



cBio-Cementation of Sandy Soil through Bacterial Processing to Precipitate Carbonate

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Abstract

Bio-cement built on microbial induced carbonate precipitation MICP, be able to consolidate the loose grains and can applied for soil reinforcement. In this study, the performing of an ureolytic *Sporosarcina Pasteurii* for sand stabilization was estimated. The *S. Pasteurii* Could effectively consolidates sand particles through urea hydrolysis and the successive production of calcite. The bio improved sands had relative great compressive strength after 60 days exposure to bacterial cells injections cycles. The compressive strength of bio stabilized sands was reliant on the utilized cell concentrations and density of urea and CaCl_2 . High bacteria cell masses decreased the compressive strength. The optimal density of cell, was OD_{600} 0.5, when cost and performance were taken into account. The study shows that bio cementation of sand built on microbial induced carbonate precipitation (MICP) has ability for the reduction of sand permeability through pore clogging with precipitated carbonate.

Keywords: Bio-Cementation, Compressive Strength, Permeability.

التعزيز الحيوي للتربة الرملية من خلال النشاط البكتيري لترسيب الكربونات
ليث كاظم شنون، محمد عبد الخالق ابراهيم

الخلاصة:

يمكن للأسمنت الحيوي الذي يعتمد على ترسيب الكربونات الناجم عن الميكروبات MICP، أن يربط جزيئات التربة الرخوة ويحدث تقوية لها. في هذه الدراسة، تم تقييم أداء بكتريا *Sporosarcina Pasteurii* لتحليل اليوريا لإحداث استقرار في الرمل. يمكن لهذه البكتريا تعزيز جزيئات الرمال الرخوة بفعالية من خلال تحليل اليوريا والإنتاج المتتالي للكالسيوم. كان للمعالج الحيوي قوة ضغط عالية نسبية بعد 60 يوماً من التعرض لدورات حقن الخلايا البكتيرية. كانت قوة الضغط المسلطة على الرمل المعالج حيويًا تعتمد على كثافة الخلايا المستخدمة وتركيزات CaCl_2 واليوريا. خفضت كثافات خلايا البكتيريا العالية من قوة الضغط. وكانت كثافة الخلية المثلى، OD_{600} 0.5، بعد أخذ النظر في الأداء والتكلفة. توضح هذه الدراسة أن التماسك الحيوي للرمل بناءً على ترسيب الكربونات المستحثة جرثومياً (MICP) له القدرة على تقليل نفاذية الرمال من خلال انسداد المسام بالكربونات المترسبة.

1. Introduction

Mineral precipitation affected by microbial action in subsurface, frequently signified to a microbial induced carbonate precipitation (MICP), can be developed for a range of engineered applications involving the restriction of groundwater contaminants Fujita et al. [1], ground strengthening or changing properties of porous materials DeJong [2]; van Paassen et al.[3]; Whiffin et al.[4], and the formation of hydraulic barriers for functions such as improved expanding storage security of CO_2 or oil recovery [Cunningham et al.[5].

Many bacteria are able of urea hydrolyzing, that can modify the moistening state of the creation water, and in the existence of calcium, may support the calcium carbonate precipitation Ferris et al.[6], 2003; Mobley and Hausinger[7]; Stumm and Morgan [8].

In earlier reports, excessive calcium carbonate precipitation was noticed nearby injection spots that could possibly lead to inhibited moving of nutrients which is undesirable influences on well injection process Fujita et al.[9]; Whiffin et al.[4]. Previously a bio mineralization mechanism can be counted field related, metal accumulation should be established to be controllable at a related scale as sustaining economic possibility Harkes et al.[10]. Controlling mineralization has been studied by estimating the reaction with transport, for example, changing injection strategies or injection speeds, operating the concentrations of reactant, expanding the number of actions, or governing the spreading of active bacteria [De Muynck et al.[11]; Harkes et al.[10]; Whiffin et al.[4], Mohammad et al.[12]. Furthermore, it has been stated that the forms and sizes of crystals shaped are influenced by the number and shape (planktonic or



attached) of cells, and so on the ecological conditions in nearby liquids can involve precipitation Achal et al.[13]; Tobler et al.[14]. Multiscale and wide cross corrective study on the probability of such a mechanism is answer for its successful application DeJong et al.[15].

