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ORIGINAL PAPER

Optimization of the biological soil improvement procedure

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Abstract
Biocalcification is a developing method in the realm of bio-geotechnics, potentially invaluable for soil stabilization. The method is based on microbial-induced calcite precipitation. Hydrolysis of urea by the urease enzyme discharging from bacteria in the presence of Ca^{2+} is one of the most notable methods for calcium carbonate precipitation. However, partial clogging may occur as a result of premature bacterial activity that hinders free flow of the mixture, prohibiting spatial homogeneity of the sediment formation, thus limiting the extent of calcification. In order to circumvent clogging, bacterial activity was suppressed in this study prior to injection by lowering the temperature of the suspension and the reaction to 3 °C prior to mixture, delaying $CaCO_3$ precipitation and thus allowing more uniform dispersion of the mixture. *Sporosarcina pasteurii* and *Arthrobacter crystallopoietes* were cultured in two different media and injected with reactant agent into samples of non-cohesive sand. The effect of culture media and temperature was studied on the rate and volume of $CaCO_3$ precipitation. Furthermore, the effect of cementation of each batch on the shear strength of the treated soil was evaluated in unconfined compression test. Compressive strengths in excess of 400 kPa were recorded for samples that were injected in two phases, an hour apart. Whereas the highest compressive strength obtained from a single-phase injection at room temperature was approximately 80 kPa. By lowering the temperature of the bacterial suspension and the reactant solution prior to injection, the compressive strength of the sample treated in a single phase was increased to 230 kPa.

Keywords Bacterial activity · Biocementation · Inhibitor factor · Sediment distribution · Single-phase injecting · Unconfined compressive strength

Introduction

Applications of various biological-induced processes in engineering have received much attention in recent years; biological clogging of porous materials (Nemati and Voordouw 2003; Rusu et al. 2011) biological remediation of contaminated environment (Fujita et al. 2000; James et al. 2000; Warren et al. 2001; Fujita et al. 2010; Li et al. 2013), wastewater treatment (Hammes et al. 2003) biological remediation of concrete and cement mortar (Ramachandran et al. 2001; Abo-El-Enein et al. 2012, 2013) and microbial-induced cementation of porous materials (Rong et al. 2012; Cheng et al. 2014; Montoya and DeJong 2015; Sel et al. 2015) are a few of the more recent attempts to utilize this potential for the advancement of technology. Promising results of the accelerated calcification through microbial-induced calcite precipitation (MICP) methods have emerged from the studies of a number of researchers (Whiffin 2004; DeJong et al. 2006; Dick et al. 2006; Whiffin et al. 2007; Al-Thawadi 2008; Sarda et al. 2009; van Paassen et al. 2009b; Meyer et al. 2011; Cheng and Cord-Ruwisch 2012; DeJong et al. 2013; Achal and Kawasaki 2016; Salifu et al. 2016). The immediate aim of the latter references has been to improve the mechanical properties of porous materials.

The biological-induced cementation method is based on managing chemical reactions by increasing the speed of the reaction and forming stable sediments. In other words, microbial components play a mediating role for increasing reaction speed. In the presence of proper nutrients, microorganisms can catalyze the chemical reactions, and consequently precipitate the inorganic mineral substances and improve the mechanical properties of soil (by binding soil particle with mineral bridge).

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Table 1 Possible chemical-induced reactions proposed for biological stabilization of soils

