 mm/min (Sharma and Ramakrishnan, 2016). The entire experimental program is listed in Table 3.

Table 3. Experimental Program

S.No	Type of microbe	External cementing solution	Soil type	Molarity in 'M'	Curing periods	Description
1.	*S. Pasteurii*	Egg Shell solution	CL	0.25	1,3,7 days	0.25M ES
2.		Egg Shell solution		0.50	1,3,7 days	0.50M ES
3.		Egg Shell solution		0.75	1,3,7 days	0.75M ES
4.		Egg Shell solution		1.00	1,3,7 days	1.00M ES
5.		$CaCl_2$solution		0.25	1,3,7 days	0.25M CC
6.		$CaCl_2$solution		0.50	1,3,7 days	0.50M CC
7.		$CaCl_2$solution		0.75	1,3,7 days	0.75M CC
8.		$CaCl_2$solution		1.00	1,3,7 days	1.00M CC
9.		Egg Shell solution	CI	0.25	1,3,7 days	0.25M ES
10.		Egg Shell solution		0.50	1,3,7 days	0.50M ES
11.		Egg Shell solution		0.75	1,3,7 days	0.75M ES
12.		Egg Shell solution		1.00	1,3,7 days	1.00M ES
13.		$CaCl_2$solution		0.25	1,3,7 days	0.25M CC
14.		$CaCl_2$solution		0.50	1,3,7 days	0.50M CC
15.		$CaCl_2$solution		0.75	1,3,7 days	0.75M CC
16		$CaCl_2$solution		1.00	1,3,7 days	1.00M CC
17.	*L. Fusiformis*	Egg Shell solution	CL	0.25	1,3,7 days	0.25M ES
18.		Egg Shell solution		0.50	1,3,7 days	0.50M ES
19.		Egg Shell solution		0.75	1,3,7 days	0.75M ES
20.		Egg Shell solution		1.00	1,3,7 days	1.00M ES
21.		$CaCl_2$solution		0.25	1,3,7 days	0.25M CC
22.		$CaCl_2$solution		0.50	1,3,7 days	0.50M CC
23.		$CaCl_2$solution		0.75	1,3,7 days	0.75M CC
24.		$CaCl_2$solution		1.00	1,3,7 days	1.00M CC
25.		Egg Shell solution	CI	0.25	1,3,7 days	0.25M ES
26.		Egg Shell solution		0.50	1,3,7 days	0.50M ES
27.		Egg Shell solution		0.75	1,3,7 days	0.75M ES
28.		Egg Shell solution		1.00	1,3,7 days	1.00M ES
29.		$CaCl_2$solution		0.25	1,3,7 days	0.25M CC
30.		$CaCl_2$solution		0.50	1,3,7 days	0.50M CC
31.		$CaCl_2$solution		0.75	1,3,7 days	0.75M CC
32.		$CaCl_2$solution		1.00	1,3,7 days	1.00M CC

2.6 Urease Test

The microbes used in this study were subjected to the urease test to ascertain the capacity of microorganisms in hydrolyzing urea and to generate ammonia and carbon dioxide. This urease test was conducted using Christensen's Urea Agar Base (UAB). This urease test medium comprises of urea 20 g/l, NaCl 5 g/l, peptone 1 g/l, glucose 1 g/l, KH_2PO_4 2 g/l, phenol red 0.012 g/l and agar 15 g/l. The urease medium was filter sterilized and the remaining components of medium were autoclaved. UAB slants were inoculated with *L. fusiformis* and *S. pasteurii* bacteria fresh culture, and then kept at 30°C for incubation. When urea was hydrolyzed, ammonia accumulates in the medium and makes it alkaline (Hammad et al., 2013). This rise in pH causes the indicator to change from yellow to deep pink and is a positive test for urea hydrolysis. Control sample and urease negative samples having yellow colour and urease positive samples having deep pink colour which is shown in Fig. 1.

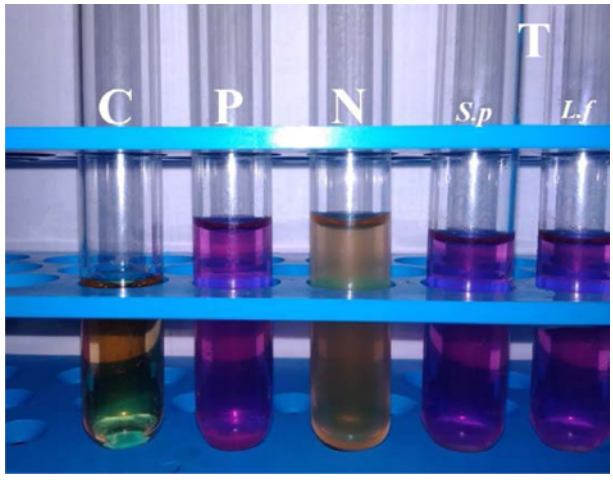

Fig. 1. Urease Test of Microbes (C – Control, P – Positive, N – Negative T – Test Samples) (S. p – *Sporosarcina pasteurii* and *L.* f – *Lysinibacillus* fusiformis)

2.7 Urease Activity of Microbes

The urease activity in supernatant microbe solution was determined by calculating the generation of ammonia from urea by using Nessler's method. The reaction solution contained 0.1 ml of supernatant microbe solution, 1 ml of urea (0.2 M) and 0.9 ml of Tris-acetate buffer (0.05 M) with adjusted pH of 7.5 and was incubated at 30°C for 10 minutes. After 10 minutes, the reaction was stopped by inactivating the enzyme with 1 ml of 10% trichloroacetic acid. Then the mixture was centrifuged at 12800 rcf for 10 minutes. Adding 1 ml of Nessler's reagent, the solution was made up to 50 ml with deionized water while swirling. The yellow color produced was measured at 405 nm and the urease activity was determined from a standard graph of ammonium chloride. This standard curve was prepared earlier by making different dilutions of ammonium chloride solution (1 mg/ml). 1 unit of urease activity is the amount of urease that produces 1-micron mole of ammonia in one minute at 37°C.

