An Overview of the Factors Affecting Microbial-Induced Calcite Precipitation and its Potential Application in Soil Improvement

Wei-Soon Ng, Min-Lee Lee, and Siew-Ling Hii

Abstract**—**Microbial-induced calcite precipitation (MICP) is a relatively green and sustainable soil improvement technique. It utilizes biochemical process that exists naturally in soil to improve engineering properties of soils. The calcite precipitation process is uplifted by the mean of injecting higher concentration of urease positive bacteria and reagents into the soil. The main objective of this paper is to provide an overview of the factors affecting the MICP in soil. Several factors were identified including nutrients, bacteria type, geometric compatibility of bacteria, bacteria cell concentration, fixation and distribution of bacteria in soil, temperature, reagents concentration, pH, and injection method. These factors were found to be essential for promoting successful MICP soil treatment. Furthermore, a preliminary laboratory test was carried out to investigate the potential application of the technique in improving the shear strength and impermeability of a residual soil specimen. The results showed that both shear strength and impermeability of residual soil improved significantly upon MICP treatment. The improvement increased with increasing soil density.

Keywords**—**Bacteria, biocementation, bioclogging, calcite precipitation, soil improvement.

I. Introduction

NOWADAYS, new construction on weak soils has become inevitable owing to the growing worldwide scarcity of land. The weak soil deposits are commonly characterized by low strength and high compressibility [1]-[3]. Soils in tropical regions, like Malaysia, experience further softening owing to the intense and prolonged downpours.

Soil improvement techniques require evolution in order to ensure effective and efficient improvement, and at the same time possess sustainable and environment friendly characteristics. Chemical grouts available in the market such as Portland cement, lime, asphalt, sodium silicate, and etc have proven successful in soil improvement [4]-[8]. However, the use of these artificial injection formulas often modifies the pH level of soil, contaminates soil and groundwater attributed to their toxic and hazardous characteristics [4], [9].

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In recent years, a relatively green and sustainable soil improvement technique, termed as *Microbially Induced Calcite Precipitation (MICP)* has been introduced. The technique utilizes biochemical process in soil to improve its engineering properties (i.e. strength, impermeability). The applications of this technique have shown promising achievement in diverse fields, i.e. improvement of concrete strength and durability [10], [11], brick durability [12], soil (or sand) strength [13]-[16], sand impermeability [17], [18]. Ivanov and Chu performed an approximate cost comparison between the raw materials for microbial grouting and the conventional chemical grouting [19]. They suggested that the cost for microbial grouting ($0.5 – $9 / m^3 of soil) is significantly cheaper than that of chemical grouting ($2 – $72 / m^3 of soil).

The main objective of this paper is to provide an overview of the factors affecting the MICP in soil. Furthermore, a preliminary laboratory test is carried out to investigate the potential application of the technique in improving the engineering properties of soil (i.e. shear strength and impermeability). The effectiveness of the MICP soil improvement technique is determined by comparing the shear strength and permeability of the control and the MICP treated soil specimens. The calcite formed in the treated soil is further examined using Scanning Electron Microscope (SEM).

II. Biocementation and Bioclogging

Biocementation improves the shear strength of soil through the production of soil particle-binding materials, as the result of introducing bacteria and cementation reagents into the soil. The soil cementation materials are mostly carbonates, silicates, phosphates, sulphides and hydroxides [19]. Calcium carbonate (calcite) is an attractive element to be studied in biocementation because calcite formation is commonly found in nature. In addition, urease positive bacteria are widespread in the environment, and this made the in situ soil treatment does not likely require the introduction of foreign ureolytic bacteria [20]. The native ureolytic bacteria can be multiplied through nutrient injection until their growths reach desired concentration.

Bioclogging is a process where the soil void is filled by the product from microbial-induced biochemical process. The clogging of soil restricts the water flow through the soil, and hence reduces its permeability. Vandevivere and Baveye [21] and Abdel Aal et al. [22] found that the permeability of soil

reduced significantly through the accumulation of biomass and production of exopolymeric substances. The accumulation can occur at soil pore throat or uniformly on soil particle surface.

