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Properties of Biomineralization Process in Various Types of Soil and Their Limitations

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Weak and problematic soils affect stability and safety of structures founded on them. The problems occur due to limitation or absence of shear strength or over shear stress applied on the soil during loading which then lead to large settlement and consequently failure to the founded structures. Replacing the soil with better materials would be very costly as these types of soil are normally extended to a great depth under the ground surface. The proposed solution for such kind of soils is curing weak soils instead of replacing them. One of the proposed treatment methods is bio-grouting in which the conditions and the scales of the application differs according to the soil types and limitations. Reviews on previous researches have shown that treatment results by bio-grouting method are controlled by several factors, such as size of pores, value of pH, duration of treatment, presence of water and electro-kinetic effect, which give impact to treatment results quality and quantity. The outcome can go as far as killing the bacteria which then reduce the microbial growth if it was not controlled. Understanding of bio-grouting process and its application will help in improving the engineering properties of the weak soils and its applications.

Keywords : Bio-grouting, Biomineralization, Electro-kinetic treatment, soil improvement, soil treatment, soft marine clay.

I. INTRODUCTION

Soil can be categorized as problematic when it is not capable to carry loads applied on it and caused large settlements. This affects the safety and the efficiency of the structures constructed on this type of soils. The weakness is due to high water content and the absence or the limited values of shear resistance. The presence of weak soils such as soft marine clay within coastal areas and riverbanks can extend to a huge depth which makes it impossible or, very costly to be replaced or constructing special foundation system for the structures. Therefore, some treatment methods by using micro-organisms products such as Biomineralization process [1,2] and Bio-Enzymatic Stabilization [3] were proposed to overcome the problems.

Injections of micro-organisms into soil is a process proposed for treatment or curing weak soils which also known as bio-grouting. The process is based on injecting the soil with ureolytic bacteria which then producing inorganic minerals into the soil for treatment. The treatment occurs when the medium around the bacteria is helpful for reduplicating cells of the bacteria and producing the urease enzymes that ureolysis the urea and help in forming the cementation crystals that increase the strength of the treated soil. The process of forming the cementation crystals by the bacteria is called biomineralization [4,5]. Minerals synthesis is divided into two types: Biologically controlled mineralization (BIM) and Biologically controlled mineralization (BCM) [5,6]. Minerals formed outside the cell (extracellular) due to metabolic activity for BIM whereas the minerals formed and directly synthesized at an exact location for BCM process under certain conditions [7].

Microbial induced calcium carbonate precipitations (MICCP) are aimed to be technically applicable for revolutionary treatment on different tenders by researchers [7]. It can also been used for treating weak soils [8–13] and cracks of the concrete structure elements by the $CaCO_3$ precipitation [14,15].

II. BIOMINERALIZATION PROCESS

Microbial induced calcium carbonate $CaCO_3$ precipitation MICCP is formed by bacteria on steps inside bacteria's cells forming carbonates ions CO_3^{2-} and on the cells surface forms calcium carbonate $CaCO_3$. The urea $CO(NH_2)_2$, through ureolysis process on steps, decomposes to form carbonates ions CO_3^{2-} as shown in chemical Equations 1 and 2.

$$\mathbf{CO(NH_2)_2 + 2H_2O \xrightarrow{Bacteria} NH_2COOH + NH_3} \quad (1)$$

$$\mathbf{NH_2COOH + H_2O \xrightarrow{Bacteria} NH_3 + H_2CO_3} \quad (2)$$

The outer surface of the bacteria is electrically charged by negative charge that attracts the positive ions of the calcium Ca^{2+}. During this process the formation of cementation matter of calcium carbonate $CaCO_3$ occurs, see Figure 1 [9]. The precipitation of the calcium carbonate $CaCO_3$ develops on the cell surface of the bacteria externally [16,17]. The biomineralization of $CaCO_3$ using the reaction between urea and calcium chloride $CaCl_2$ at the cell's surface follows the chemical equation 3.

$$\mathbf{CaCl_2 + 2NH_3 + H_2CO_3 \longrightarrow CaCO_3 + 2NH_4Cl} \quad (3)$$

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The biomineralization depends on the activity of the bacteria. Bacteria's activity is mainly based on cell reduplication and the production rate for the urease enzyme. Both are controlled by the pH value of the medium around the bacteria.

