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Full Length Research Paper

Cementation in a matrix of loose sandy soil using biological treatment method

Ayhan Gurbuz[1], Yasin Dursun Sari[1]*, Zehra Nur Yuksekdag[2] and Berat Cinar[2]

[1]Civil Engineering Department, Engineering Faculty, Atilim University, 06836, Ankara- Turkey.
[2]Department of Biology, Science and Arts Faculty, Gazi University, 06500, Ankara-Turkey.

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Man-made materials varying from cement-based to chemical-based have been injected into soils to improve their engineering properties (shear strength, compressibility, permeability, bearing capacity etc.). Soil type in general plays important role in determination of treatment material and method. Materials used for soil treatment might have side effects in terms of air pollution, soil or water contamination etc. during manufacturing or application. An alternative, environmentally friendly soil treatment method that is based on the use of bacteria present in soils and named Biological Treatment Method (BTM) has been used by researchers to bond particles of loose sandy soils via creation of calcite ($CaCO_3$) generated by bacteria using urea to influence the precipitation of calcium carbonate. This study presents the results of bacterial induced cementation (BIC) in matrix of loose sandy soil. A bacterium used in this study is *Sporosarcina pasteurii* that is naturally present in soils and is aerobic type. The bacteria grown in laboratory environment were injected to the matrix of loose sandy soil. Subsequent nutrient mediums were introduced to specimens to accelerate the development of cementation level. Number of bacteria, pH level, temperature and amount of $CaCO_3$ were measured during the duration of testing. Images of Scanning Electron Microscope (SEM) showed that creation of cementation from precipitation of $CaCO_3$ on the surface and pores of soil matrix were observed for only sand samples into which nutrient was flushed on sequence of arbitrary time.

Key words: Biological treatment, sand, soil treatment.

INTRODUCTION

Demand to civil engineering structures continues to boost as population on the World increases at 1.1 to 2% per year. Both new construction sites and renovation sites on weak soil require soil improvement techniques to come with need of human for civil engineering structures. Worldwide, more than 40,000 soil improvement projects take place at cost of US$ 6 billion per year (DeJong et al., 2010). A wide range of products is available on the market for the improvement of soil properties (permeability, internal friction angle, bearing capacity etc). Some of the products used for treatment of soil are not considered as environmentally friendly due to the pollution effect and poison effect during manufacturing and application.

Promising results of novel techniques based on bacterial induced cementation (BIC) have let many researches to use bacteria in the improvement of the soil properties and concrete properties (Martin et al., 1996; Dennis and Turner, 1989; Seki et al., 1998; Ramakrishan et al., 1998, 2001; DeJong et al., 2006; Perkins et al., 2000; Ghosh et al., 2005; Tittelboom et al., 2010).

The main focus of this study was to determine the BIC in matrix of loose sandy soil while the effects of amount of nutrient and sequence of nutrient, temperature, pH level on BIC in matrix of loose sandy soil were being studied. Number of bacteria, pH level, temperature and amount of calcite ($CaCO_3$) were measured during the duration of testing. BIC was detected by Scanning Electron Microscope (SEM) and measured by the EDTA Titration Method.

*Corresponding author. E-mail: ydsari@atilim.edu.tr. Tel: 90 532 2558775, 90 312 5868333.

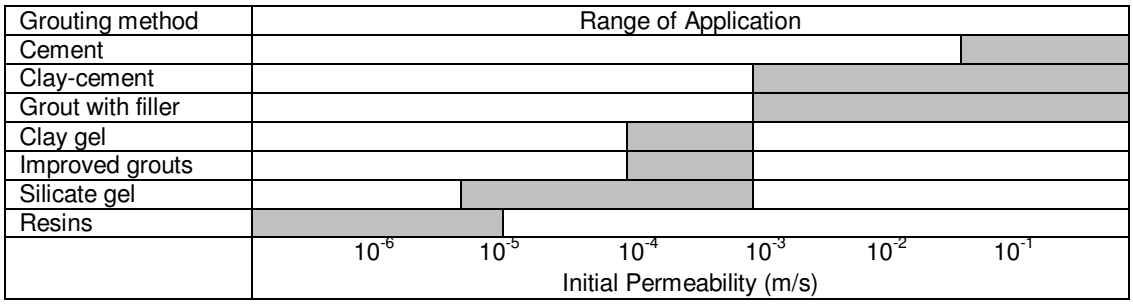


Figure 1. Applications of grouts for granular soils.