Conversion type	Catabolic reaction
Urea hydrolysis*,†	$CO(NH_2)_2 + CaCl_2 + 2H_2O \rightarrow 2NH_4Cl + CaCO_{3(s)}$
Aerobic oxidation*	$Ca(C_2H_3O_2)_2 + 4O_2 \rightarrow CaCo_{3(s)} + 3CO_2 + 3H_2O$
Iron reduction†	$CH_3CO_2^- + 8Fe(OH)_{3(s)} + 6HCO_3^- + 7H^+ \rightarrow 8FeCO_{3(s)} + 20H_2O$
Sulfate reduction*	$Ca(C_2H_3O_2)_2 + 2CaSO_4 \rightarrow 2H_2S + H_2O + 3CaCO_{3(s)} + CO_2$
Nitrate reduction*	$5Ca(C_2H_3O_2)_2 + 8Ca(NO_3)_2 \rightarrow 13CaCO_{3(s)} + 8N_2 + 7CO_2$
Ammonification‡	Amino acids + Ca^{2+} + source + phosphate source → calcium phosphate + by-products

*van Paassen et al. (2010a, b)

†DeJong et al. (2010)

‡Akiyama and Kawasaki (2012a, b)

Natural microbial cementation is usually based on calcium, magnesium, iron, manganese, and aluminum sediments, which precipitate as carbonates, silicates, phosphates, sulfides, and hydroxides (e.g., iron hydroxides) (Ivanov and Chu 2008). So far various types of natural cementation of soils have been reported: e.g., it is found at the roots of wetland plants, iron-reducing bacteria mediate cementation soil around of roots (Weiss et al. 2005).

In the recent year, a number of different processes have been tested by researchers including urea hydrolysis,[1] aerobic oxidation of calcium acetate, iron/sulfate/nitrate reduction, and deamination[2] of amino acid. Possible biochemical reactions proposed for soil stabilization to date are listed in Table 1 (DeJong et al. 2010; van Paassen et al. 2010a; Akiyama and Kawasaki 2012a, b).

Recent laboratory investigations on the accelerated processes have been concentrated on the precipitation of calcite by hydrolysis of urea. Chemically hydrolyzing of urea (when there is no catalyzer) is a slow and lengthy process, while in the presence of urease[3], it would be about 10^{14} times faster than the usual process (non-catalyzed reaction) (Benini et al. 1999). In other words, MICP in sandy soils is an accelerated "diagenesis" process resulting in "weakly" cemented sandstone.

It is noteworthy that in situ deep stabilization of sand (e.g., mitigation of liquefaction potential) requires injection of reagents that permeate through the pores of the soil. The size and viscosity of the reagent constituents is a limiting factor in the permeation process. Generally fine grained soils filter cement particles and cause clogging at the injection point, so deeper penetration of cement grout requires higher injection pressure to cause hydraulic fracture, even then the grout does not permeate beyond the fracture surface. Thereupon ordinary cement can only be used for permeation grouting into coarse granular soils. Only micro-fine cement and chemical grouts such as silicate, lignin, acrylate, urethane, and resin grouts may permeate deeper in sandy soils (Engineers 1995). Bacterial suspension with reactant agent also permeates readily in sandy soils at low hydraulic heads since its viscosity is close to water, and all constituents are in the order of micrometer (Vos et al. 2011; Whitman et al. 2012). However, early bacterial activity upon introduction of the bacteria suspension together with the reagent solution causes premature calcium carbonate precipitation and consequence clogging especially at the point of injection (Al-Thawadi 2008; van Paassen et al. 2010a) that hinders free diffusion of the stabilizing fluid, result in non-homogeneity of calcium carbonate precipitation. Hence, although attaining complete uniformity may not be possible, it is important to develop a method to achieve the highest possible relative homogeneity.

Researchers have made a number of attempts to overcome this deficiency. Multiple injection of the stabilizing fluid has yielded very high strengths; Yang and Cheng (2013) reported uniaxial compressive strengths in excess of 55 MPa using above-mentioned method (Yang and Cheng 2013). Mass stabilization in the order of a 100 cubic meter of sand was attempted by Van Paassen et al. (2009a, b), in these tests, injecting and circulating the bacteria followed by a fixation fluid which dispersed it throughout the mass prior to injection of the reactant agent, circumventing clogging and concentration of sediments near points of entry. By this technique, a relatively uniform cementation has been reported. However, the mentioned procedures may be viable only in a laboratory column or enclosed mass, whereas recirculation and multiple injections in open ground may prove inefficient. Natural downward draw in unsaturated soils and dilution by ground water in saturated soils, and the restriction of the injection well numbers in urban areas prohibits efficient circulation. Multiple injections are also costly and time-consuming. Therefore, attainment of sufficient cementation

[1] Urea hydrolysis is the conversion of urea to carbonate and ammonium ions in the presence of water.