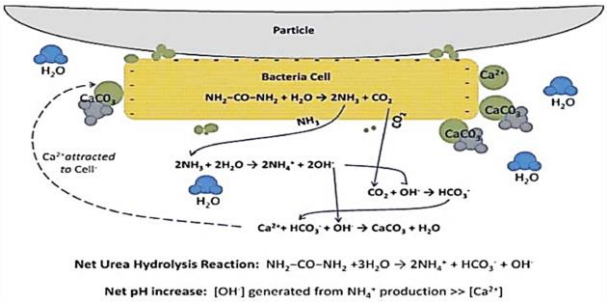

Fig. 1.Perception of calcium carbonate process on sand particles by the MICCP bacteria [9]

III. METHODOLOGY

The wide range of grains size of soils makes the treatment method differentiates from coarse to fine soils. The rate of curing, duration and required amount of cementation material also differ based on soil particle size. Treatment took place in coarse soils (sandy soils and crashed aggregates) [8–11,18–22] and fine soils (kaolinite) [12] showed a very high improvement of shear strength and the resistance of the treated soil. The treatment was observed enhancing suction and shear strength, and reducing the void ratio, permeability, liquid limit and index of plasticity [23,24].

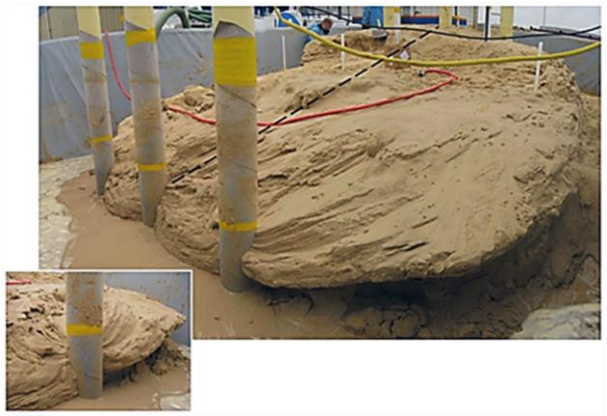

Fig. 2.Shape of treated sand part showing the variation of treatment in both longitudinal and transversal directions and the presence of treatment effect at the corners [10]

Production of $CaCO_3$ can be managed mechanically through controlling the pressure of the injection point (wells) and the outlets. Large scale experiment of the treatment shows a variety in both longitudinal direction (direction of the flow from the inlets to the out lets) and the transversal direction (the perpendicular direction to the flow direction from inlet to the outlet). The variations in longitudinal direction are due to speed of the flow at the inlet location but these variations decrease towards the outlet. The flow speed increases because of time, reduction in pore size and microbial ureolysis activity with time. The decrease of the shear strength, content and mass of $CaCO_3$ as shown in Figures 2 and 3 are in the same direction of the flow [10,18]. The reason for the transversal variation might be the presence of the flow channels and their speed changing with the time that influences the concentration and the time of formation of the cementation matter $CaCO_3$ as shown in Figure 2 [10]. SEM done on another test shown the same results [20]. The increase in the speed of the flow inside the channels makes the formation of $CaCO_3$ difficult due to the clogging which caused by the bacteria for the precipitation of $CaCO_3$ [10]. This appears in SEM scan that types of $CaCO_3$ crystals are different which is reflected the disturbance of bacteria spreading inside treated soil as shown in Figure 4 [19].