3. Results and Discussions

3.1 Effect of *S. pasteurii* and *L. fusiformis* Microbes on CL and CI Soils (7 days strength)

Figures 2 and 3 represent the unconfined vertical stress versus strain for CL and CI stabilized with *S.pasteurii* microbe. The UCS of untreated CL and CI soil are 199 kPa and 94 kPa respectively. After treatment with *S.pasteurii* and eggshell as a cementing solution, the UCS of CL soil is increased from 199 kPa to 499 kPa. The improvement ratio is calculated as per Eq. (3).

$$\text{Improvement Ratio (IR)} = \text{UCS}_{\text{treated}} / \text{UCS}_{\text{untreated}} \quad (3)$$

The improvement ratio of treated CL soil is 2.51 times greater when compared to untreated CL soil. Similarly, the soil treated with S. *pasteurii* and $CaCl_2$ as a cementing solution, the UCS of CL soil is increased from 199 kPa to 434 kPa. The improvement order of treated CL soil is 2.18 times greater when compared to untreated CL soil. In untreated CI soil also, the addition of *the S.pasteurii* microbe was observed to increase the UCS. Maximum strength was recorded when the CI soil was stabilized with *S. pasteurii* and eggshell as a cementing solution and the improved strength was from 94 kPa to 212 kPa. The increased strength was 2.26 times greater when compared to untreated CI soil. Similarly, the CI soil treated with *S. pasteurii* and $CaCl_2$ as a cementing solution, the improved UCS strength was recorded in the range from 94 kPa to 171 kPa. The improvement ratio was 1.82 times greater when compared to untreated CI soil.

Figures 4 and 5 show the unconfined vertical stress versus strain for CL and CI stabilized with *L.fusiformis* microbe. While using eggshell as a cementing medium, the UCS is increased from 199 kPa to 372 kPa for CL soil and for CI soil the increased strength was from 94 kPa to 186.71 kPa. The improvement ratio was 1.87 and 1.98 for CL and CI soil, respectively. Similarly, for $CaCl_2$ cementing medium, the UCS value was increased from 199 kPa to 247 kPa for CL soil and for CI soil the strength was improved