[2] Amino acid deamination is the removal of an amine group from a molecule.

[3] Urease is an enzyme which catalyzes the hydrolysis of urea.

Table 2 Composition of the culture media

Culture medium	Compound				pH
Caso*	Casein peptone	Soymeal peptone	NaCl	Urea	7.3 ± 0.2
	15 g/l	5 g/l	5 g/l	20 g/l	
Y-Ext*	Yeast extract	NH_4Cl	$NiCl_2$		8.5 ± 0.2
	20 g/l	10 g/l	10 µM		

is highly desirable in many of the engineering projects; liquefiable sandy soils, for example, require minimal premature cementation to overcome this potential.

The main objective of this study was to optimize the procedures described by Whiffin et al. (2007), Al-Thawadi (2008), van Paassen et al. (2009b) and Okwadha and Li (2010) for the attainment of maximum cementation with a single shot of injection. This objective was achieved by retarding the precipitation process by lowering the temperature of the injection mixture (reactant solution and bacterial suspension) as a novel approach. Thus, following the precedent set by the aforementioned authors, in this study, the urea hydrolysis process was selected for the experiment (Table 1), and for this purpose, the *Sporosarcina pasteurii* bacterium was used too for microbial catalyze of urea hydrolysis in sandy soil. Furthermore, *Arthrobacter crystallopoietes* was also tested under similar conditions to evaluate its capabilities. It, too, demonstrated more or less a similar capacity for urea hydrolysis. Preliminary tests on the urease activity of the bacterium and the effect of culture media as well as the soil were carried out.

In the present study which was conducted at K.N. Toosi University during 2013 and 2014, single as well as double phase injections of reactant agent with bacterial suspension were tested. Two different culture media and two different ambient temperatures were used. The calcium carbonate sediment in the treated samples was examined by scanning electron microscopy (SEM) and X-ray diffraction (XRD).

Firstly, precipitation condition was examined ex situ, and finally the practicability of this procedure was evaluated in improvised soil columns. In order to study the effect of calcium carbonate sediment on mechanical properties of the treated soil, the unconfined compressive strength (UCS) test was employed.

Materials and methods

Microorganisms and culture media

The two bacteria used in this study were *S. pasteurii* (PTCC = 1645) and *A. crystallopoietes* (DSM 20117^T), a gram positive, aerobic, rod shape, non-spore forming, and urease positive bacterium (Funke et al. 1996).

The former bacterium was obtained from the bacteria collection of the Iranian Center for Scientific and Industrial Researches, and the latter was isolated[4] from soil samples taken from Eshtehard region of Karaj with cooperation and support of the Agriculture and Natural Resources College of Tehran University.

Two sterile culture media under batch aerobic condition at 30 °C were tested in this study;

- Caso plus 2% of urea, here referred to as Caso*
- Yeast Extract plus Ammonium Chloride and Nickel Chloride, here referred to as Y-Ext*

The compositions of the culture media are presented in Table 2.

Since purification of bacterial cells is a time-consuming, difficult, and expensive procedure, the bacteria suspension was injected directly into culture media.

The bacteria were cultivated under aerobic condition at 30 °C. The rate of cultivation of the bacterium was monitored by spectrophotometer, and the bacteria were harvested at the end of exponential phase or the beginning of stationary phase. At this stage, mortality rate of bacteria is equal to reproduction rate and corresponds to the optical density (OD) of approximately two in this study.

Measurement of the urease activity

Considering the importance of urease activity of the bacteria, the presence and activity of the urease enzyme must be measured to identify the bacterial activity.