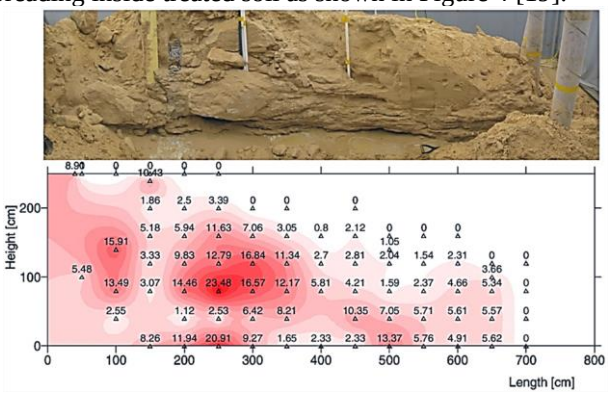

Fig. 3.Variation of the treatment size showing decrease from the inlet to the outlet. The inlet locates at 150cm and the outlet at 650cm [18]

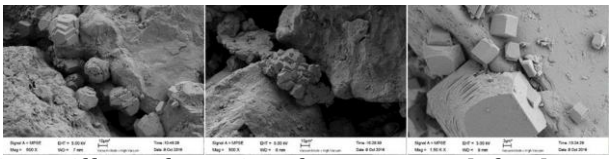

Fig. 4.Different formation of $CaCO_3$ crystals for the same treated soil sample [19]

This method showed that it can be applied easily to a large scale as followed in the experiment and can give the desired shear strength if it is controlled [10,18]. Cohesive soils have a problem of limitation of pore size. The pores are too small that it would not allow the bacteria to migrate inside the soil during the treatment. The process of the treatment would be very difficult and the required results of the treatment would be hard to achieve. Electro-kinetic effect helps in achieving the required results by forcing the input materials and the production of the bacteria to migrate inside the soil [25]. The process showed a significant improvement in the treated soil for undrained shear strength as shown in Figure 5 [12]. The pores where filled with $CaCO_3$ causing that significant improvement as shown in Figure 6. The location of the injection of the urea, calcium chloride and bacteria has influence on the undrained shear strength of the treated soil. There are two proposed methods of injection. The first method showed that the injection of the urea with the bacteria at the cathode (inlet) is more efficient than the second method where the urea with the calcium chloride were injected at anode (outlet). This might be due to enough time given for the bacteria to ureolysis the urea before reacting with the calcium chloride for forming $CaCO_3$. In addition, repeating the cycles of injection gives better resistance to erosion as precipitations interact with soil particles in pores and produce high cohesion [26]. Table I is summarizing the treatment methods based on collected data in this research.

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Table- I: Comparison of different treatment methods from previous work.

Method	Soil type	Process	Restrictions	References
Mechanical biogrouting	Cohesive less soil	- Forcing bacteria and input materials to enter soil by pressure	- Clogging at inlets - Non-uniform treatment along distance between electrodes - Applicable for course grained soil only	[10,11,18,27]
Electro-kinetic biogrouting	Coarse grained soil	- Forcing bacteria and input materials to migrate through soil pores using electro-kinetic method	- Clogging at inlets - Non-uniform treatment along distance between electrodes - Bacteria challenging migration inside pores	[1,12]
Surface percolation	Coarse grained soil	- Applying bacteria by wetting without fully saturation to guarantee full coating of soil grains by $CaCO_3$	- Weak compared to submerged method - Needs different injection locations for the same treatment zone	[13,28]
Submerged flow	Coarse grained soil	- Same as surface percolation but by applying fully submergence to the treated soil	- 3 times wasting materials compared to surface percolation method	[13,28]