Three split column tests were presented in order to: investigate the injection process that makes homogenous CaCO₃ distribution along the bed of the column with different bacteria concentration.

This study then investigates the possible use of MICP built for sand stabilization and hydraulic conductivity. The effects of bacteria density, urea and calcium concentrations on sand stabilization have been tested, as one with performance under various bacteria concentration.

2. Materials and Methods

2.1 Preparation of the Bacterial Cells.

For the MICP procedure, *Sporosarcina Pasteurii* (American Type Culture Collection ATCC 11859) *S. Pasteurii* bacteria utilized in this study were cultured under aerobically batch environments in growth medium. The medium of growth was prepared by dispersing 5 g/L peptone, 5 g/L NaCl, 4 g/L Yeast extract, 1 g/L Beef extract and 50 ml Urea Mixture in one liter of purified water. The mixture was put in autoclave at 121 °C for 15 minutes to execute any germs that might contaminate the growth of bacteria (*S. Pasteurii*).

The autoclaved solution was cooled to room temperature. Urea mixture was prepared by liquefying 10 g of urea grains in 50 ml of purified water and sterilized using 0.25 µm bacterium filter, then added to main growth solution and shaken until homogenized. The urea solution was added after autoclaving to prevent urea hydrolysis in high temperature.

2.2 Bacteria Culture

A bacteria culture was generated by transferring a small quantity of the *S. Pasteurii* lyophilized culture into 250 ml of the culture medium next the making solution cultivated in incubator (under aerobic set conditions) at 25°C for 48 hours and allowing culture growth to occur. This mixture was cooled and kept at 4°C former to its use. The main culture medium was inoculated with the cells culture (10% v/v) and incubated aerobically under agitation at 30°C for 48 hours until the cells had reached maximum population (i.e. a stationary phase). After that the cells in the culture medium was harvested at 4°C by centrifugation for 10 min at 5000 rpm. The harvested bacteria were then washed twice with buffer of 0.1 M sodium phosphate pH 7 to eliminate metabolic waste and any metabolism yielded during the bacterial growth phase. 'Metabolism' belongs to all chemical results that occurred in the bacterial cells.

Metabolic wastes are substances that bacterial cells cannot use it (i.e. excessive or have lethal effect) and must be expelled before its use.

2.3 Bacteria Counting

To regulate and control the guessed bacteria concentration, a spectrophotometer method was

used. UV-Spectrometer (Shimadzu 1800) Japan was employed for calculating bacteria. To investigate the concentration of bacteria cells, around 10⁸ cells /ml was taken by dilution utilizing distilled water and counted by measuring the absorbance (optical density) of the solution operating a spectrophotometer at 600 nm wavelength. Using Equation below is for estimation the concentration of bacteria cells suspended in the solution culture corresponding to OD₆₀₀ value (Ramachandran et al. [16].

$$y = 8.59 \times 10^7 \times Z^{1.3627}$$

Where Z is reading at DO₆₀₀, and Y is the cells concentration (mL⁻¹).

2.3 Reagent Solution

Calcium chloride, CaCl₂ and Urea in different mole concentration were used as a reagent solution or cementation and nutrient solution. The reagent materials per liter of ions free water are 3 g Nutrient broth, 10 g NH₄Cl, 2.13 NaHCO₃ (Sodium acid Carbonate), 0.25 mol = 27.75 g CaCl₂ (calcium chloride) and 0.5 mol = 30.03 g CO(NH₂)₂ (Urea), DeJong et al.[2]; Ferris et al.[17]; Y. Inagaki et al.[18].