Activity of urease enzyme can be measured by monitoring the rate of urea hydrolysis. Based on the method proposed by Whiffin (2004), the rate of urea hydrolysis was measured by the variation of electrical conductivity: the electrical conductivity increases with the level of ionic products, produced by the hydrolysis of urea under standard conditions.

Following the same pattern as Harkes et al. (2010), 1 ml bacterial suspension was added to 9 ml of 1.11 M urea,

[4] 16S rRNA gene sequences test (was done by Iranian Biological Resource Centre (IBRC)) has been used for the identification of selected bacterium. BLAST results shows that the isolated strains has phylogenetically affinities with Arthrobacter Crystallopoietes (DSM 20117 (T)), a gram positive, aerobic, rod shape, non-spore forming, and urease positive bacterium.

and the electrical conductivity is measured over a 5-min period at room temperature in terms of mS/min. 1 mS/min bacterial activity (electrical conductivity rate) is correlated to hydrolysis of 11 mM urea during 1 min (Harkes et al. 2010).

Measurement of the rate of sediment precipitation

In order to evaluate the rate of precipitation of sediments formed by bacterium cultivated in different culture media (Caso* and Y-Ext*) and at different initial temperatures (room temperature and 3 °C), an equal volume of the bacteria and the reactant solution with different molarities were combined, and the solution was allowed to precipitate at the room temperature. The precipitated sediment was separated from the solution using a centrifuge device at specified time intervals (i.e., seven measurements at 10 and 20 min, 1, 2, 4, 8 and 24 h after the injection). The sediment was then dried in an oven and weighed, and the percentage of the formed sediments was calculated.

Soil and column

Non-cohesive siliceous (36%) coastal sand from southern shores of the Caspian Sea was used for the experiments. According to sieve analysis, the soil is classified as poorly graded sand (SP) ($d_{10} = 0.08$ mm, $d_{30} = 0.09$ mm and $d_{60} = 0.12$ mm).

Samples of the sand with dry density of 1.55 g/cm^3 were prepared by tamping and tested in the standard direct shear test (100 mm box), and the internal angle of friction was measured to be approximately 33º. The pH value of the soil was also measured to be 8 ± 0.2.

PVC tubes with internal diameter 37 mm were used to prepare the treated columns of the soil: A (green) dish scrubber (Scotch layer) or a rustproof net (in order to prohibit soil particle erosion) was put at the bottom. Then, about 1-cm filter gravel was put on top of Scotch layer (soil filter). The required amount of the sand was weighed and placed in the cylinder in three layers and tamped. The final height of the prepared sample was used as a measure of the required dry density. Finally one centimeter of filter gravel and a Scotch layer was placed on top of the sample and flushed with de-aired water.

Unit weight of the dried lumps of the treated samples was measured after the uniaxial tests by sealing with paraffin wax and floating in water, providing the added weight of the calcium carbonate.

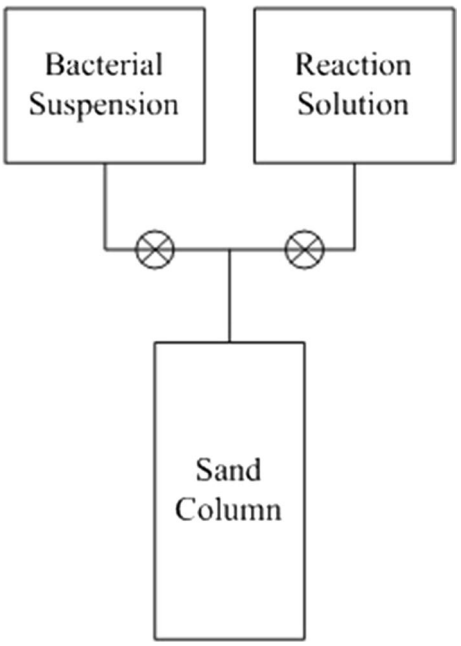

Fig. 1 Schematic of sand column setup for injection procedure

Injection procedure

Bacteria were cultivated up to the end of the exponential phase (OD ≈ 2) and then harvested and prepared for injection.