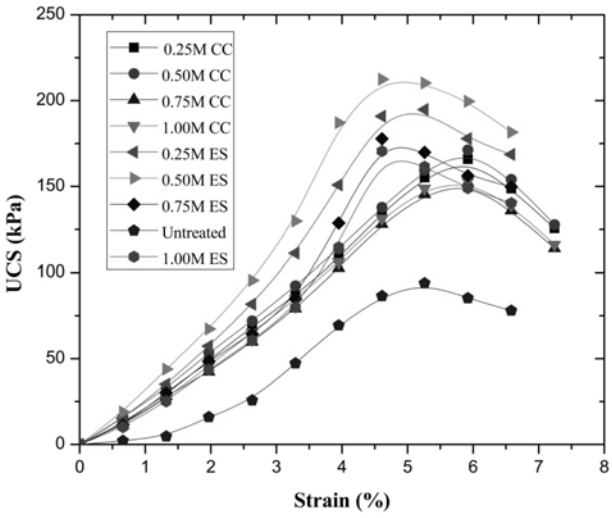

Fig. 3. Effect of *S. pasteurii* on UCS of CI Soil

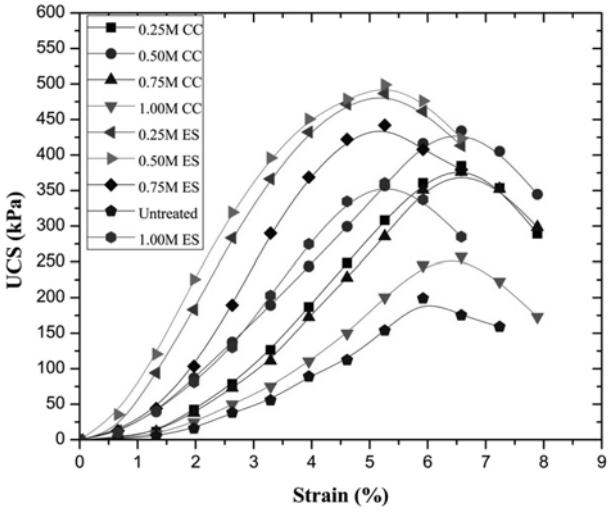

Fig. 2. Effect of *S. pasteurii* on UCS of CL Soil

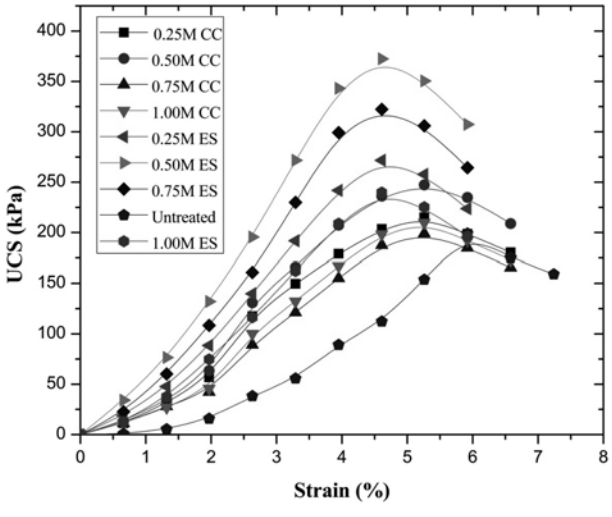

Fig. 4. Effect of *L. fusiformis* on UCS of CL Soil

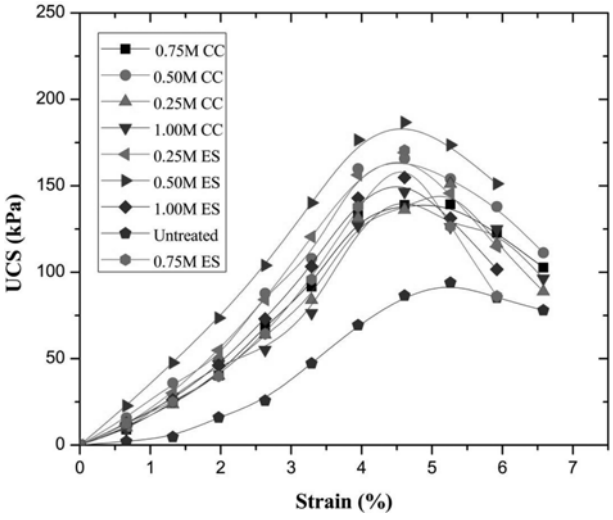

Fig. 5. Effect of *L. fusiformis* on UCS of CI Soil

from 94 kPa to 165 kPa. The improvement ratio was 1.24 and 1.75 for CL and CI soils, respectively.

The interpretation of the above results concluded that both microbes enhance the strength behavior of the soil but it was found that *S. pasteurii* microbe treated soil gives higher strength when compared to *L. fusiformis* microbe treated soil. This trend was based on the urease producing activity of the microbes. The urease producing activity of *S. pasteurii* microbe was approximately 450 U/ml and for *L. fusiformis* microbe, it was 370 U/ml. The urease activity is directly proportional to the strength improvement of soil i.e., as the urease activity of microbe increases then the strength of the soil is also increased. So the *S. pasteurii* microbe gives higher strength for both CL and CI soils because of high urease activity compared to *L. fusiformis*. The maximum compressive strength achieved was 499 kPa. This high strength increment for soil shows that the generation of bio-calcification process which reflects a treated soil sample matrix with more calcium carbonate minerals in the voids and thus enhancing the bonding capacity of the soil particles.

Asgari et al. (2015) presented that the UCS of CL soil treated with 3% lime content was improved from 395 kPa to 900 kPa and also Harichane et al. (2011) mentioned that the UCS of CL soil treated with 8% lime content was increased from 222 kPa to 460 kPa. In this current study, the CL soil stabilized with *S. pasteurii* microbe and eggshell as a cementing solution has the UCS improvement in the range of 199 kPa to 499 kPa. In our current study for CL soil treated with *S.Pasteurii* and eggshell (0.5 M) cementing medium gives $CaCO_3$ precipitation as 9.59% and for $CaCl_2$ cementing medium (0.5 M) cementing medium gives $CaCO_3$ precipitation as 9.32%. For CI soil treated with *S. Pasteurii* and eggshell (0.5 M) cementing medium gives $CaCO_3$ precipitation as 8.87% and for $CaCl_2$ cementing medium (0.5 M) cementing medium gives $CaCO_3$ precipitation as 8.39%. For CL soil treated with *L. Fusiformis* and eggshell (0.5 M) cementing medium gives $CaCO_3$ precipitation as 8.56% and for $CaCl_2$ cementing medium (0.5 M) cementing medium gives $CaCO_3$ precipitation as 6.10%. For CI soil treated with *S. Pasteurii* and eggshell (0.5 M) cementing medium gives $CaCO_3$ precipitation as 8.73% and for $CaCl_2$ cementing medium (0.5 M) cementing medium gives $CaCO_3$ precipitation as 7.94%. Choi et al. (2016) shows that the maximum value of calcite precipitation for Ottawa sand treated with calcium chloride and eggshell cementing medium was 6.6% and 7.7% respectively which is lesser than our current study. In present study the maximum value of calcite precipitation for CL soil treated with calcium chloride and eggshell cementing medium was 9.32% and 9.59%, respectively.