2.4 Soil

The compatibility concerning the soil particle characters and the size of bacteria cells is significant factor for MICP process. The soil pores should have adequate size to permit the transportation of bacteria which 0.5–3.0 µm in length, Mitchell and Santamarina [19], with 50–400 µm stated as the best soil grain size range for bacterial movement in the pores, Rebata-Landa [20]. In the present study Karbala clean loose sand was used as a porous media in the batch samples and bench scale experiments.

Sand with mean particle size D₅₀ equals to 0.403 mm, the uniformity coefficient C_u equals to 2.497 and the curvature coefficient C_c equal to 1.224. The sand particle size distribution curve was obtained from sieve analysis in accordance with ASTM D6913-04, is shown in Figure (1). The Figure (2) represents grains photo in scanning electron microscopy (SEM), which displays a regular sub rounded shape and the size of particles were medium to fine as tested at Physics department collage of science Al-Nahrain University. The specific gravity (G_s) of sand particles is determined using the pycnometer method as specified by ASTM D854-10 with value equals to 2.63 The values of minimum and maximum void ratios were tested according to ASTM D4254 and ASTM D4253, and it were for e_{min} and e_{max} equal to 0.588 and 0.857 respectively. Table (1) summarized the physical properties of the sand were used. The chemical properties and mineral compositions of the sand used were illustrated in Table (2).



Table (1): Physical properties of Karbala sand utilized in tests.

Parameter	Value	Test Method
Shape	Sub rounded	SEM
Mineral composition	>89.5% Quartz	EDS
Effective particle size, D_{10} (mm)	0.189	ASTM D6913
Effective particle size, D_{30} (mm)	0.507	ASTM D6913
Mean particle size, D_{50} (mm)	0.412	ASTM D6913
Effective particle size, D_{60} (mm)	0.46	ASTM D6913
Coefficient of Uniformity, C_u	2.43	ASTM D6913
Coefficient of Curvature, C_c	1.08	ASTM D6913
Specific gravity, G_s	2.637	ASTM D854-10
Minimum void ratio, e_{min}	0.587	ASTM D4253
Maximum void ratio, e_{max}	0.856	ASTM D4254
Coefficient of permeability (m/sec)	0.00158	ASTM D5856-15
Bulk density (g/cm ³)	1.69	ASTM D7263
Porosity, n (%)	35.9	ASTM D7263-09
Organic matter (%)	0.197	ASTM D2974 - 14