III. Biological Processess of MICP

The urease enzyme, supplied directly into soil or produced in situ by bacteria, decompose urea ($CO(NH_2)_2$) in the soil through a chemical reaction known as hydrolysis of urea:

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

The ammonium (NH_4^+) released from urea hydrolysis results in local pH rise and commences the precipitation of calcium carbonate. The high pH at localised area increases the tendency for bacteria itself to serve as nucleation site for calcite crystal. Calcite is precipitated through the combination of carbonate ions (CO_3^{2-}) from the hydrolysis of urea and the calcium ion (Ca^{2+}) from supplied calcium chloride:

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

The calcite ($CaCO_3$) generated from the chemical reactions is responsible for the biocementation and bioclogging of soil specimens.

Many bacteria are capable of producing urease enzyme from their microbial activity [23]. The study performed by Bachmeier et al. has shown that urease from the microbial activity is crucial for MICP to take place [24]. The calcite precipitation process depends essentially upon four elements: calcium ion concentration, dissolved inorganic carbon (DIC) concentration, pH, and availability of nucleation sites [25], [26]. Nevertheless, the environmental conditions (e.g. salinity, temperature, nutrient, etc) also have their influences on MICP performance [26].

IV. Factors Affecting the Performance of MICP

A. Nutrients

Nutrients are the energy sources for bacteria, and hence it is critical to provide proper and sufficient nutrient for calcite producing bacteria. Nutrient is supplied to bacteria during culture stage and soil treatment stage. The common nutrients for bacteria include CO_2, N, P, K, Mg, Ca, Fe, etc [27]. The lack of organic constituents in soil is a limitation for bacteria growth. The supply of nutrient into soil specimen during soil treatment process is essential. Numerous previous reported studies have included 3 g/l of nutrient broth into the treatment solution to sustain the growth and viability of urease producing bacteria [9], [28], [29]. The supply of nutrient is to ensure the bacteria can sustain long enough to support calcite precipitation in order to achieve the desired level of improvement.

B. Types of Bacteria

The bacteria type that suitable for MICP application should be able to catalyst the urea hydrolysis and they are usually urease positive bacteria. The typical urease positive bacteria are genera *Bacillus*, *Sporosarcina*, *Spoloactobacilus*, *Clostridium* and *Desulfotomaculum* [30]. The aerobic bacteria are preferable as they release CO_2 from cell respiration, and CO_2 production is paralleled by the pH rise due to ammonium production.

Bacillus sp. is a more common type of bacteria used to precipitate calcium carbonate in their micro-environment through catalytic conversion of urea to ammonia and carbon dioxide [26], [31]. The common types of bacillus used in previous studies were B. sphaericus in repairing or improving the durability of concrete [10], [32], B. Megaterium in improvement of concrete strength and durability [11], [33], and B. Pasteurii in concrete and soil improvement [12], [34], [35]. However, the amount of calcite produced in MICP varied with the types of Bacillus strains [36].

C. Geometric Compatibility of Bacteria

Bacteria are the most abundant microbes in soil [37], [38]. Their sizes are mostly ranging from 0.5 to 3.0 μm [27]. Soil microbes transport across the soil through the pore throat between soil particles, either by self-propelled movement or by passive diffusion. The geometric compatibility of urease-producing bacteria is critical whenever the transportation of bacteria within the soil is required for soil treatment. Small pore throat size would limit their free passage, depends on the size of microbes and soil composition. As an example, bacteria with size ranging from 0.3 to 2 μm can move freely within sandy soil with particle size of 0.05 to 2.0 mm [39]. Significant amount of silt and clay in soil would have inhibitory effect on bacteria's movement. This inhibitory effect obstructs the bacteria distribution in soil. It is thus essential to take into considerations the type of soil, its pore throat size, and size of bacteria when selecting the appropriate type of bacteria for MICP treatment.

D. Bacteria Cell Concentration

Higher bacterial cell concentration supplied to the soil sample would certainly increase the amount of calcite precipitated from MICP process [40]. The rate of urea hydrolysis has a direct relationship with the bacterial cell concentration, provided sufficient cementation reagents are available. High concentration of bacteria produces more urease per unit volume to commence the urea hydrolysis.