Sharma and Ramakrishnan (2016) reports a UCS value of 345 kPa for CI soil treated with *S. pasteurii* microbe and calcium chloride cementing medium. In this research, the maximum UCS of CI soil with *S. pasteurii* and calcium chloride solution was 171 kPa. It is noteworthy that the maximum strength of treated soil achieved by Sharma and Ramakrishnan (2016) is higher than current study results. This may be because of the fact that the percentage of fine-grained soils in the current study (74.1%) which is greater than (53.6%) Sharma and Ramakrishnan (2016) might have influence the bacterial movement. The microorganisms are having the capacity of moving freely in the void spaces available in the coarser materials by passive diffusion or self – propelled movement, while in finer materials the free movement and entry of microbes is hindered. Mitchell and Santamarina (2005) reports that the bacteria were not entering into the void spaces where the pore size of soil less than 0.4 μm. In this study, the particle having size less than 0.4 μm in CL and CI soils was 40.3% and 42.9%, respectively. The CL soil has a high amount of coarse sized materials than CI soil, and hence the bacterial movement was good enough to enhance the strength of CL soil. But in *L. fusiformis* microbe stabilization, the improvement ratio of CI soil (for eggshell 1.98 and $CaCl_2$ 1.75) was more when compared to CL soil (for eggshell 1.87 and $CaCl_2$ 1.24). The fact that the size of the *L. fusiformis* microbes was quite small as compared to *S. pasteurii* microbe, promoted permeating ability to generate densely packed particle to particle contact so that this bacterium could offer more specific surface area for bond generation of calcium carbonate precipitation.

The results of Gomez et al. (2014) were comparable to the current study as the estimated calcite content in both studies were almost equal (Calcite content is 9.8% in Gomez et al. (2014) and 9.6% in current study). The unconfined compressive strength of cushion sand (Poorly graded sand) treated with MICP technology was 3,950 kPa and the estimated average calcite content was 9.8% in Gomez et al. (2014). In our current study the unconfined compressive strength of Low compressible clay soil was 499 kPa and the estimated average calcite content in our current study was 9.59%. The unconfined compressive strength obtained from the current study was smaller than the Gomez et al. (2014). The difference was caused mainly by the difference in soil type, bacteria, cementing medium and BCCP procedure followed in these studies.

Young's modulus or elastic modulus is referred to as the ratio between the stress to the strain in elastic range of soil behavior. The

Table 4. Young's Modulus of Bacterial Treated Soils

S.No	Bacteria type	Soil type	External cementing solution	Molarity of cementing medium in 'M'	Young's modulus (E) in MPa
1.	*S. Pasteurii*	CL	Eggshell	0.25	11.91
2.				0.50	15.19
3.				0.75	10.86
4.				1.00	8.48
5.			$CaCl_2$	0.25	8.46
6.				0.50	9.18
7.				0.75	8.87
8.				1.00	7.81
9.		CI	Eggshell	0.25	3.22
10.				0.50	4.37
11.				0.75	2.64
12.				1.00	2.93
13.			$CaCl_2$	0.25	2.79
14.				0.50	3.02
15.				0.75	2.39
16.				1.00	2.58
17.	*L. Fusiformis*	CL	Eggshell	0.25	6.18
18.				0.50	8.42
19.				0.75	7.95
20.				1.00	5.63
21.			$CaCl_2$	0.25	4.49
22.				0.50	6.24
23.				0.75	7.08
24.				1.00	4.86
25.		CI	Eggshell	0.25	3.75
26.				0.50	4.78
27.				0.75	3.91
28.				1.00	2.99
29.			$CaCl_2$	0.25	2.51
30.				0.50	3.42
31.				0.75	3.06
32.				1.00	2.52

Young's modulus of CL and CI soils treated with *S. pasteurii* and *L. fusiformis* microbes are tabulated in Table 4. It can be seen that the highest Young's modulus value is achieved as 15.19 MPa when the CL soil was treated with *S. pasteurii* microbe. Strozyk and Tankiewicz (2016) reports a Young's modulus of clay soils lies between 4 MPa to 16.7 MPa. In our current study, the Young's modulus lies between 2.39 MPa to 15.19 MPa. It is concluded that Strozyk and Tankiewicz study gives slightly higher Young's modulus than our current study results. The fact beyond that was higher percentage of clay fraction present in our current study than Strozyk and Tankiewicz study (Casey et al., 2016).

3.2 Effect of Molarity of Cementing Medium

For all the experiments, the urea concentration in the cementing medium was fixed as 0.25 M whereas the concentration of externally applied calcium source i.e., both eggshell solution (ES) and calcium chloride solution (CC) was used as 0.25, 0.50, 0.75 and 1.00 M. Figs. 6 and 7 show the results of UCS of CL and CI soils for varying molarity of cementing solutions. The maximum strength was achieved in 0.5 M of the eggshell (ES) cementing medium for both the soils treated with the *S.pasteurii* microbe (BP). The high molarity of cementing medium tends to reduce or inhibit the urease activity of microorganisms which leads to a decrease in calcium carbonate precipitation ratio. The bar chart representation shows that the calcium carbonate precipitation ratio was higher for 0.50 M of cementing medium because the amount of urease in the solution hydrolyzed almost all the urea.