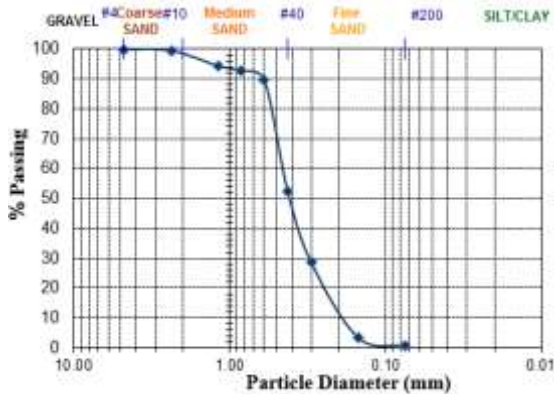


Figure (1): Sieve analysis grain size curve of sand used in this study

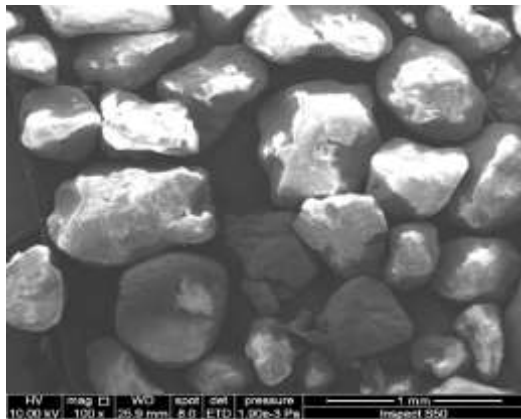


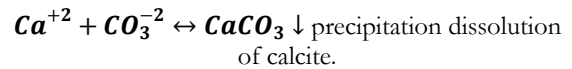
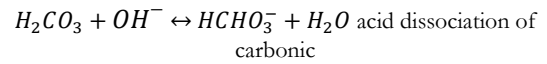
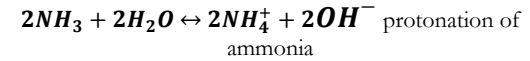
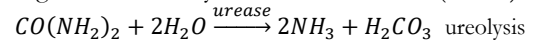
Figure (2): Photo represents the grains of sample in SEM

Table (2): Chemical properties and mineral composition of sand used.

Characteristics	Value	Analysis method
SiO ₂ (%)	98.3	XRD
CaCO ₃	0.042	XRD
Fe ₂ O ₃	1.37	XRD
MgO ₂	0.019	XRD
Al ₂ O ₃	0.15	XRD
K ₂ O	0.074	XRD
Na ₂ O	0.027	XRD
P ₂ O ₅	0.018	XRD

3. Chemical Reactions

During the existence of the urease enzyme, urea is hydrolyzed to provide carbonic acid and ammonia. The bacteria *S. Pasteurii* is able to produce great quantities of urease Ciurli et al.[21]. The successive protonation of ammonia to ammonium initiates pH increase, changing the balance of calcite precipitation/dissolution reacting to precipitation via rising the accessibility of the carbonate ion (CO₃²⁻).



The separation reactions are quick paralleled to ureolysis, precipitation and dissolution. Therefore, these are supposed to follow immediately and are included with balance coefficients. Slow on the uptake reactions are defined by utilizing rate expressions.

4. Experimental methods

4.1 Specimen preparation

To perform a bio remediation and soil characteristics development, bacterial prepared solution was injected into soil sample. Clear acrylic tube columns (48 mm ID x 103 mm long, 186-mL) packed with sand. The tube columns were covered at both ends with solid O-ringed acrylic blocks (80 mm x 80 mm x 10 mm thick) which were connected by four threaded steel rods. Fluid sampling ports were placed on top and the bottom blocks with an interior mesh deposit to avoid sand drip as shown in Figure (3). The insertion of solution to the fully saturated soil sample should be attained a uniform distribution along the specimen by using staged injection with retention period and it was accomplished through influent opening by following order O'Kelly et al.[22]:

- De-aired the sand specimen by deionized water passing of 2 volume pore voids, V_v which about 140 ml.

- 1.5 V_v of bacterial cell solution was injected with a pressure head of 1 m with dropping slightly and drainage rate 10 ml/min from bottom specimen by a peristaltic pump as shown in Figure (4 a).

- After completely saturated specimen with bacterial cell, the flow ended for 12 hours period to allow the bacterial cells attached on sand particles. Once the retention time ended detach the peristaltic pump to discharge the solution by gravity.

- A vessel of reagent solutions (Urea-CaCl₂) which is lighter density than bacterial solution, were placed directly over the specimen (no pressure head) with drainage 3 ml/h from the bottom of the specimen as shown in Figure (4 b).

- After reagent solution insertion of volume 1 V_v , the flow was ceased for 24 hours to let bacteria react with the reagent solution. Next, the peristaltic pump was detached from the line and accepts the solution



to discharge by gravity and repeat the later for various time and concentrations.



Figure (3): Clear acrylic column for soil treatment

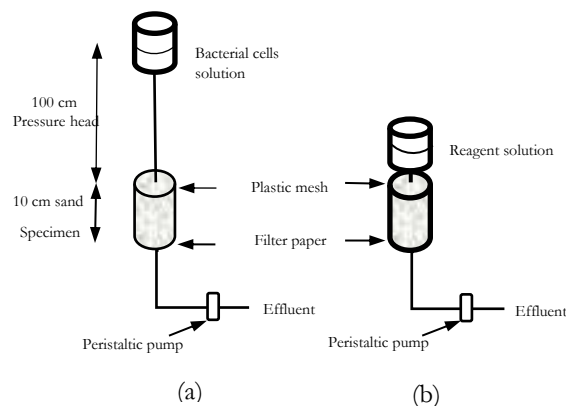


Figure (4): The bacterial and reagent solutions injection protocol: (a) injection of bacterial cells solution; (b) injection of reagent solution.