The volumes of the injected bacteria and of the reactant solution were nearly equal to the pore volume. The reaction solution contained 1 M urea and calcium chloride.

The injection procedure proposed by Whiffin (2004) was adapted; the reaction solution and the bacteria suspension were mixed just prior to injection (Fig. 1).

Due to high activity of bacteria at room temperature, precipitation begins immediately after combination of reactant solution and bacteria suspension. This hinders the injection and in order to subdue this effect, the two fluids were mixed 5 cm above the entry point into to the sand column, and a head of 1 and 0.6 m was provided, respectively, for the bacteria (*S. pasteurii*) in Caso* and Y-Ext* media for the flow in room temperature.

Reduction of temperature of the reactant solution and the bacteria suspension (up to 3 °C) proved to be more effective, and the premature activity of the bacteria was subdued. As a result, a lower head was employed to inject mixture of reaction solution and *S. pasteurii* suspension (Y-Ext*@3 °C), approximately 0.2 m head was provided.

At room temperature, five samples for each variation were prepared. Three samples for each variation were prepared at lowered temperature. All 52 samples were successfully prepared and tested.

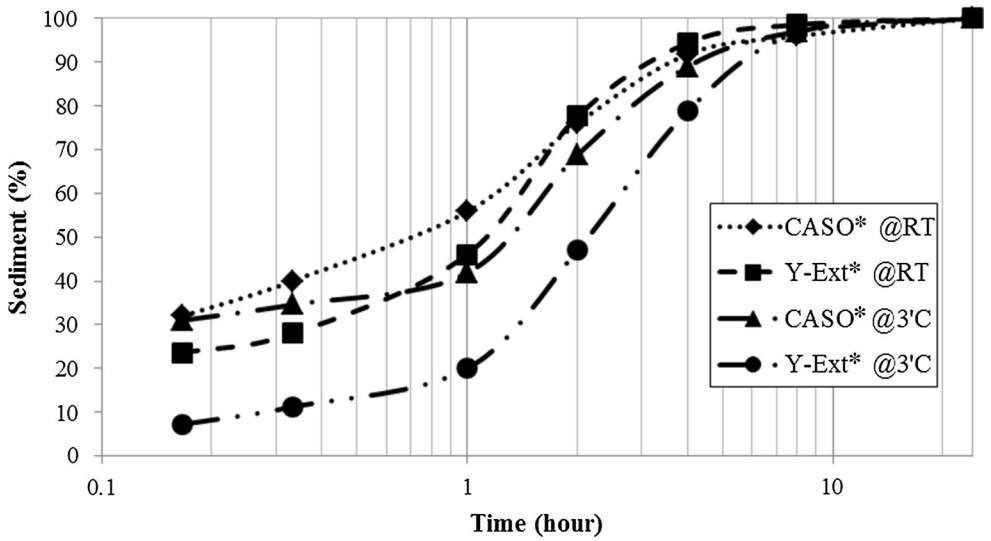

Fig. 2 Precipitation rate produced by *Sporosarcina pasteurii* (**RT* room temperature)