Choi et al. (2016) reports the UCS of Ottawa sand treated with eggshell and calcium chloride cementing solution as 418 kPa and 370 kPa, respectively, whereas in current research, the UCS of CL soil treated with eggshell and calcium chloride cementing

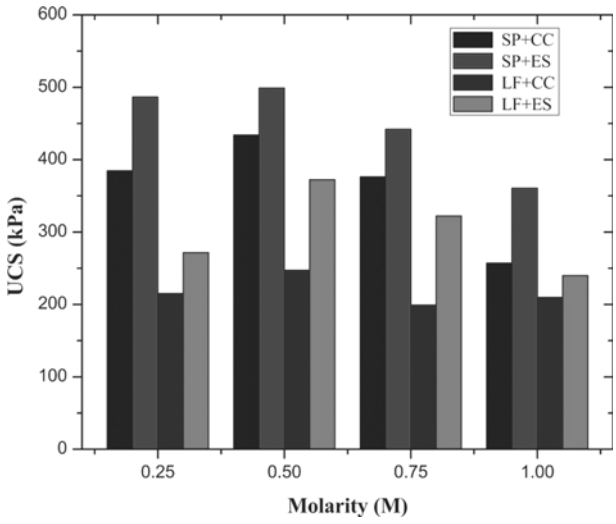

Fig. 6. Effect of Cementing Medium with Different Molarities on CL Soils

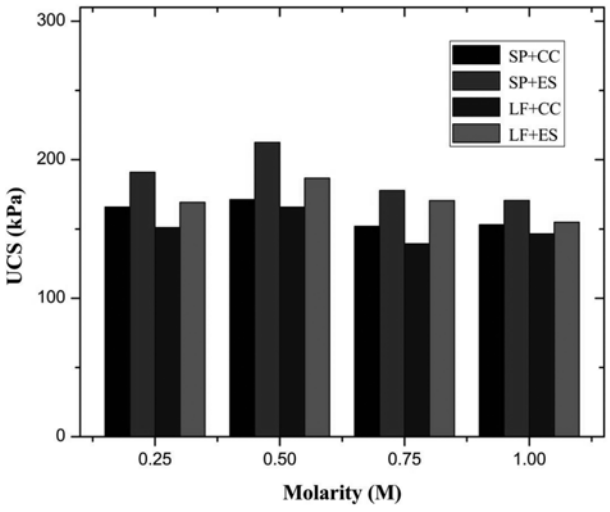

Fig. 7. Effect of Cementing Medium with Different Molarities on CI Soils

solution is obtained as 499 kPa and 434 kPa, respectively. By comparing the above two cases, it can be concluded that the eggshell solution used as a cementing solution gives higher strength when compared to calcium chloride solution for both the soils. This may be because of the fact that the eggshell solution has more calcium carbonate 94% (% of ash content present in the eggshell was 94%) than calcium chloride solution which increases the strength of soil (Lechtanski, 2000; Choi et al., 2016). In current study, same molarity of the both eggshell and calcium chloride cementing medium was used (0.25 M, 0.50 M, 0.75 M and 1.00 M). But calcium source present in the eggshell and calcium chloride cementing medium was different. The calcium source present in the eggshell cementing medium was 35% and the calcium source present in the calcium chloride cementing medium was 24%. Therefore the strength enhancement was high in eggshell treated clay soil specimens when compared to calcium chloride treated clay soil specimens.

3.3 Effect of Curing Period on Soils

The UCS of CL and CI soils treated with different microbes and also different molarity of cementing medium for varied curing period has been presented in Tables 5 and 6. The test data revealed that the curing period makes a major influence on the UCS of treated soils. The unconfined compressive strength of the microbial treated soils is improved by increasing the number of curing days.

CL soil treated with *S. pasteurii* microbe with eggshell as

Table 5. Effect of Curing Period on CL Soil

S.No	Type of microbe	Cementing medium	Different molarity of cementing medium in 'M'	Unconfined compressive strength (kPa)		
				1 day curing	3 days curing	7 days curing
1.	*S. pasteurii*	Egg shell solution (ES)	0.25	442.57	463.60	486.61
			0.50	464.98	481.06	499.09
			0.75	398.93	414.98	442.04
			1.00	319.62	340.53	360.55
		$CaCl_2$ solution (CC)	0.25	337.57	356.62	384.71
			0.50	408.05	421.09	434.16
			0.75	330.01	350.18	376.20
			1.00	216.32	235.12	257.18
2.	*L. fusiformis*	Egg shell solution (ES)	0.25	223.04	243.64	271.68
			0.50	328.21	341.30	372.34
			0.75	289.05	307.23	322.32
			1.00	213.52	227.76	239.85
		$CaCl_2$ solution (CC)	0.25	179.63	195.09	215.12
			0.50	202.16	220.15	247.22
			0.75	158.96	173.99	199.06
			1.00	173.67	188.73	209.76

Table 6. Effect of Curing Period on CI Soil

S.No	Type of microbe	Cementing medium	Different molarity of cementing medium in 'M'	Unconfined compressive strength (kPa)		
				1 day curing	3 days curing	7 days curing
1.	*S. pasteurii*	Egg shell solution (ES)	0.25	181.92	184.27	190.93
			0.50	198.37	205.82	212.46
			0.75	157.78	163.25	177.79
			1.00	152.58	160.94	170.61
		$CaCl_2$ solution (CC)	0.25	146.81	155.08	165.88
			0.50	153.17	159.32	171.23
			0.75	135.92	143.94	151.97
			1.00	132.95	147.76	153.04
2.	*L. fusiformis*	Egg shell solution (ES)	0.25	156.15	162.43	169.22
			0.50	178.63	185.90	186.71
			0.75	152.41	161.66	170.52
			1.00	140.79	147.56	154.82
		$CaCl_2$ solution (CC)	0.25	133.05	140.39	151.07
			0.50	146.72	156.02	165.78
			0.75	123.27	131.80	139.28
			1.00	129.38	135.12	146.50