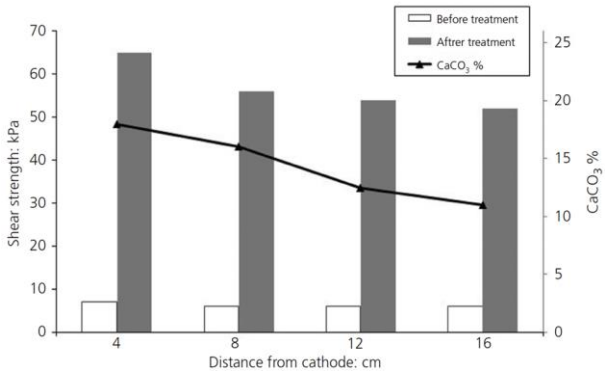

Fig. 5.The achieved undrained shear strength for treated 65% of kaolinite soil [12]

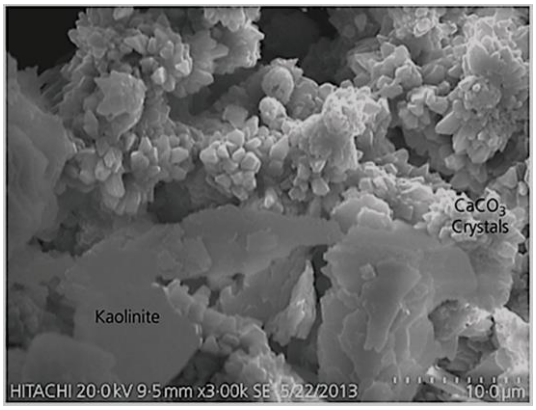

Fig. 6.SEM showing $CaCO_3$ crystals beside in the pores between the kaolinite particles after treatment process [12]

IV. CONTROLLING FACTORS

Biomineralization process in the soil controlled by several factors; mineralogy of the soil, pores size, pH values that influences both microbial ureolysis and microbial growth, application of electro-kinetic, and duration of treatment. These factors control the quality and the quantity of the treatment during the biomineralization process.

A. Pore size

Soil pores size control the permeability of the soil. The amount of the $CaCO_3$ that will be formed during the treatment process depends on the rate of the migration of input materials through the soil texture [29]. The hydrolytic restrictions would be a reason of declination in bacteria activity by restraining bacteria and its products to flow through soil pores [30,31] and interrupts the precipitation process of $CaCO_3$. Furthermore, the permeability of the soil can change during the treatment. It decreases with time due to the clogging occurrence during the formation of the $CaCO_3$ inside the pores. This clogging disturbs the flow of water through pores of treated soil [32]. This can be an advantage in targeting a reduction of permeability [23,24,33,34] but disadvantage for extending the treating zone and must be considered during treatment process. For optimizing the treatment process, microbe size can be controlled relatively with pores size [29].

As getting far away from the inlets, the amount of the $CaCO_3$ decreases by time due to clogging that causes the reduction of pores size near zones to inlets. This clogging makes; a limited treated zone measured along the distance from the inlets and disturbance in the flow inside the soil with time which affects the quality of the treatment in the section perpendicular to the flow direction [10,11,15,18,31,35–37]. The pores and its size also regulate the time and the process of the migration of the bacteria inside the soil. Generally, the coarse soils allow the bacteria to migrate easily compared to the cohesive soils.

The crystal size of precipitations increases with increasing of solution concentration. As size of crystals increases clogging pore occurs. Clogging during treatment process will reduce the permeability of the treated soil. This reduction in permeability can be as high as 20% for 2% $CaCO_3$ of treated sample's weight [30,35]. To overcome the problem of clogging, more injection zones for bacteria were proposed to achieve the targeted and uniform strength as shown in Figure 7 which is based on processing of microbial injection, see Figure 8 [28].