Materials used for classical soil treatment

Due to weak properties of soils, topographic conditions and increase in population in the world, new construction sites are required to meet with the needs of population. Therefore, soil grouting techniques have been used to improve the weak properties of soils by filling uncemented voids, increasing shear strength of soil and decreasing the permeability and amount of consolidation of soils. The first step in selecting a type of grout is to determine the general category of soil type to be improved. The most of soil improvement techniques uses man-made materials (cement, or petroleum–based materials), which causes either air pollution or containment during production and application besides being expensive.

Relation between the coefficient of permeability of soil and type of grouting material is presented in Figure 1. Type of grouting material becomes a petroleum-based material as the coefficient of permeability of soil decreases. Potential danger of damaging adjacent structures is the use of high grouting pressure while man-made materials are being injected in soils. Non-displacement grouting techniques (permeation) are preferable methods.

Biological treatment method (BTM)

A new and alternative treatment method of soil has appeared in the use of bacteria, already naturally present in soils, with nutrients as an energy source. This treatment method is called biological treatment method (BTM). BTM is more environmentally friendly than man-made materials. Bacteria grown in laboratory media and nutrient required for energy source to bacteria are injected in matrix of soil to form cementation among the particles of soil. Cementation in soil matrix is created utilizing microbiologically induced precipitation of calcite ($CaCO_3$). Bacteria have been used in other fields such as remediation of concrete, containment etc.

Characteristics of bacteria

A kilogram of soil contains 10^9 to 10^{12} organisms (Mitchell et al., 2005). However, numbers of bacteria living in soil become less as depth from surface increases. Bacteria have no membrane-enclosed nucleus with a simple cell structure. Bacteria are round shaped, rod-like or spiral. Size of bacteria varies between 0.5 and 3 μm (Madigan and Martinko 2003; Mitchell et al., 2005). Cell growth and reproduction of bacteria need carbon to form the molecule in the cells and energy to sustain life. Bacteria live in aerobic and anaerobic environment, and are heterotrophic. Aerobic bacteria live in the environment having free or dissolved oxygen in contrast to anaerobic bacteria. The semi-permeable cell membrane controls the transport of chemicals and electrical charges in and out. Precipitation of carbon dioxide on the bacteria surface takes place on the boarder range of soil varying from clay to gravel. Chemical process of biological treatment from the reaction of soil, nutrient and bacteria can be classified as inorganic precipitation, organic precipitation and gas generation. The products of inorganic precipitation, organic precipitation and gas generation can be considered bio mineralization, biofilm formation, and biogas generation, respectively.

Previous research on applications of biological treatment method

BTM can be classified as an artificial soil stabilization method for uncemented soil deposits of sandy soils. BTM might be used to change the permeability, stiffness, compressibility, shear strength and volumetric response of soils. Previous researches on biological treatments vary from remediate groundwater or water contaminated to improvement of the soil properties and concrete properties. However, researches on the improvement of engineering properties of soils using BTM are few. Martin et al. (1996) conducted laboratory hydraulic conductivity tests and strength tests on biologically treated clayey silt using biopolymers, and found that permeability was

Table 1 . Use of bacteria in different applications of biological treatment.

Researchers	Area of Research
MacLeod et al. (1988)	To investigate the microbiological mechanisms to reduce the hydraulic conductivity of the layers to improve the efficiency of oil extraction
Cunnigham et al. (1991)	To assess the effect of biofilm accumulation on porous media hydrodynamics
Nelson and Launt (1991)	To study the recovery of reservoir oil
Yang et al. (1993)	To develop biological shields for zonal remediation
Ferris et al. (1996)	To use bacteria as a mineral plugging agent
Martin et al. (1996)	To investigate the hydraulic conductivity and strength of clayey silt
Seki et al. (1998)	To study the hydraulic conductivity of soils on laboratory samples
Dennis and Turner (1998)	To study the hydraulic conductivity of soils on laboratory samples
Ramakrishan et al. (1998, 2001)	To remediate cracks in concrete structures
Komlos et al.(1998)	To examine the effects of thick biofilms in porous media under radial flow conditions
Ceribasi (2000)	To remove heavy metal from wastewaters
Perkins et al. (2000)	To evaluate the biofilms on shear strength of dense Ottawa sand
Etemadi et al., 2003	To study the stabilization of metals
Nemati and Voordouw (2003)	To study the modification of porous media permeability
Khachatoorian et al. (2003)	To study environmental stabilization of contaminated soils
Whiffin (2004)	To study the effects of microbial precipitation of calcium carbonate on the physical properties of sands
Dutta et al. (2005)	To remediate groundwater contaminated with nitrate using in-situ bio-barrier
Ghosh et al. (2005)	To improve the strength of cement mortar
GeoDelft (2006)	To evaluate the effect of biosealing
DeJong et al. (2006, 2010)	To evaluate the effects of calcium carbonate precipitation on the shear strength of sands.
Fujita et al. (2008)	To study in-situ immobilization of metal contaminants
Mastromei et al. (2008)	To use the bacteria on restoration of masonry structures
Shen and Cheng (2008)	To use the bacteria on restoration of masonry structures
Muynck et al. (2008)	To study the durability of mortar specimens
Tittelboom et al. (2010)	To investigate the use bacteria to repair cracks in concrete