cementing medium solution showed the maximum strength improvement. The strength improvement ratio is 2.34 times greater for a 1-day cured sample and 2.51 for 7 days cured sample than untreated soil. This result confirmed that the bio-calcification process is much faster in the early curing stages i.e., the improvement ratio of a 7-day cured sample was only 0.17 times higher when compared to a 1-day cured sample. This scenario was observed for both CL and CI soils treated with *S.pasteurii* microbe and *L. fusiformis* microbe. Park et al. (2014) report the UCS of poorly graded sand (SP) treated with 8% calcium carbonate for 7 days curing as 251.43 kPa. In the present study, the maximum strength of CL soil treated with *B.pasteurii* microbe and eggshell cementing medium for 7 days curing was 499 kPa. For the sake of comparison, it can be visualized that the untreated soil UCS strength for Park et al. (2014) was 54.42 kPa while soils considered in this research has a UCS of 199 kPa.

The improvement ratio versus curing period for CI soil treated with *S.*pasteurii and *L.fusiformis* is shown in Figs. 8(a) and 8(b). Whereas the improvement ratio versus curing period for CL soil treated with *S.pasteurii* and *L.fusiformis* are shown in Figs. 9(a) and 9(b). By comparing all the cases the maximum improvement ratio has been achieved as 2.51 for CL soil treated with *S. pasteurii* and 0.50 M eggshell cementing solution. Sidik et al. (2014) and Li et al. (2018) have shown that the growth of microbes from 0 to 10 hours would be in the lag phase while from 10 hours to 24 hours, the bacterial growth would be in the logarithmic phase

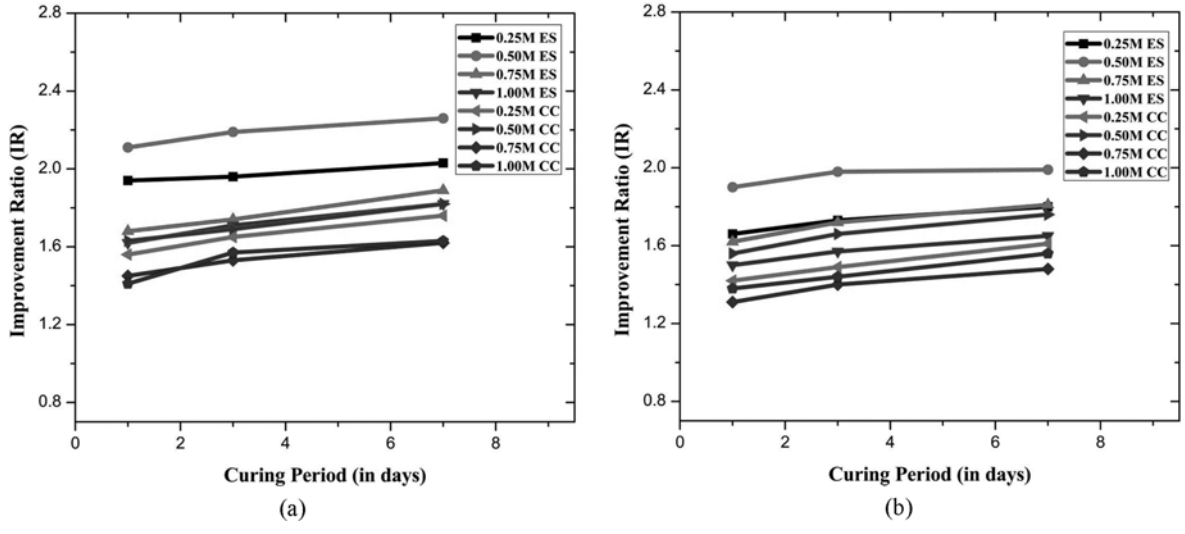

Fig. 8. Shear Strength Improvement Ratio of CL Soil: (a) Improvement Ratio vs Curing Period of CI Soil Treated with *S. pasteurii* Microbe, (b) Improvement Ratio vs Curing Period of CI Soil Treated with *L. fusiformis* Microbe