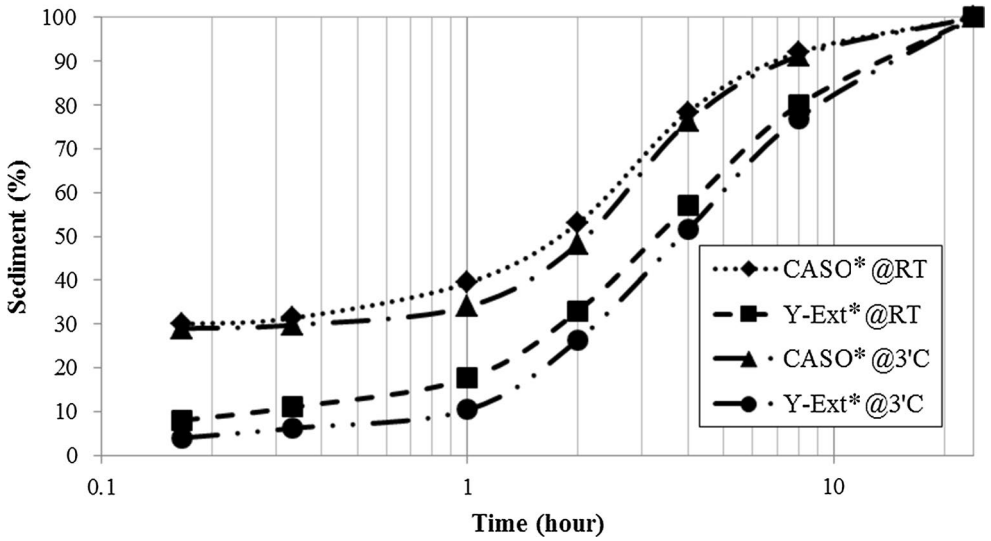

Fig. 3 Precipitation rate produced by *Arthrobacter crystallopoietes* (**RT* room temperature)

Results and discussion

Urease activity

Following the procedure described by Harkes et al. (2010), urease activities of the bacteria (*S. pasteurii* and *A. crystallopoietes*) were evaluated by measuring the electrical conductivity of the solution along 5 min after mixture. The urease activity of *S. pasteurii* in Caso* and Y-Ext* was about 42.6 and 19.0 mM urea/min, respectively, while the urease activity of *A. crystallopoietes* in Caso* and Y-Ext* was about 0.5 and 0.4 mM urea/min, respectively.

Sporosarcina pasteurii proved to be more active than *A. crystallopoietes.* No bacterial activity (for either bacterium) was recorded at 3 °C temperature.

Precipitation rate

Formation of sediments in the both bacteria, both culture media and with different initial temperatures (i.e., room temperature and 3 °C) was measured in a 24-h period after mixture. Figures 2 and 3 show the trend of sediment formation for 1 mol of reactant solution during this time for *S. pasteurii* and *A. crystallopoietes*, respectively.

The relative sediment used in Figs. 2 and 3 is a ratio of sediment mass at any time "*t*" to the mass obtained at the end of reaction (i.e., $t_f = 24$ h).

The total amount of sediment produced after 24 h was the same for all samples (0.5 g), irrespective of culture media, initial temperature, or bacterium used.

The presence of urea in Caso* culture media leads to the increase of CO_3^{2-} concentration in cultivated batch (at the end of cultivation time), and as a result, it leads to a more sediment production in Caso* media at the outset of the precipitation in the both bacteria. Therefore, a relative retardation in the sediment formation was observed in Y-Ext* batch of both bacteria.

Approximately 30 and 55% of the final sedimentation for *S. pasteurii* and 30 and 40% of the final sedimentation for *A. crystallopoietes* were formed during the first 10 and 60 min, respectively, in the Caso* culture media at room temperature. For samples prepared at the initial temperature of 3 °C in the Y-Ext* culture media, this rates were greatly reduced. About 7 and 20% of final sedimentation for *S. pasteurii* and 4 and 10% of final sedimentation for *A. crystallopoietes* were formed during the first 10 and 60 min, respectively, in the Y-Ext* culture media at 3 °C.

The subsequent adjustment of the temperature for the samples prepared at 3 °C causes a rapid increase in sediment formation and after a few hours reaches the amount formed by samples otherwise prepared.

Like all living organisms, bacterial growth and enzyme activity diminishes away from optimum temperature. Considering the other reaction conditions, urease activity of *S. pasteurii* is optimum at temperature ranges from 20 to 37 °C (Okwadha and Li 2010), and no noticeable activity was recorded at 3 °C. This fact led to the notion that temperature control could act as a retardant, allowing sufficient time for natural diffusion of the stabilizing fluids.