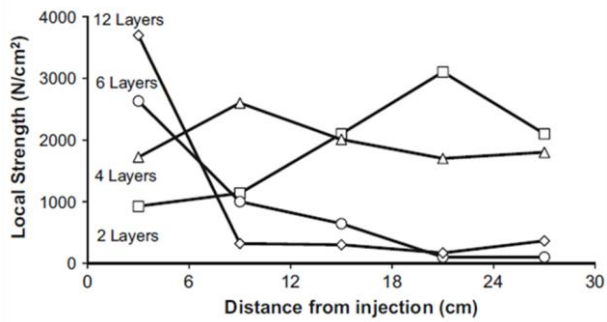

Fig. 7. Number of injection layers of bacteria on shear strength [36]

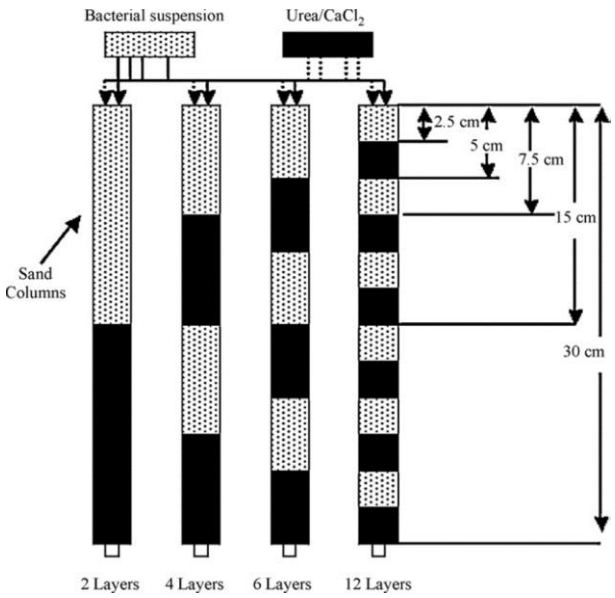

Fig. 8.Different methods of bacteria injection for different number of layers [36]

B. Value of pH

The microbial growth and the microbial ureolysis activity depend on value of soil pH. Production of urease enzyme by the bacteria increases as pH values increased. As a results, the rate of microbial growth and its ureolysis activity also increased [38]. The microbial ureolysis is similar to the curve of the microbial growth Figure 9 [39] and that affects the amount of the treatment resulted from the biomineralization process. The microbial growth must continue in stationary phase along the duration of the treatment to achieve the required improvement for the treated soil.

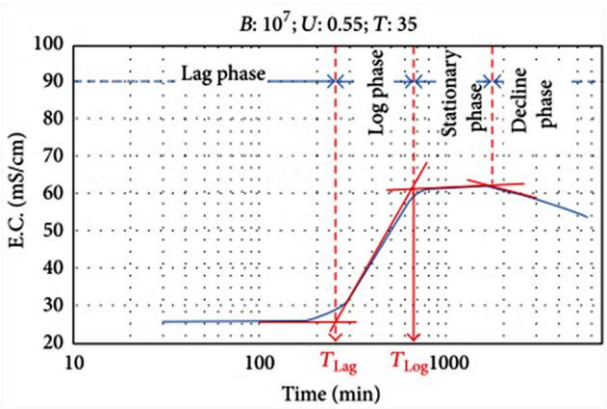

Fig. 9.The pattern of microbial ureolysis similar to the curve of the microbial growth [38].

Value of pH controls the type and the amount of $CaCO_3$. Ureolysis of urea by bacteria produced ammonia NH_4^+ and bicarbonates HCO_3^-, and this production increases the pH value [40–44]. With presence of different calcium Ca^{2+} and magnesium Mg^{2+} compounds the rate of pH increases with time as shown in Figure 10. For calcium Ca^{2+} compounds, the best is $CaCl_2$ [42]. This pH increment results in acceleration of the precipitation rate of $CaCO_3$ [38,42,52,44–51] as shown in Figure 11 [51].