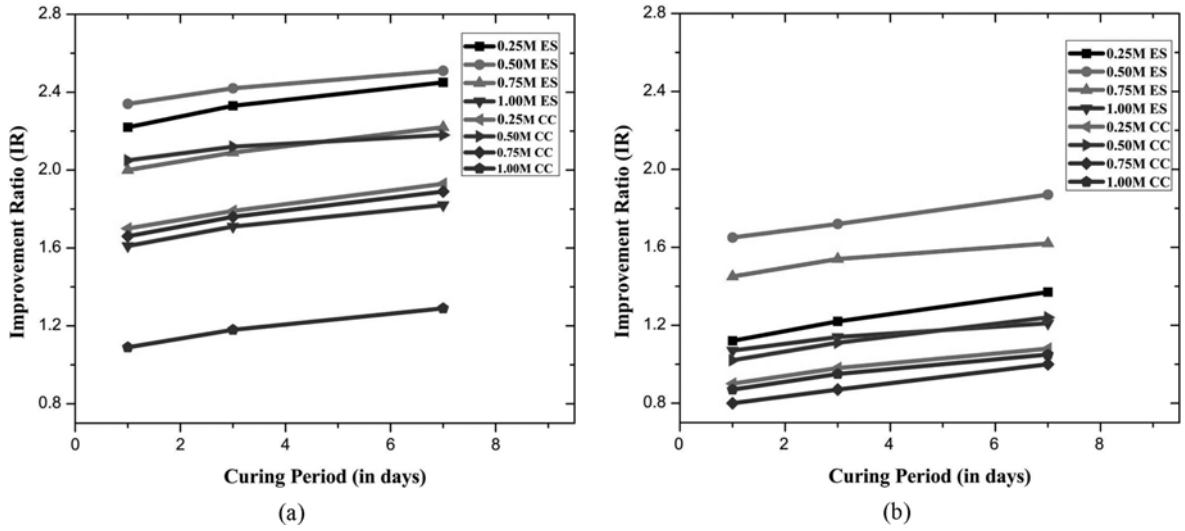

Fig. 9. Shear Strength Improvement Ratio of CL Soil: (a) Improvement Ratio vs Curing Period of CL Soil Treated with *S. pasteurii* Microbe, (b) Improvement Ratio vs Curing Period of CL Soil Treated with *L. fusiformis* Microbe

and from 24 hours to 48 hours, the bacteria growth would be in the stationary phase. It is observed that between 0 hours to 26 hours, the urease producing ability of bacteria was quite high, after which it got slightly decreased. Hence the improvement ratio was quite higher for to 1-day curing, after which it is increased by a very nominal value.

3.4 Micro Structural Analysis by SEM+EDX Images

The microstructure of bacterial treated soil specimens was characterized with the help of SEM and EDX images. Figs. 10(a), 10(b) and 10(c) show the SEM images of untreated soil, *S. pasteurii*, with $CaCl_2$ cementing medium treated soil and *S. pasteurii* with eggshell cementing medium treated the soil. These images expose the view of calcium crystals precipitated between soil particles. The untreated virgin soil sample shows the smooth particle surfaces which can observed in the Fig. 10(a).

In the SEM of soil specimen treated with calcium chloride cementing solution (Fig. 10(b)), some calcite ($CaCO_3$) crystals have been identified on the soil particles which were observed as bright white-colored calcite crystals in some places. More amounts of such calcite crystals were identified on the soil particles when the soil samples were treated with the eggshell cementing solutions which are shown in Fig. 10(c).

In addition to SEM analysis, EDX was carried out on the BCCP treated soil specimens to categorize the concentrations of different elements present in the soil specimens. EDX is an analytical method used for the analysis of elements or characterization of chemicals present in the specimens. Results of EDX analysis are represented as an EDX spectrum graph. These graphs represent the elements corresponding to each of its peak value. The larger the peak in a spectrum, higher is the concentration of the element in the specimen. Fig. 11(a) illustrates the EDX carried out on an untreated soil specimen. In this, can be was identified that the intensity of Si and O elements are in a higher percentage since they are the main elements in soil particles. Fig. 11(b) shows the

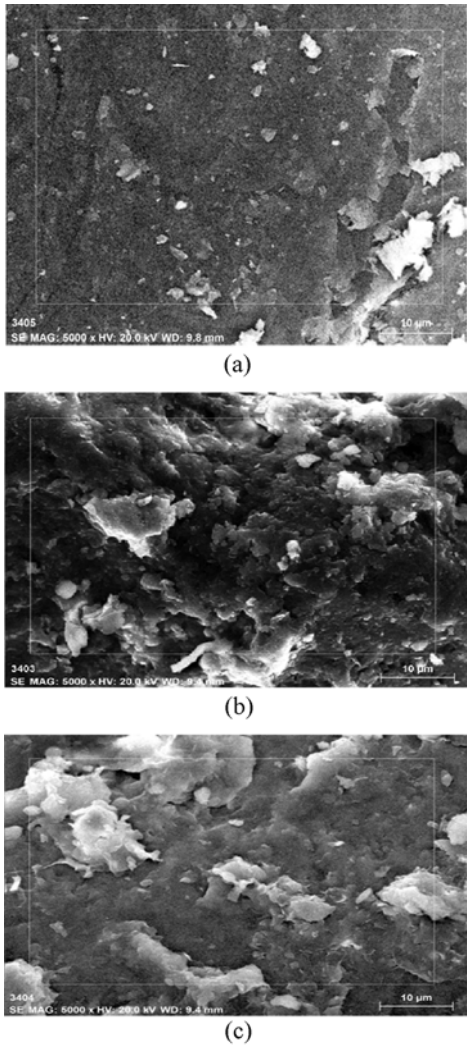

Fig. 10. Scanning Electron Microscopy Analysis of Untreated and Treated Soils: (a) SEM Image of Untreated Soil, (b) SEM Image of *S. pasteurii* and $CaCl_2$ Cementing Medium Treated Soil, (c) SEM Image of *S. pasteurii* and Egg Shell Cementing Medium Treated Soil