decreased two fold and the strength increased up to 50%. The decrease in hydraulic conductivity on biologically treated soils was studied by researchers (Seki et al., 1998; Dennis and Turner, 1998). The decrease in hydraulic conductivity was three orders of magnitude less than the initial conductivity of untreated soils. DeJong et al. (2006) carried out tests on the biologically treated sandy soil, and determined that shear velocity increased with time. Some of the reported cases on the use of bacteria for any treatment purpose of different application areas are collected, summarized, ordered in date of publication and presented in Table 1.

Factors affecting bacterial activity

Many factors affecting the growth of bacteria and the production of calcite carbonate could be listed as nutrients, water, pH, temperature, presentation of the organic contaminants and heavy metals, space of solids, the concentration of dissolved organic carbon, concentration of calcium ions, presence of nucleolus sites (Tittelboom et al., 2010; Mitchell et al., 2005; DeJong et al., 2006, 2010). Nutrients are energy source of bacteria to sustain life. Therefore, the growth of bacteria depends on available nutrient type and amount of nutrient in system. Some of the nutrients are ammonia acids, CO_2, N, P, K, Mg, Se etc. (Mitchell et al., 2005). Nutrient transport, precipitation in chemical reactions, the type and amount of soluble materials, the environment pH, aeration control and thermal stability are dependent on water availability. Calcium carbonate precipitation reached peak at pH level of 8 (Stocks-Fisher et al., 1999). In lower concentration of enzyme (0.03 g/1), an increase of temperature from 20 to 50°C improved

Table 2. Summary of formulation for BMT.

Stage	Biological treatment of the solution
Activation	2% bacteria (*Sporosarcina pasteurii* DSM 33) 5 ml Tripton Soy Broth (Merck) 2 and 3% urea [$NH_2(CO)NH_2$] End of 48 h, concentration of bacteria measured (600 nm)
Incubation period	Concentration of cell of bacteria (*Sporosarcina pasteurii* DSM 33) 2 and 3%urea [$NH_2(CO)NH_2$] $CaCl_2$ [Nutrient Broth (Merck), 3 g; NH_4CL (Merck), 10 g; $NaHCO_3$ 2.12 g (25.2 mM)] pH = 8 Centrifuged at end of 48 h for two times
Development part	Concentration of cell of bacteria (*Sporosarcina pasteurii* DSM 33) 2 and 3% urea [$NH_2(CO)NH_2$] 25.2 mM $CaCl_2$ at room temperature to mimic the natural environmental conditions.

the production of $CaCO_3$ (Nemati and Voordouw, 2003).

Cementation process of BTM

Cementation *in-situ* takes place in matrix of soils via either deposition due to chemical weathering process or from water saturated with calcium carbonate. Degree of cementations has big effect on the stress-strain and strength behavior of cohesionless soils. Carbonates, silicates and ion oxides are usually natural commentating agent. Natural cemented soils are found all over the world.

The bacteria used in this study consume urea (NH_2-CO-NH_2) for energy source and produces ammonia (NH_3) which increases pH level and carbon dioxide (CO_2) in Equation (1). In presence of water, ammonia produced by bacteria is converted to ammonium (NH_4) in Equation (2). CO_2 reacts with hydroxyl ions (OH^-) in Equation (3), resulting in the formation of carbonate required for the precipitation of calcite ($CaCO_3$) in Equation (4).

$$NH_2\text{-}CO\text{-}NH_2 + H_2O \rightarrow 2NH_3 + CO_2 \quad (1)$$
$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (2)$$
$$CO_2 + OH^- \rightarrow HCO_3 \quad (3)$$
$$Ca^{2+} + HCO_3\text{-} + OH^- \rightarrow CaCO_3 + H_2O \quad (4)$$

Since the cell wall of bacteria is negatively charged, the bacteria pull cations from the environment, including Ca^{2+}, to deposit on their cell surface in Equation (4), leading the precipitation of ($CaCO_3$) which serves as a nucleation site (Kroll, 1990; DeJong et al., 2010).