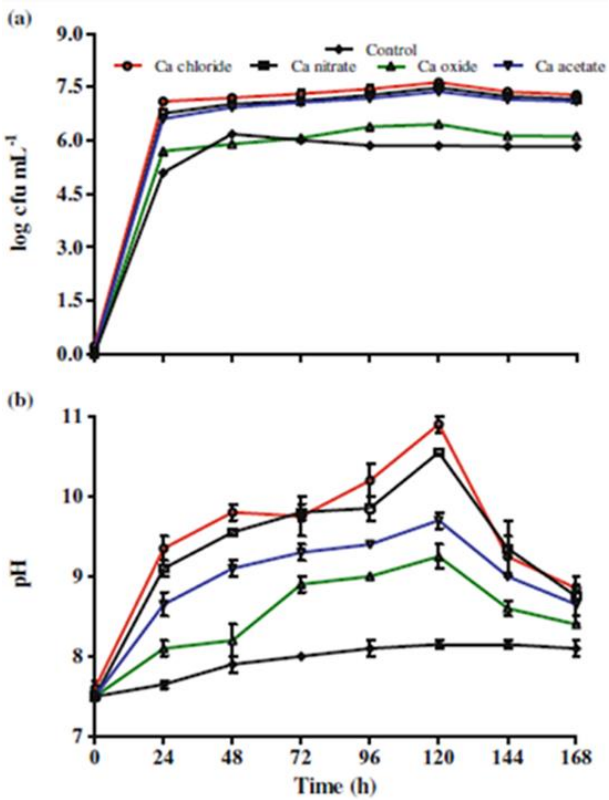

Fig. 10. (a) relationship between cfu (colony forming unit) and time, and (b) pH changing with time for Bacillus sp. CR2 cells. All related to media containing different sources of calcium [41]

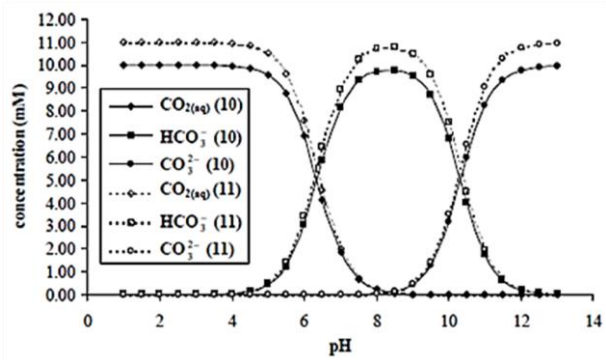

Fig. 11. Inorganic carbon Dissolved speciation as a factor of pH for a closed system [50]

Fig. 12.

The increase in pH value influences the amount of negative charge on the membrane of bacteria's cell [53]. Thus increase causes high extracellular Ca^{2+} concentration and low concentration of protons is required for the progress of treatment [53]. As the precipitation is a function of the ionic strength and cells number that makes the precipitation mechanism complex [48,54]. Simply, due to the increase in the concentration of positive ions Ca^{2+} around the bacteria no more Ca^{2+} will go towards the cell and the precipitation will decrease gradually until it stops. the ionic strength of cells membrane (negative charge on bacteria cells) increases with pH increase.

The urease enzyme production rate starting to increase at pH value of 6 until 10 and its peak is at pH value of 8. pH value also controls the solubility of $CaCO_3$ in either water or medium [15]. Keykha et al. [55] found that strength increases steadily with pH increasing from 5 to 9. Fine soils require regulator that can regulates the pH and makes it to be 9.0 or higher to ensure that the microbial growth will continue for the full duration of the curing process [12,38,53]. Most of Ca^{2+} precipitates forming $CaCO_3$ at pH value of 9 [15]. It is important especially when using electro-kinetic due to the occurrence of electrolysis reactions. Experiment done by Keykha et al. [12] shown in Figure 12 explains the setup of the pH regulators.