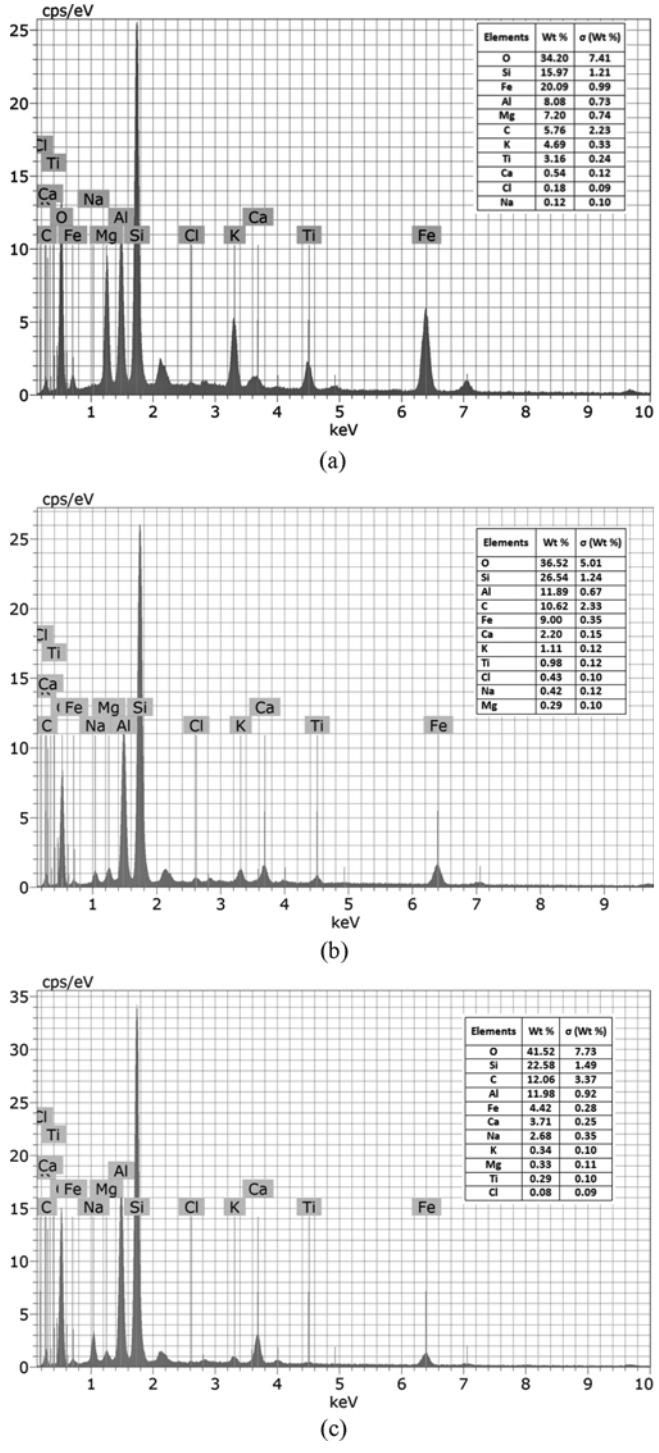

Fig. 11. Energy Dispersive X-Ray Spectroscopy Analysis of Untreated and Treated Soils: (a) EDX on Untreated Soil Specimen, (b) EDX on Soil Treated with $CaCl_2$ Cementing Medium with *S. pasteurii,* (c) EDX on Soil Treated with Eggshell Cementing Medium with *S. pasteurii*

EDX carried out on a soil sample treated with $CaCl_2$ cementing medium with *S. pasteurii*. The intensity of the elements such as Silica, Oxygen, Carbon, and Calcium are high when compared to the untreated soil specimens. Fig. 11(c) shows the EDX carried out on a soil specimen treated with eggshell cementing medium with *S. pasteurii*. The three main elements in calcite ($CaCO_3$) namely Calcium, Carbon, and Oxygen are seen to be high among all the elements along with Silica and Oxygen. The EDX images also indicated the traces of chlorine when the soil treated with eggshell cementing medium.

4. Conclusions

Totally 32 sets of the experimental program was conducted to study the ability of BCCP in increasing the shear strength of CL and CI soils. The following conclusions are drawn based on the experimental study:

1. Both *S. pasteurii* and *L. fusiformis* microbe increase the UCS of CL and CI soils. But the *S. pasteurii* microbe gives higher strength increment for both the soils due to the higher urease activity when compared to *L. fusiformis*.
2. The CL soil shows higher strength than CI soil due to the presence of higher coarse sized particles. In concise, a high strength improvement ratio is observed in CL soil, which is 2.51 times than the untreated soil.
3. The unconfined compressive strength improvement ratio of the CI soil (for eggshell - 1.98 and $CaCl_2$ - 1.75) was more when compared to that of CL soil (for eggshell - 1.87 and $CaCl_2$ - 1.24) since CI the soil particles treated with *L. fusiformis* microbes are smaller size and the permeating ability of the microbe is quite high.
4. The eggshell cementing medium gives higher strength for both types of soil than the calcium chloride cementing medium due to their naturally available high calcium source. Also, the excess calcium chloride present in the soil is harmful and hence the eggshell solution is best suited for BCCP technique.
5. The 0.50 M of cementing solution concentration was confirmed as the optimum dosage along with the urea concentration of 0.25 M. The higher concentrations of cementing solution reduce the urease ability of both the microbes.
6. As the curing period is increased, the corresponding UCS of both CL soil as well as CI soils is marginally increased. This result concluded that the bio-calcification process is much faster in early curing stages i.e., the improvement ratio of 7-day cured sample was only 0.17 times higher when compared to 1-day cured sample.
7. The SEM and EDX images confirmed the calcite precipitation and also highlighted the increased intensity of main components of $CaCO_3$.

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