4.2 Permeability Examination

The relative change of the hydraulic conductivity was monitored through the same sand column. Falling head test has been utilized to measure the coefficient of permeability development through experiment duration (ASTM D 5856-15). Water flows across the sand sample via stand pipe joined to the top of sample cell column; as shown in Figure (5).

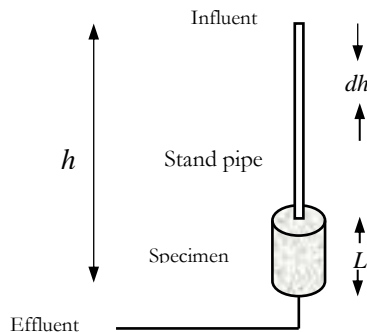


Figure (5): schematic draw for soil sample hydraulic conductivity test (variable head).

The water head (h) change over with time as flow take place across the sand specimen. Head of water is recorded at various times as follow:

$$K = \frac{(a \times L)}{A(t_2 - t_1) \ln \left(\frac{h_1}{h_2} \right)}$$

Where: t = time; L = length of sand column; A = cross section area of sand; a = cross section area of stand pipe and K = coefficient of permeability.

5. Results and Discussion

5.1 Bacteria Concentration

Three groups of sand columns specimens were injected in different *S. Pasteurii* bacteria cells concentration solution. The OD_{600} values were high (1.3), medium (0.5) and low (0.2) to find out the optimum bacteria cells concentration to achieve biocementation. Specimens were tested for three days and the results as shown in Figure (6), the pH value for high concentrated bacteria (1.3) has convex trend due to fermentation of microorganisms which raise the acidity as well dissolve carbonates and hydroxides binding sand grains. Also the high concentration of organic species may produce the slime which lubricates sand particles and caused reduction in internal friction angle. The reduction in hydraulic conductivity causes microorganisms accumulation between sand pores rather than calcite precipitation. Low bacteria cells concentration (0.2) treatment pretends no significant change in tests results. However, the OD_{600} value of 0.5 shows sufficient urease activity represented by increasing in electrical conductivity also the internal friction angle of treated specimens which tested by direct shear method and was enhanced due to calcite precipitation on sand particles and reduces the permeability as shown in Figure (6).

5.2 Bacteria Number of Injections

A comparison was carried out to investigate the suitable time's number of bacteria cells injection influence on sand specimens. Two injection criteria was adopted in the experiment which lasted four days; single bacteria cells injection at the beginning of the test and multi injection times, dual injection, conducted every 48 hours along testing duration. Results were illustrated that multi injection procedure has notable development for the same experiment duration (four days). A slight decreasing in hydraulic conductivity attributed to the development of calcite precipitation indicated by the increasing of urease activity (electrical conductivity). The pH values were almost coupled; however the internal friction angle has increased fairly as shown in Figure (7). The multi injection procedure will keep the average of urease production at levels initiate continuous cementation by calcite precipitation on sand particles. However, the injection of bacteria cells without nutrient to prevent increasing in biomass, which could potentially plug the pores by biomass instead of calcite precipitation, Tobler et al. [14]. The plugging occurs by biomass will prevent the distribution of bacteria cells. Furthermore, the growth of biomass may reduce the pH solution and may delay urea hydrolysis and mineral precipitations as demonstrated in Figure (6).