MATERIALS AND METHODS

Preparation of solution of BTM

Sporosarcina pasteurii DSM 33 was used in this study. The *S. pasteurii* DSM 33 was grown at 30°C in Tripton Soy Broth (TSB) (Oxoid) and urea (2%) (Merck) for 48 h. $CaCO_3$ precipitation experiments were carried out in liquid medium (urea- $CaCl_2$). *S. pasteurii* DSM 33 was grown at 30°C under aerobic conditions. Broth cultures were centrifuged (Sigma Z-15) at 4500 rpm for 15 min. The supernatant was removed at the end of centrifuge. The cells were washed with saline. The cells were suspended in urea-$CaCl_2$. The solution consisting of bacteria, nutrient and $CaCl_2$ are presented in Table 2 for the stage of activation, incubation period and development part, respectively. Bacteria can move freely through the soil particles if the size of pore throats within soil particles is bigger than the size of bacteria. This study was carried out on the natural sand (Table 3) taken from a river side. It is believed that pore throats provide enough size to bacteria to move freely.

Experimental method to determine the cementation

The main focus of the first part of this study was to determine the BIC in matrix of loose sandy soil while the effects of nutrient and temperature on BIC were being investigated. Four type of test were carried out: (1) sand with bacteria, (2) sand with bacteria at 37°C, (3) sand with bacteria and nutrient, and (4) sand with bacteria and nutrient at 37°C. The second part of this study, based on the results of first part of study, was initiated to study the effects of amount of nutrient, temperature, pH on BIC in matrix of loose sandy soil.

Sand was put into caps having 86 mm in diameter and 15 mm in height (Figure 2). Specimens were prepared in loose state conditions. The bacteria were grown in laboratory environment, then, were injected to the matrix of sand using needle of injection to create BIC among particles of loose sandy soil. Subsequent nutrient mediums were introduced to specimens at arbitrary time durations to accelerate the development of cementation level.

Monitoring of cementation in matrix of loose sandy soil

Number of bacteria, pH level, temperature and amount of calcite ($CaCO_3$) were measured during the duration of testing.

Table 3. The properties of sand used in the research.

D_{50} (mm)	C_u	C_c	G_s	e_{min}	e_{max}	Shape
0.4	2.21	1.54	2.62	0.86	1.39	rounded