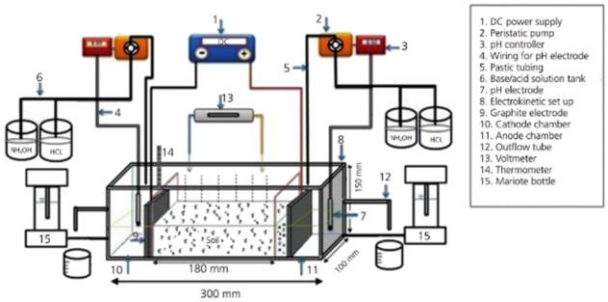

Fig. 13. The setup of the pH controller system to assess the pH value for the survival of the bacteria during the treatment process [12]

C. Duration of treatment

The duration of treatment process influences the treatment quality. The duration depends on the permeability of the soil. The duration is the required time for transferring matters needed for the treatment and based on the targeted quality. The microbial growth and the microbial ureolysis are influenced by the treatment duration where both decreased as time increased. Compared to chemical treatment, MICP treatment normally is slower and complex due to the effect of the surrounding environmental factors on the treatment process [32]. Shear strength was also observed to slightly increase with the increase of treatment duration where the shear strength after 7 days of treatment was 90 kPa increased to 92 kPa after 14 days at the same pH value. In addition, Wang et al. [56] found that most of strength was gained in the first three days and the rate of strength increment slowly decreased with treatment duration for bricks treatment according to XRD and SEM results. This was due to the consumption of most of the input materials in the first 3 days of the treatment which getting lessen with time [56]. But this slightly difference in shear strength could be widen and enhanced by increasing the amount of input material and elongating the duration of microbial activity.

D. Water presence

The treatment only work in the region of water [10]. The presence of water as the medium that contains bacteria and input chemicals, as $CaCl_2$ and $CO(NH_2)_2$, are compulsory for the treatment process for the formation of the $CaCO_3$ occurrence. The water presence is necessary for the ease of migration of the input materials and the output products inside the soil and as a part of the chemical reaction during the process as mentioned in the chemical equations 1 and 2.
The flow speed inside soil pores influences the mechanical properties of treated soil. Obviously, the shear strength resulted for submerged treated sample was significantly high compared to continuous percolation method as shown in Figure 13 and according to method of water flow in sandy soil samples as shown in Figure 14 [28]. The reason of reduction in percolation process would be due to prevention of precipitation of $CaCO_3$ on soil particles during the treatment process as water flows inside the pores.

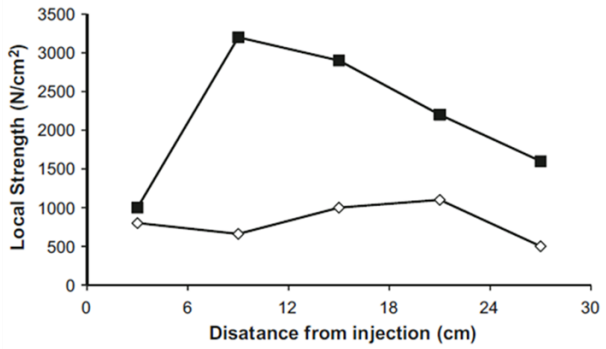

Fig. 14. Strength of cemented column at local points treated by submerges flow (■) and surface percolation (◊) [36]

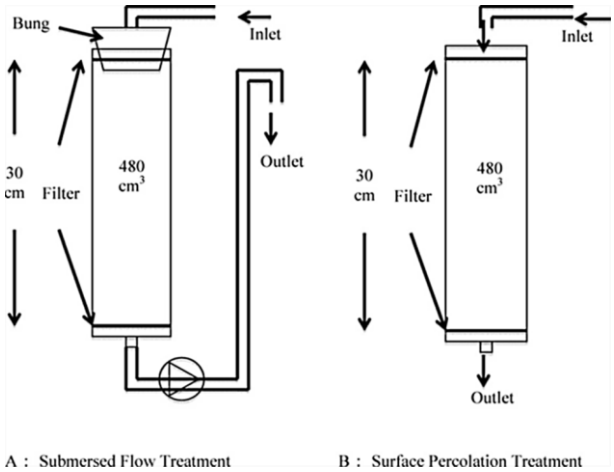

Fig. 15. Process of applying water to the treated soil samples submerged flow and surface percolation method [36]