Li et al. [41] and Stocks-Fischer et al. [28] both suggested that bacteria cell served as nucleation sites for calcite to precipitate in biochemical reaction. Lian et al. [42] studied the crystallization by *Bacillus Megaterium*. They identified from SEM images that nucleation of calcite takes place at bacteria cell walls. The availability of nucleation sites is one of the key factors for calcite precipitation [43]. Stocks-Fischer et al. [28] also demonstrated that calcite precipitation is associated with the concentration of *Bacillus Pasteurii*, one of the urease positive bacteria.

E. Fixation and Distribution of Bacteria in Soil

The urease positive bacteria should be distributed evenly and fixed in place when they are injected into the soil. Improper method of injection might cause the bacteria to be located only in certain part of soil or be flushed out from the soil. Harkes et al. [44] studied on the methodologies to dispense bacteria and settle them over a 18cm long sand bed. They found that injection of undiluted bacteria suspension, followed by the one pore volume of high salinity fixation fluid (50 mM of calcium chloride) could successfully retain almost all bacteria suspension in the sand bed.

High salinity solution encourages flocculation, and this promotes the adsorption of bacteria and retention in sand column [45], [46]. Nevertheless, low salinity solution (e.g. fresh surface water) has its advantage where homogenous distribution of bacteria is required at large sand body. Low ionic strength and adsorption strength of bacteria in low salinity solution allow them to transport over great distances [44]. Last but not least, fixation fluid in higher flow rate flushes bacteria cell over larger distances compare to lower flow rate.

F. Temperature

The microbial activity and growth are less sensitive to the temperature within the range of 20 to 30 °C. The rate of urea hydrolysis is marginally higher in 30 °C, as compare to 20 °C. Increment in temperature after 30 °C does not promote the decomposition rate any further [18]. It is, however, impractical to alter or control the soil temperature while the MICP treatment is performed on soil specimen or in situ.

It is suggested to select a calcite forming bacteria that live optimum in soil temperature. The soil temperature varies with latitude, altitude, incident solar radiation, moisture content, conduction, type of soil, depth of soil and etc [47]-[49]. As an example, Abdul Rahim Nik et al. performed a study on soil temperature in Malaysia at open area and forest [50]. They found that the average soil temperature for open area (from depth 0 to 30 cm) is approximately 30 °C throughout the year. This makes *Bacillus Megaterium* suitable for MICP application in Malaysia considering the optimum growth temperature for this bacterium is also 30 °C [51]-[53]. Other than that, the temperature of injected cementation solution will affect the ambient temperature in soil, given that the specific heat of water is higher than soil [48].

In terms of urease enzyme, Sahrawat [54] stated that the optimum temperature for urease activity lies at approximately 60 °C. Urease activity increased with increasing temperature from 10 °C and reached peak at 60 °C, the activity was inhibited at 100 °C when temperature is raised further. The optimum temperature reported by Sahrawat [54] is consistent with the findings from Liang et al. [55] and Chen et al. [56]. This optimum temperature for urease activity, however, is impractical to be applied for soil treatment either on site or in laboratory.

G.Reactant Concentration

Refer to (1) and (2), the products from 1 mole of urea and 1 mole of calcium chloride would react to form calcite. A solution contains equimolar of both reactants would provides better conversion to calcite [18]. In terms of weight, the stoichiometric ratio of 2.5 for urea and calcium chloride is critical in order to achieve complete production of calcite, considering the molecular weights of urea ($CO(NH_2)_2$) and calcium chloride ($CaCl_2 \cdot 2H_2O$) are approximately 60 g/mole and 147 g/mole, respectively.

The concentration of reagents and the salinity have their influences on the MICP process [57]. The effects of reagents (e.g. urea and calcium chloride) concentration on calcite precipitation were studied by Nemati et al. [18]. Higher concentration of urea and calcium chloride extends the amount of composited calcite [18], [40]. This phenomenon is further supported by De Muynck et al. [58], where the weight gain of soil sample due to carbonate precipitation was higher with higher concentration of reagents.

This statement, however, is only valid for certain concentration of reagents. High salinity has inhibitory effect on microbial activity and calcite precipitation [59]. The salinity of cementation fluid is mainly contributed by calcium salt. Urea and calcium chloride with lower concentration contributes to a satisfied level of urea decomposition into ammonia. Microbial activity might be retarded by high salinity, thus limiting or eliminating the urease production from ureolytic bacteria [18], [60]. In the other case, urease is still available for MICP process at high salinity but the ratio of calcite precipitated and theoretically calcite composition decreased with increasing reactants' concentrations [17], [58], [61].