5.3 Strength Development

One of the intentions of this study is to examine the potential of *S.Pasteurii* to perform as an ingredient for making bio cement. Soft rocks were made by dispersing the bacteria cells in situ within 48 hours treatments of successive injecting of bacteria pursued by cementation solution (urea/calcium)

every 9 hours. Remarkably, the gotten strength was attached to the point-to-point contact of CaCO_3 crystals that made bonds among the adjacent sand particles as seen in Figure (8). The creation of strength caused by this kind of contact was approved by Sharma and Fahey [23].

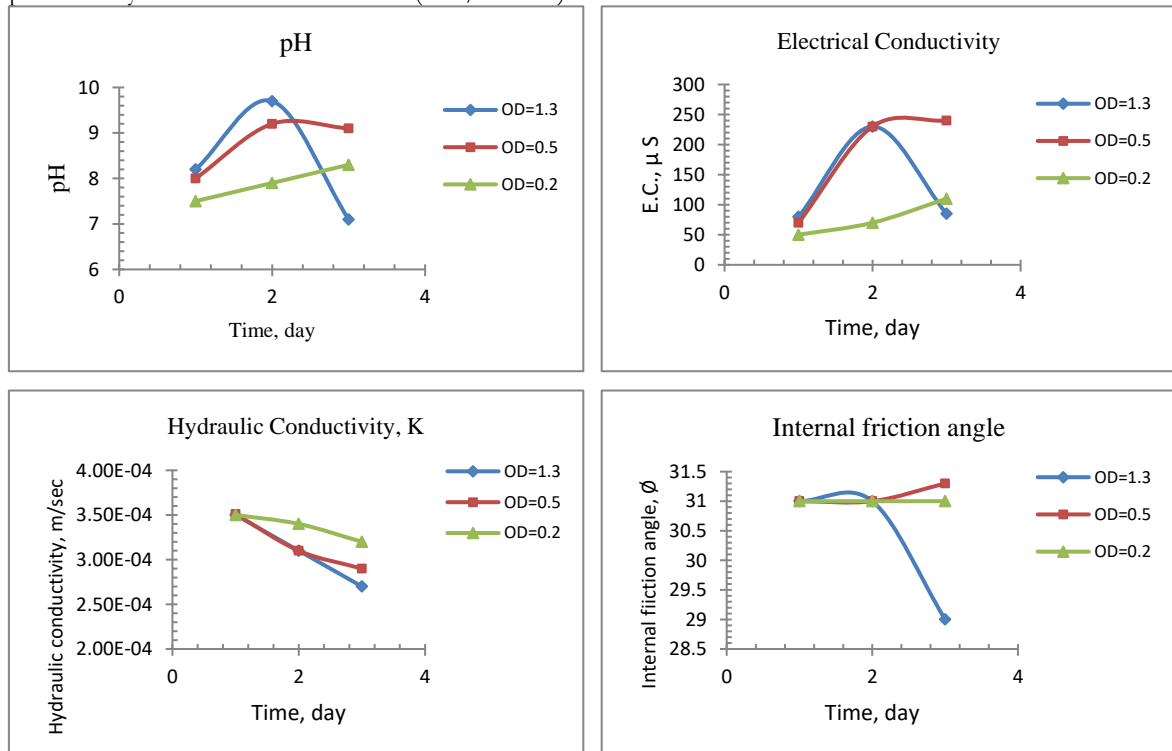


Figure (6): Results of pH, electrical conductivity, permeability and internal friction angle for sand column specimens treated by three different bacteria concentrations.