Degree of saturation also influences the shear strength achieved after treatment [31]. Low water content soil gained higher local strength compared to saturated one [28]. This is because of bacteria resistance to mechanical action is high enough to accumulate in the thin film of water around soil particles [57]. This forms $CaCO_3$ precipitation as a coating to soil particles for unsaturated samples due to water surface tension compared to scattered agglomerated rhombohedral crystals formation for saturated samples [13].
Salinity rate controls the produced amount of $CaCO_3$ [38]. The presence of salts in water also accelerates the treatment and increases the strength more than fresh water as shown in Figure 15 [58,59]. This is because chemical bonds in weaker sodium based precipitation are broken by fresh water [59].

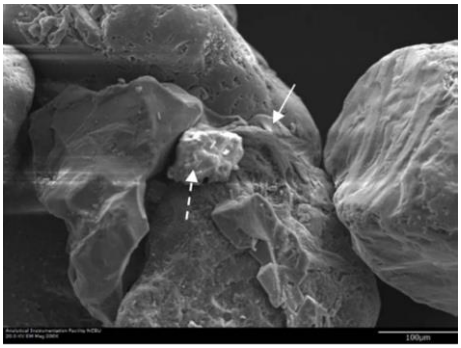

Fig. 16. SEM after treatment applied to sand dunes using slat water dashed arrow shows salt particle [57]

E. Electro-kinetic

The electro-kinetic EK is a physical phenomenon happens after applying electric current in solutions. The phenomenon is playing on the potential difference between the two electrodes. This phenomenon is a combination between electroosmosis, electromigration and electrophoreses phenomena. Electroosmosis is the migration of soil water from anode to cathode of an electrolytic cell. Electromigration is the migration of ions to the opposite charge electrode. Electrophoresis is the migration of charged particles by an electric field influence. EK phenomenon is very important to help in forcing bacteria to move in a medium which is difficult such as in cohesive soil [33]. It is mostly operative in clayey soils either at saturated and unsaturated conditions. The electrolysis reactions only occurs with help of EK which demonstrates the pH change in soil [25,60]. This happens due to production rate of hydrogen ions difference at cathode and anode which leads to increase in pH near cathode and decrease near anode [61]. This explains the variation in shear strength along the distance between anode and cathode done by Keykha et al. [1] where bacteria is more active near cathode than anode because of high pH value generated by EK.

V. CONCLUSIONS

Soil treatment by biogrouting process can be applied for various types of soils with the consideration of their limits for each case. The scale of the application differs based on the soil types and its pores size. The pores size controls and specifies the method of the treatment by biogrouting; mechanical process such as applying pressure is essential for coarse soils (high permeability soils) and applying EK is essential for the fine soils (low permeability soils). Furthermore, the pores size specifies the amount of the input materials such as $CaCl_2$ bacteria and CO(NH2)2 required for the treatment. Besides pores size and soil types, pH of soils also plays an important control to the process. It influences the cementation production rate as it could kill the bacteria or reduce the microbial growth if it was not controlled. The microbial ureolysis activity is managed by the microbial growth and both have influence on the rate of the biomineralization cementation production. Additionally, time controls the production of urease enzyme and the microbial growth. It also has an influence on the quality of the treatment and its progress. Cementation cannot precipitate above water surface. Therefore, the presence of water is very important for the occurrence of chemical reaction and the migration of the input materials and output products through the soil. In addition, degree of saturation controls the rate, amount and crystalize shape of cementation matter. EK is very important to the treatment process as it helps on transferring the ions through the soil during the curing process. This is achieved by increasing the current voltage. It can be concluded that biomineralization helps to give a significant improvement on the strength of the treated soils and can be applied on large scale for real cases at sites.

VI. ACKNOWLEDGEMENT

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