The variation of calcite precipitation in high salinity can be explained by the halophilic characteristic of bacteria, where salinity has less inhibitory effect on moderately halophilic bacteria compare to those with non-holophilic. Moderately halophilic bacteria capable to grow at wide range of salinity, and should be used in the soil treatment if environment with high salinity is expected [57]. Several moderately halophilic bacteria were studied for their calcite precipitation capability in salinity environment, and they showed different response towards increasing concentration [62]-[64].

High composition of calcite by high concentration of reagents can be achieved provided urease enzyme is introduced to the soil but not produced in situ by the ureolytic bacteria. Besides, repeated injection of reagents into the soil would increase the composition of calcite [18], [58].

H.pH

The calcite precipitation commences when urea is decomposed by urease enzyme. The urease enzyme is produced by microbial metabolic activities and as a result, urea hydrolysis is preferable around the cell. Like all other enzymes, urease enzyme only active at certain range of pH. Stocks-Fischer et al. [28] stated that the optimum pH for urease enzyme is in the range of 7.5 to 8.0, and this finding is

further supported by the works of Evans et al. [65] and Arunachalam et al. [66].

Stocks-Fischer et al. [28] found that the urease activity increased rapidly from pH 6.0 to 8.0. Urease activity reached its peak at pH 8.0 and decreased gradually at higher pH. Nevertheless, promising level of urease activity is still available at pH 9.0. The pH of reactant medium will increase gradually during the MICP process. The ammonia produced by urea hydrolysis will increase the pH of medium. Carbon dioxide from the urea hydrolysis and microbial respiration, on the contrary, acts as buffer to the pH rise. The species of calcite forming microbes should adapt to this range of pH in order to perform well in producing urease enzyme for urea decomposition.

I. Injection Method

Studies pertaining to the favorable and proper treatment method of MICP can be found in abundance. Most researches on MICP were performed by injection method which is similar to the grouting of artificial material for soil improvement. Martinez et al. [67] [1] performed an investigation to identify the suitable injection method to obtain uniform calcite distribution in sand column. The stopped-flow injection of cementation fluid allowed uniform calcite precipitation along the 0.5 m sand column. On the other hand, continuous injection method promoted abundant calcite precipitation near the injection port, but the calcite content decreased over distance from the injection point. Similar finding was obtained from the numerical modeling developed by Barkouki et al. [68]. The stopped-flow injection is capable of distributing cementation fluid evenly in sand column before the composition of calcite.

V. Preliminary Tests on MICP Soil Treatment

Most previous reported studies on MICP soil treatment have proven successful in improving the engineering properties of sand. In the present study, the engineering properties (shear strength and permeability) of residual soil is investigated, as more than 70% of peninsular Malaysia land areas comprise residual soil.

A. Materials

Bacillus Megaterium was chosen as the calcite forming bacteria in this study since its application has been proven in improving the strength of concrete. The factors affecting the performance of MICP, as discussed previously, are made constant in this preliminary test.

The residual soil specimens were prepared in three different densities, i.e. 85% of MDD, 90% of MDD, and 95% of MDD. The physical properties of the residual soil were tested in the laboratory, as tabulated in Table I.

TABLE I
Residual Soil Composition

	Residual Soil
Composition	
Gravel (%)	0
Sand (%)	29
Silt (%)	55
Clay (%)	16
Liquid Limit (%)	58.0
Plastic Limit (%)	44.3
Plasticity Index	13.7
Soil Classification BSCS	MHS (Sandy Silt)
Maximum Dry Density (MDD)	1563 kg/m^3

B. Reactant Concentration

The Bacillus Megaterium was grown in nutrient broth at temperature of 37°C under aerobic condition. The grown culture (5×10^7 cfu/ml) was harvested at late exponential phase and mixed with air-dried soil specimens. The cementation fluid contained cementation reagents, 3 g nutrient broth, 10 g NH_4Cl, and 2.12 g $NaHCO_3$ per litre of deionized water [9], [28], [29], [69]. The cementation reagents employed in this study are urea ($CO(NH_2)_2$) and calcium chloride ($CaCl_2 \cdot 2H_2O$) prepared at a concentration of 0.25 M.