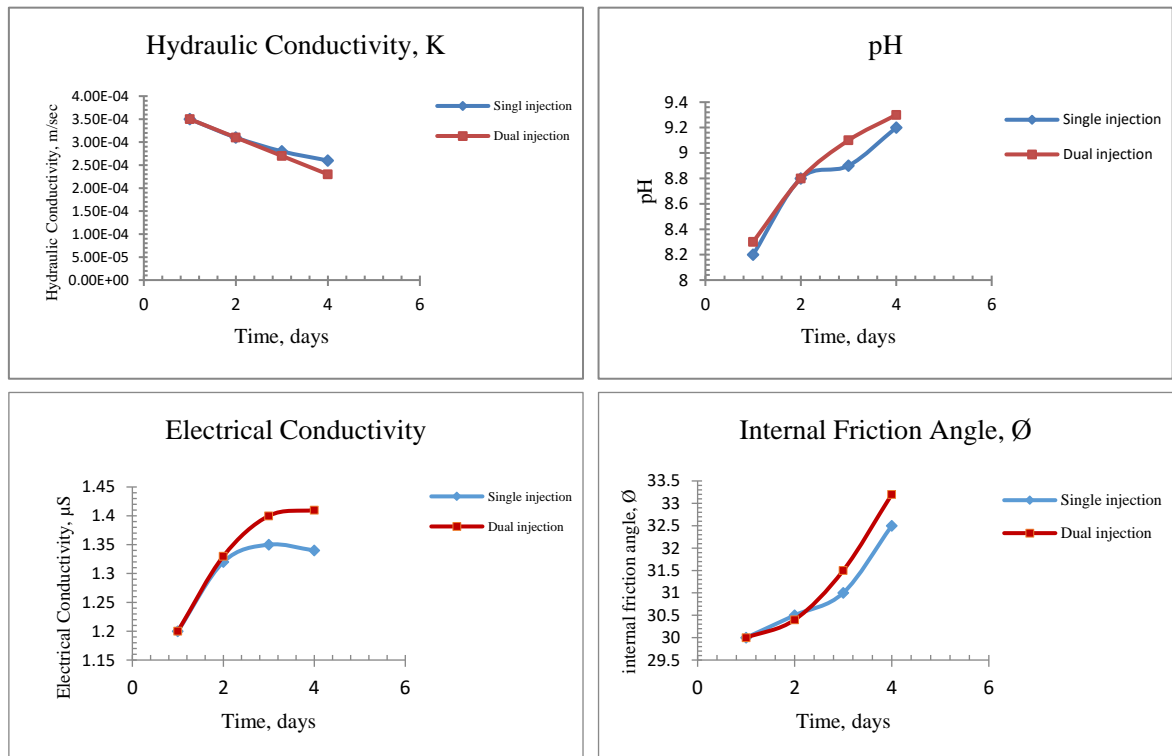


Figure (7): Results of pH, electrical conductivity, permeability and internal friction angle for sand column specimens with different injection criteria



Unconfined compressive strength test was carried out on column specimens to verify the mechanical properties of cemented sand. A bacteria cells concentration with $OD_{600} = 0.5$ was adopted as tested previously. The reagent solution concentration was 1M urea and 1M calcium chloride (Urea molarity 1.85 times that of calcium chloride) as best ratio accepted from previous literatures Whiffin et al.[4], O'Kelly et al.[22]. As shown in Figure (9) the sand strength grows exponentially with treatment time with maximum strength 1.03 MPa in 60 days. The classification system implemented in this study was built on that established by Shafii and Clough [24], where softly cemented sand was described as requiring a UCS of fewer than 0.3 MPa, moderate cemented sand was described as holding a UCS between 0.4 MPa and 1 MPa and solidified sand was for that greater than 1 MPa.

Figure (10) shows the strength variance between specimens of sand prepared with two different injected substances corresponding bacteria cells with reagent solution ($CaCl_2$ /urea) only, and without the reagent solution (bacteria cells only) for period of 5 days.

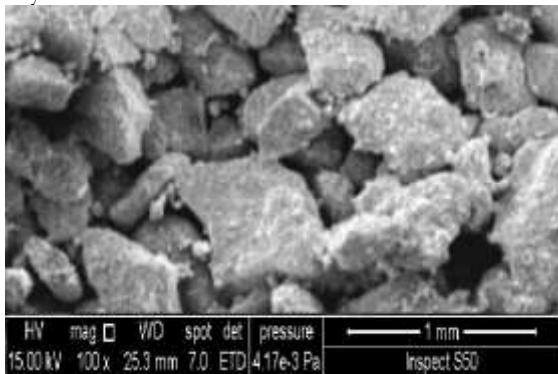


Figure (8): SEM image for bond point-to-point contact between adjacent sand particles made by calcite precipitation.