Compressive strength of treated sand

The treated sand columns were properly capped and prepared for unconfined compression strength test according to ASTM D 2166. Calcium carbonate contents were also measured as explained before.

Variation of UCS with calcium carbonate content for all samples prepared at room temperature is presented in Figs. 4 and 5. Some of the samples prepared with a single shot of

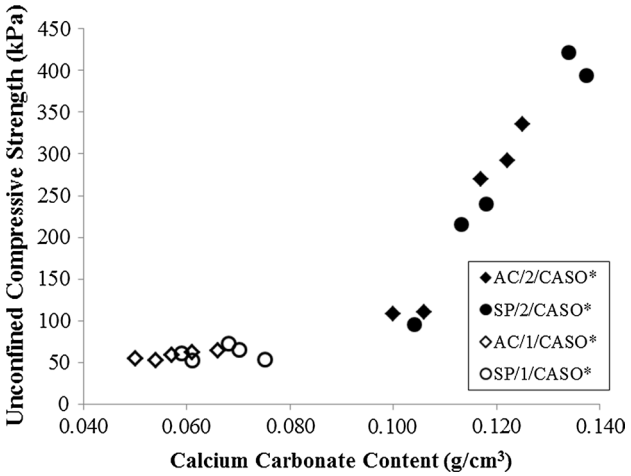

Fig. 4 Compressive strength versus $CaCO_3$ of treated column by bacteria cultivated in Caso* at room temperature

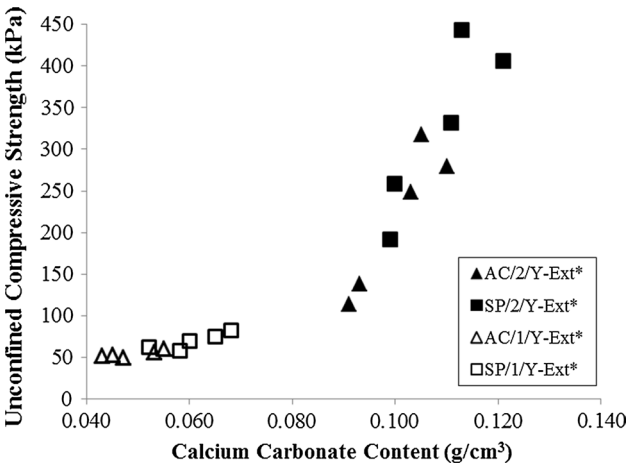

Fig. 5 Compressive strength versus $CaCO_3$ of treated column by bacteria cultivated in Y–Ext* at room temperature

injection at room temperature could not be retrieved or prepared for test. The results presented here have been obtained from successful attempts.

Almost all of the samples subjected to double injection attained compressive strengths and calcium carbonate content in excess of 100 kPa and 0.1 g/cm^3, respectively, whereas the compressive strength and calcium carbonate contents of samples subjected to a single shot were all well below the stated figures.

For all samples ($CaCo_3 > 0.1$ g/cm^3) subjected to double injection, an approximate gain of 100 kPa in compressive strength per 0.01 g/cm^3 increase in calcium carbonate content may be noted.

The UCS of samples treated with a single shot of the reactant solution at room temperature was well below the 100 kPa mark with an average value of about 60 kPa.

Table 3 Mean values of UCS of samples prepared and injected at room temperature

Bacterium	Injection	Culture media	$CaCO_3$ (g/cm^3)	UCS (kPa)
Sporosarcina pasteurii	Single	Y-Ext*	0.06	70
		Caso*	0.07	61
	Double	Y-Ext*	0.11	326
		Caso*	0.12	273
Arthrobacter crystallopoietes	Single	Y-Ext*	0.05	54
		Caso*	0.06	59
	Double	Y-Ext*	0.10	219
		Caso*	0.11	223

The calcium carbonate contents were also in the order of 0.04–0.07 g/cm^3 (Table 3).