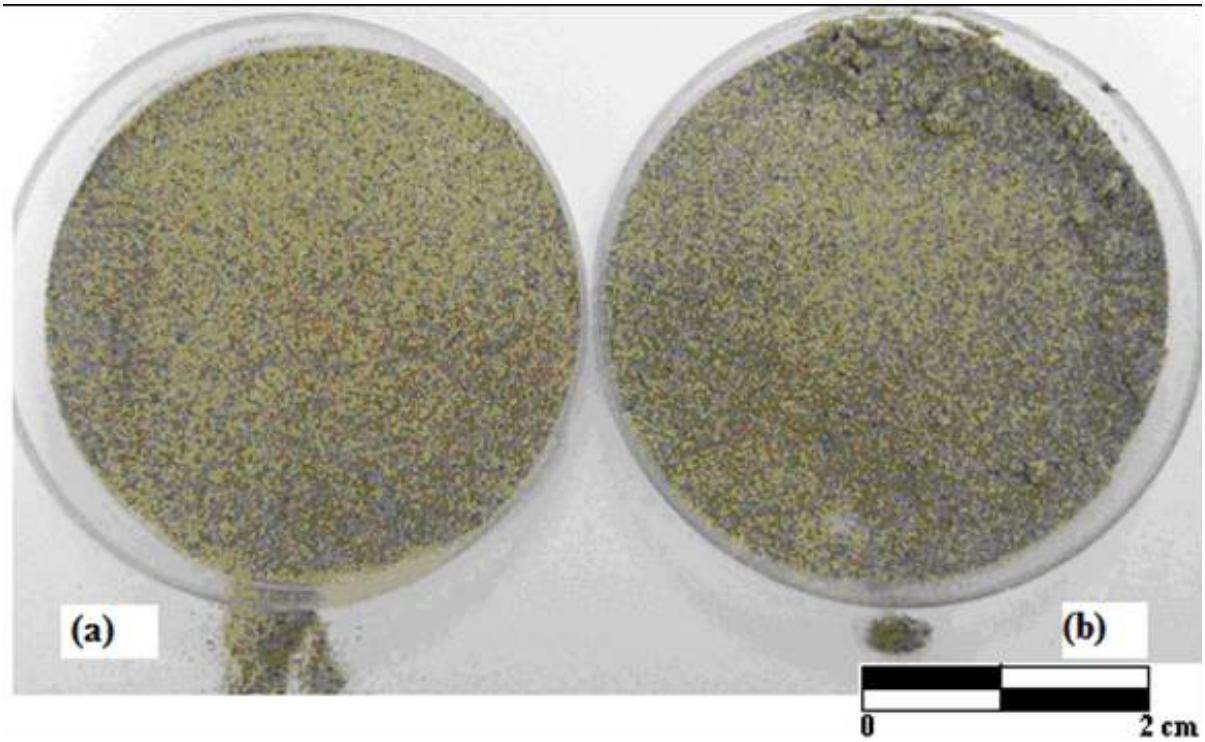


Figure 2. Soil samples: (a) untreated loose sandy soil, (b) biologically treated sandy soil.

BIC was detected by Scanning Electron Microscope (SEM) and measured by the EDTA Titration Method (Achal et al., 2009). The number of viable cells was determined as Colony Forming Units (CFU). Serial decimal dilutions of each sand sample were plated in duplicate onto urea-CaCl2 agar.

RESULTS AND DISCUSSION

Biologically induced cementation

Cementation in matrix of loose sandy soil occurred when sand, bacteria and nutrient came together. The number of bacteria (colony forming units) and $CaCO_3$ were measured by taking samples from the biologically treated loose sandy soil. The measure-ments of the first part of the study were presented in Figure 3. The number of bacteria and $CaCO_3$ versus time graph was divided into two zones based on the growth of bacteria in matrix of loose sandy soil. Zone 1 defines duration of time from bacteria injected into matrix of loose sandy soil to injection of nutrient into matrix of soil, while Zone 2 is the region starting from the injection of nutrient to the end of tests. The test results indicate that an initially rapid growth of bacteria and the formation of $CaCO_3$ took place for all biologically treated samples in Zone 1. A slow increase in growth of bacteria and the formation of $CaCO_3$ were observed for all biologically treated samples, followed by decrease in Zone 2. It might be concluded that an injected nutrient into soil can accelerate the growth of bacteria and production of $CaCO_3$. Effect of temperature on the number of bacteria and calcite was not clear. The presence of calcite on the face of sand grains and pores of sand for all four tests were detected by SEM and are presented in Figure 4. The images of SEM show that the bond of cementation on the face of sand grains and pores of sand existed clearly on the matrix of loose sandy soil with bacteria and nutrient (Figure 4d and 4e).

The effects of amount of nutrient (2 and 3%), nutrient flushes, temperature, pH on the BIC in matrix of loose sandy soil were studied more elaborately in the second part of the study by counting the number of live bacteria, measuring the pH level, amount of $CaCO_3$. The number of bacteria, amount of $CaCO_3$ and pH level with time were