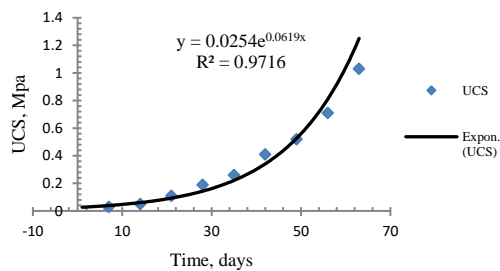


Figure (9): strength developments with time for the sand specimens' column

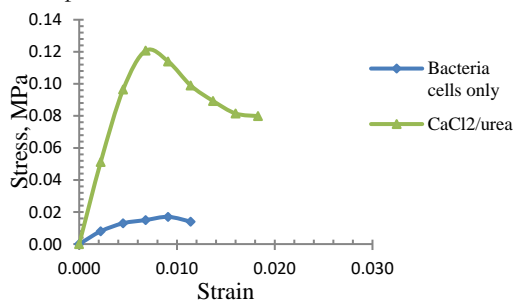


Figure (10): UCS results for different injected materials with *S.Pasteurii* bacteria cells in column sand specimens.

From the preceding figure it is obviously clear that the effect of reagent solution presence enhances about 5 times the cementation procedure due to calcite bio precipitation on sand particles.

5.4 Hydraulic Conductivity

Permeability tests were carried out on a set of compacted sand column specimens. The bacteria and reagent solution injection procedure were the same as mentioned previously. The results illustrated a significant reduction with specific specimens permeability during the period of test which last for 14 days, whereas another specimens barely their permeability is reduced. Figure (11) shows the reaction variance of bacteria cells when some species added to the reagent solution, while no mentioned drop in permeability in the absence of reagent solution. The specimens injected with bacteria cells only has no action except the accumulation of some cells body in pore spaces due to lack of urease production to complete the precipitation action. However, the existence of reagent solution catalyzes the *S. Pasteurii* bacteria cells to produce urease. Calcium or lead free ions start to precipitate as carbonates on pores throat of sand grains in which reduces the flow through these minor channels. The decrease in hydraulic conductivity of processed sand specimens was about 3.6 times with the presence of reagent solution.

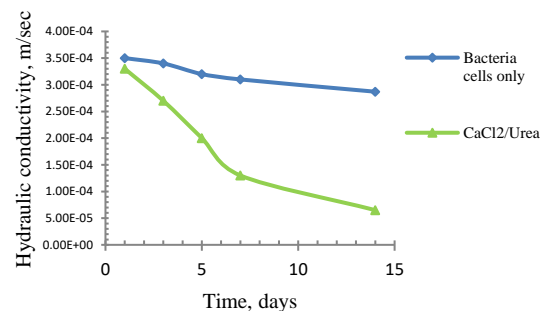


Figure (11): The permeability variance of sand column specimens with different UCS reagent solution combinations.

6. Conclusion

According to the results attained from the experimental work, some conclusions can be extracted:

The column specimen results show that the best bacteria concentration OD_{600} was 0.5 for durable bio precipitation process by injecting live bacteria cells only without adding nutrient to prevent uncontrolled colonies augmentations. Further minerals precipitation occurs when the bacteria had multiple injection number with medium OD_{600} than one with high OD_{600} which may raise the site acidity and affect inversely on precipitation process (lowering the pH value less than 7). The results of UCS strengths determined in this paper have revealed significant increment with time from zero up to 1.03 MPa with controlled environment in column specimen, pH, and temperature Results of hydraulic conductivity



illustrate a good reduction due to calcite precipitation and clogging the pore space between sand grains.

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