The residual soil was compacted into desired density within a fabricated mould (50 mm diameter and 150 mm in length). The MICP treatment was performed by injecting one pore volume of cementation fluid into the soil specimens at an interval of 6 hours for treatment duration of 48 hours. The flow velocity was controlled at approximately 1.7×10^{-5} m/s. These treatment configurations were remained constant for all the soil specimens.

Besides the soils treated with bacteria and cementation fluids, another two sets of control specimens were also prepared, i.e. untreated specimens, and specimens treated with cementation fluids only. The treatment with cementation fluids only was used as an indirect indicator for the existence of naturally inhibited calcite forming bacteria in the soil specimens.

The soil specimens that underwent different treatment conditions were tested for their shear strength and permeability. The shear strength was obtained by performing the unconfined compression test on 50 mm diameter saturated specimen. The permeability was determined from the falling head permeability test. All the test procedures were in compliance with the British Standard (BS 1377) [70].

C. Result and Discussion

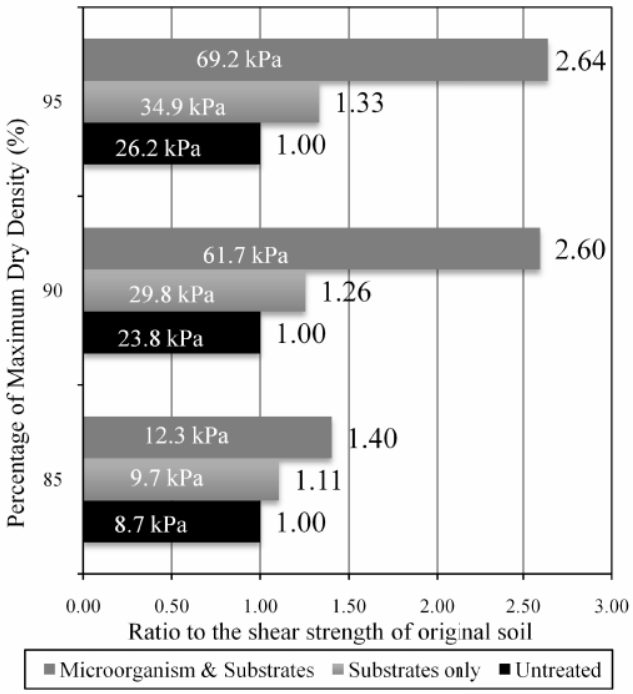

Fig. 1 Shear strength results of residual soil specimens

Fig. 1 presents the shear strength results of the residual soil specimens. It was evident that the MICP treatment with the introduction of foreign bacteria was capable to improve the shear strength of the residual soils for all densities. The shear strength improvement ratios increased with the increasing soil density, i.e. 1.41 (41%), 2.59 (159%) and 2.64 (164%) for specimens of 0.85MDD, 0.90MDD and 0.95MDD, respectively.

Fig. 2 shows the permeability results of the residual soil specimens. The saturated permeability of MICP-treated soil was markedly reduced for all densities. Consistent with the results of shear strength, the reduction in saturated permeability of soil also increased with density. The greatest reduction in permeability occurred in the densest specimen where the ratio of saturated permeability between treated and untreated specimens was 0.26 (a reduction of 74%). The reduction ratios for 0.90MDD and 0.85MDD specimens were 0.40 (60%) and 0.45 (55%), respectively.