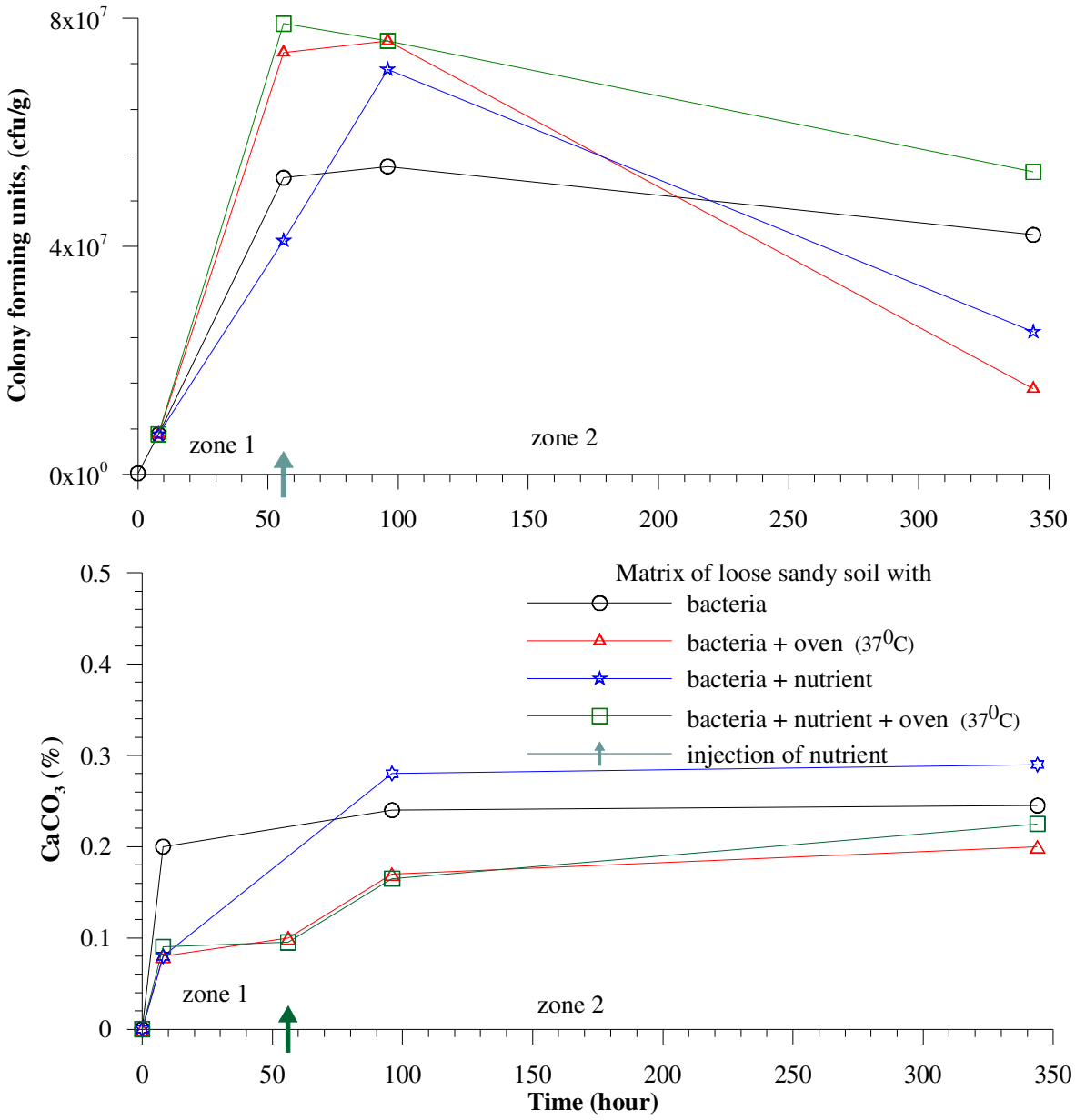


Figure 3. The development of number of bacteria and calcite in matrix of loose sandy soil.

measured and presented in Figure 5. The graph was divided into three zones based on the development of bacteria during duration of the test. Zone 1, 2 and 3 define the duration of time from the first injection of nutrient in the matrix of sand and second injection of nutrient, duration from the second to the third injection of nutrient and duration from third injection of nutrient to the end of the test, respectively. Injection of the first nutrient (2 and 3%) into matrix of soil accelerated the number of bacteria and amount of $CaCO_3$ in Zone 1. The injection of the second nutrient (3%) did not affect the amount of bacteria in Zone 2 as compared to the 2% of nutrient. The third injection of nutrient into matrix of loose sandy soil with bacteria did not accelerate the number of bacteria in Zone 3 due to the fact that the number of bacteria had already become lessened. Mea-sured amount of $CaCO_3$ increased during the testing and reached equilibrium after the number of bacteria and pH level decreased with urease enzyme. It can be concluded that increase in the amount of nutrient from 2 to 3% did not change the number of bacteria due to the fact that bacteria could not consume nutrient which were more than its need. The effect of temperature on the production of living bacteria was not so clear. The presence of $CaCO_3$ on the face of sand grains and pores of sand were detected by SEM and are

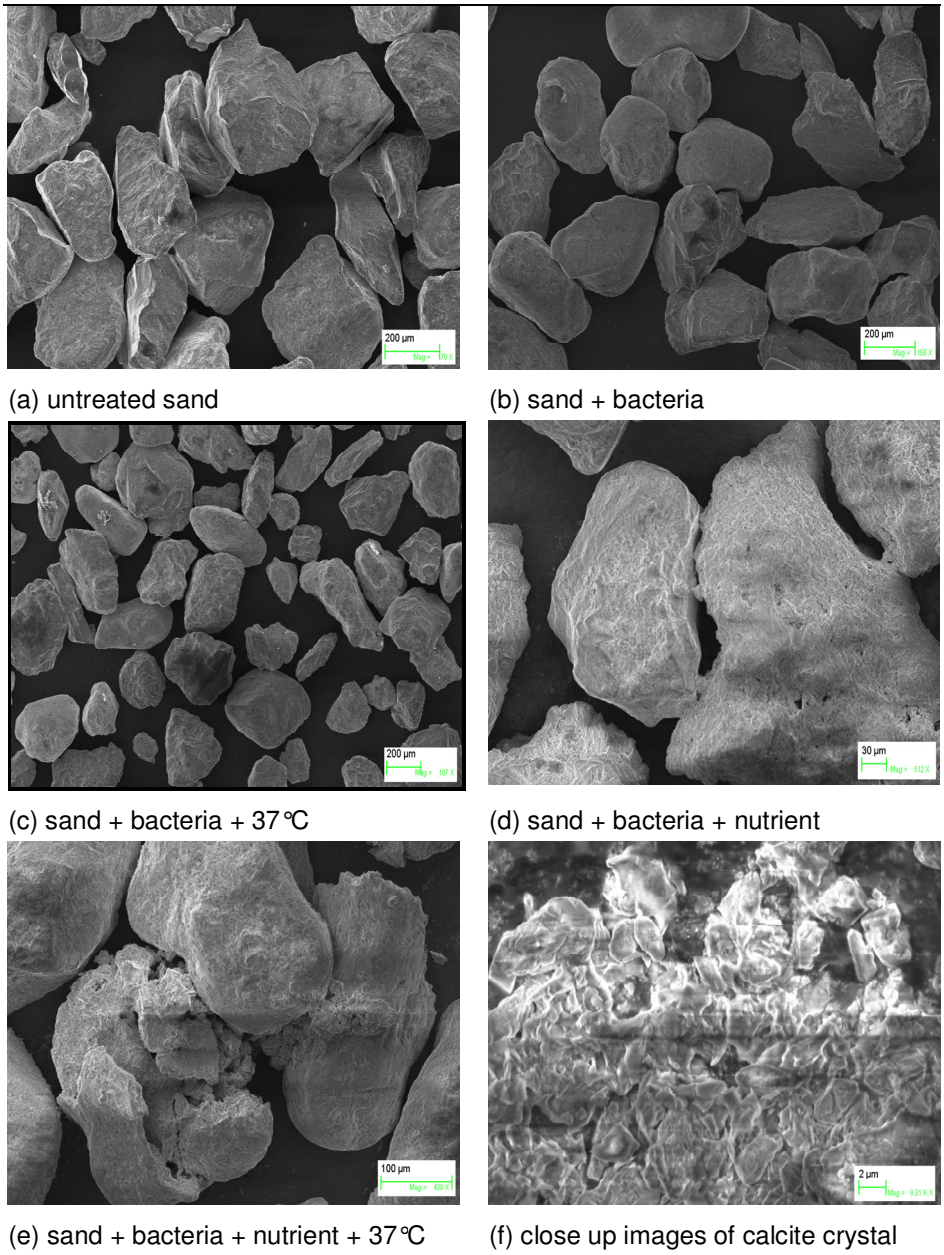


Figure 4. Images of (a) untreated sand (b) sand + bacteria (c) sand + bacteria + 37°C (d) sand + bacteria + nutrient (e) sand + bacteria + nutrient + 37°C and (f) close up images of calcite crystal.

presented in Figure 6. SEM images show that bond of cementation on the face of sand grains and pores of sand were clearly detected for the bacterially treated samples into which nutrient was injected. The presence of $CaCO_3$ was confirmed by EDTA Titration Method.

Conclusions

Biologically calcite cementation was achieved with *S. pasteurii* bacteria. Success of this method lies in nutrient treatment flushes, pH level, temperature and formation of bacteria. Biologically induced cementation was observed by images of SEM on the samples into which nutrient was injected on the arbitrary sequence of time. Effect of temperature was not clear on the creation of cementation. Therefore, the amount of cementation would be related to the time sequence of nutrient injection and pH level in this study.