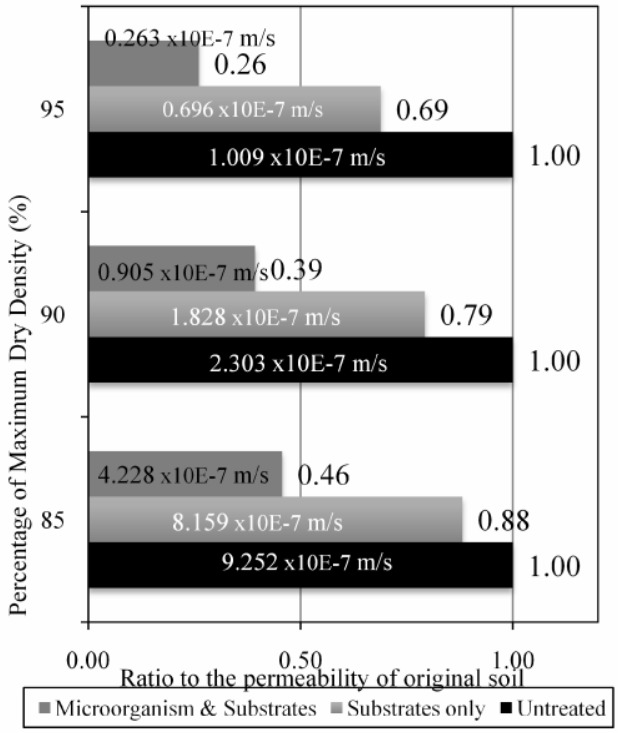

Fig. 2 Permeability results for residual soil specimens

For the soil specimens that treated with cementation fluids only, the shear strength and permeability exhibited slight alterations in the absence of foreign bacteria. The results implied that MICP was triggered by the urease positive bacteria exist naturally in the soil deposits. The improvement, however, were comparatively lower than those specimens treated with the introduction of *Bacillus Megaterium* into soil specimen.

Fig. 3 demonstrates the SEM images of the residual soil specimens that treated under the three treatment conditions, i.e. untreated, treated with cementation fluids only, and treated with bacteria and cementation fluids. A relatively smooth particle surface was observed in the untreated specimen (Fig. 3a). Upon the treatment with cementation fluids only (Fig. 3b), some calcite crystals were found on the particle surface. The formations of calcite crystal were found in abundance for the specimen treated with bacteria and cementation fluids (Fig. 3c). On closer observations, rod-shaped bacteria were found in intimate contact with these calcite crystals.

VI. Conclusion

In the present preliminary test, the MICP showed improvement in both shear strength and impermeability of residual soil. The improvement increased with increasing soil density. For the soil specimens that treated with cementation fluids only, the shear strength and permeability exhibited slight alterations due to the presence of native urease positive bacteria.

The results implied that native urease-forming bacteria can be utilized in MICP soil improvement, with sufficient and appropriate nutrient provided. This work showed its importance as this green technique can be employed effectively on residual soil improvement. Hence, it can be utilized as soil improvement method in Malaysia once this technique is mature on site application.

In addition, the engineering properties of residual soil were improved significantly, and this provides essential information to author in further research. A study is currently carried out on the optimum conditions (e.g. reagents' concentration, treatment duration, etc) in residual soil improvement to makes this technique one step closer to site application.

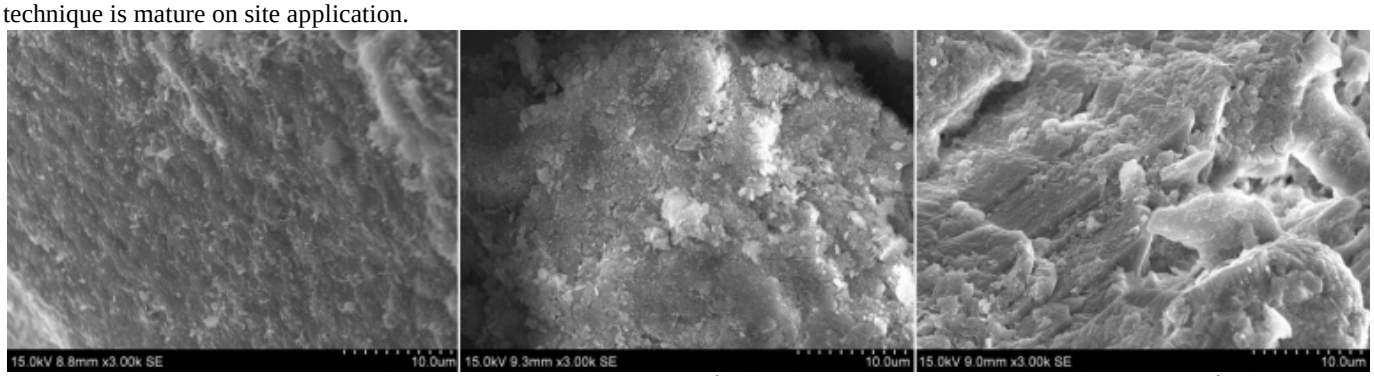

Fig. 3 SEM images (a) untreated soil; (b) treated with cementation fluids only; (c) treated with bacteria and cementation fluids

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