The development of cementation can alter the behavior of loose sandy soil in term of strength,

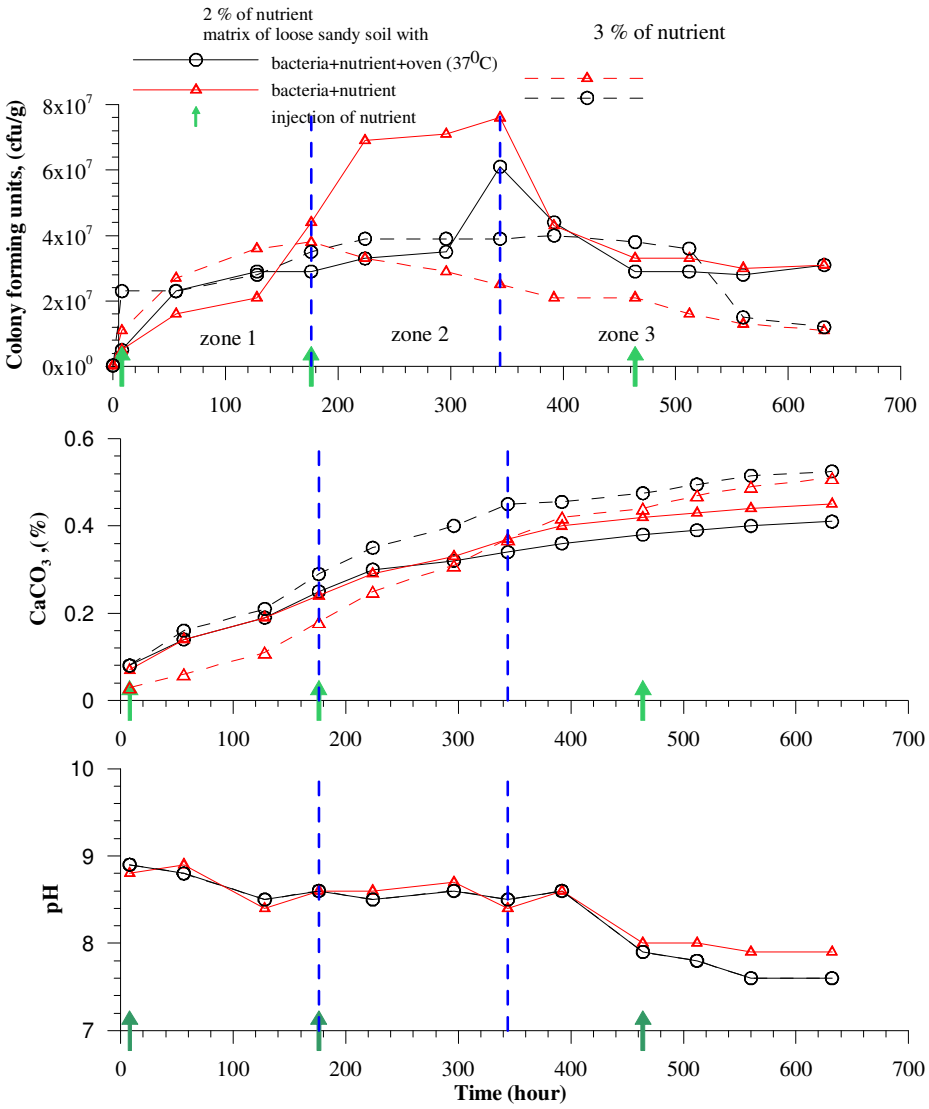
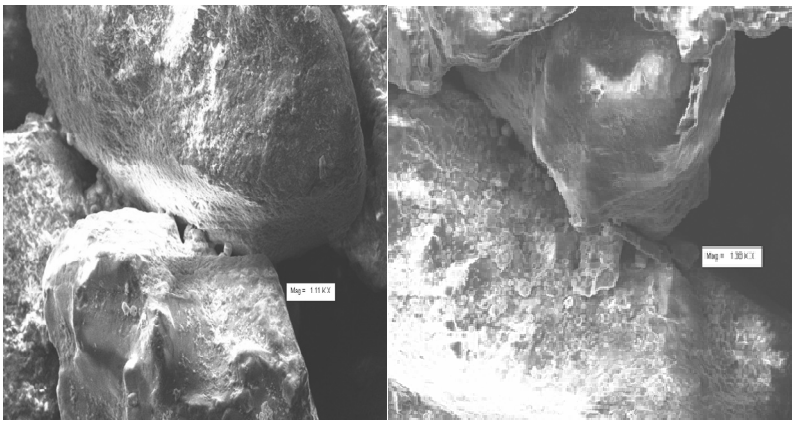


Figure 5. The development of number of bacteria and calcite in matrix of loose sand.



(a) sand + bacteria + nutrient (b) sand + bacteria + nutrient + 37°C

Figure 6. Images of (a) sand + bacteria +nutrient and (b) sand + bacteria + nutrient + 37°C.

liquefaction etc. Therefore, future studies on the biologically treatment method will open an alternative door to soil treatment method.

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