Despite the fact bacterial activity were consistently higher in SP/Caso*[5], the culture media appears not have any meaningful bearing on the compressive strength of the treated samples. Due to the high bacterial activity and the high concentration of CO_3^{2-} in the cultivated Caso* culture media, the sediment formations were formed premature, and therefore, partial clogging at the entry point and heterogeneous precipitation were occurred. Similar trend was also observed in AC/Caso*. Regardless of the low bacterial activity of *A. crystallopoietes*, the early sediment formation led to clog at the injection point and prohibit spatial homogeneity of sediment formation. As a result, the injection head must be increased to conquer inlet clogging.

The mean strengths of the samples treated with SP/2/Caso*[6] and SP/1/Caso*[7] were approximately 273 and 61 kPa, respectively, whereas the samples treated with AC/2/Caso*[8] and AC/1/Caso*[9] were approximately 223 and 59 kPa, respectively (Table 3).

As the urease activity of bacteria is reduced, the consumed time to gain an intended degree of cementation is increased. Hence, at a specified treatment time, the degree of the cementation in the lower urease activity bacteria is reduced (Fig. 5). As it is seen, the mean calcium carbonate contents and the mean UCS of the sample treated with AC/2/Y-Ext*[10] and AC/1/Y-Ext* are the lowest amount among the other treatment method (Fig. 5).

Although the urease activity of the SP/Y-Ext* is lower than SP/Caso*, its activity is not low enough to eliminate premature precipitation completely (Fig. 2), thereupon a lower degree of clogging at the injection point was occurred. It nearly improves the homogeneity of $CaCO_3$ distribution and the degree of the cementation. The mean UCS and the mean $CaCO_3$ contents of the samples treated with SP/2/Y-Ext* and SP/1/Y-Ext* were 326 and 70 kPa, respectively.

The correlation tentatively noted between the compressive strength and $CaCo_3$ content for samples with double injection does not hold for results of samples subjected to single-phase injection.

Mean values of the measured compressive strength and calcium carbonate content for samples prepared at 3 °C with a single shot of injection are listed in Table 4.

All of enzymatic reactions are temperature dependent (Okwadha and Li 2010). The reduction of the temperature is led to the reduction of the urease activity (Whiffin 2004; Okwadha and Li 2010). This activity discount is temporary, and it is enhanced by the growth of the temperature (Figs. 3, 4).

As respects the activity of the both AC/Caso* and AC/Y-Ect* suspension is too low, reduction of temperature intensify it. Therefore, the decrement of the temperature in *A. crystallopoietes* bacterial suspension increases the time of precipitation process too much. As a result, the degree of cementation is reduced at the same conditions of the performed tests, and a low $CaCO_3$ content and compressive

Table 4 Mean values of UCS of samples prepared with single shot injection at 3 °C

Bacterium	Culture media	$CaCO_3$ (g/cm^3)	UCS (kPa)
Sporosarcina pasteurii	Y-Ext*	0.06	230
	Caso*	0.06	85
Arthrobacter crystallopoiete	Y-Ext*	0.04	–*
	Caso*	0.05	53

*Could not be prepared for test

[5] Refer to a suspension which contain *Sporosarcina pasteurii* bacteria cultivated in Caso* culture media.

[6] SP/2/Caso* refer to a double shot treatment using *Sporosarcina pasteurii* bacteria cultivated in Caso* culture media.

[7] SP/1/Caso* similar to the SP/2/Caso* trend using a single shot.

[8] AC/2/Caso* refer to a double shot treatment using *Arthrobacter crystallopoietes* bacteria cultivated in Caso* culture media.

[9] AC/1/Caso* similar to the AC/2/Caso* trend using a single shot.

[10] AC/2/Y-Ext* similar to the AC/2/Caso* trend using Y-Ext* culture media, etc.

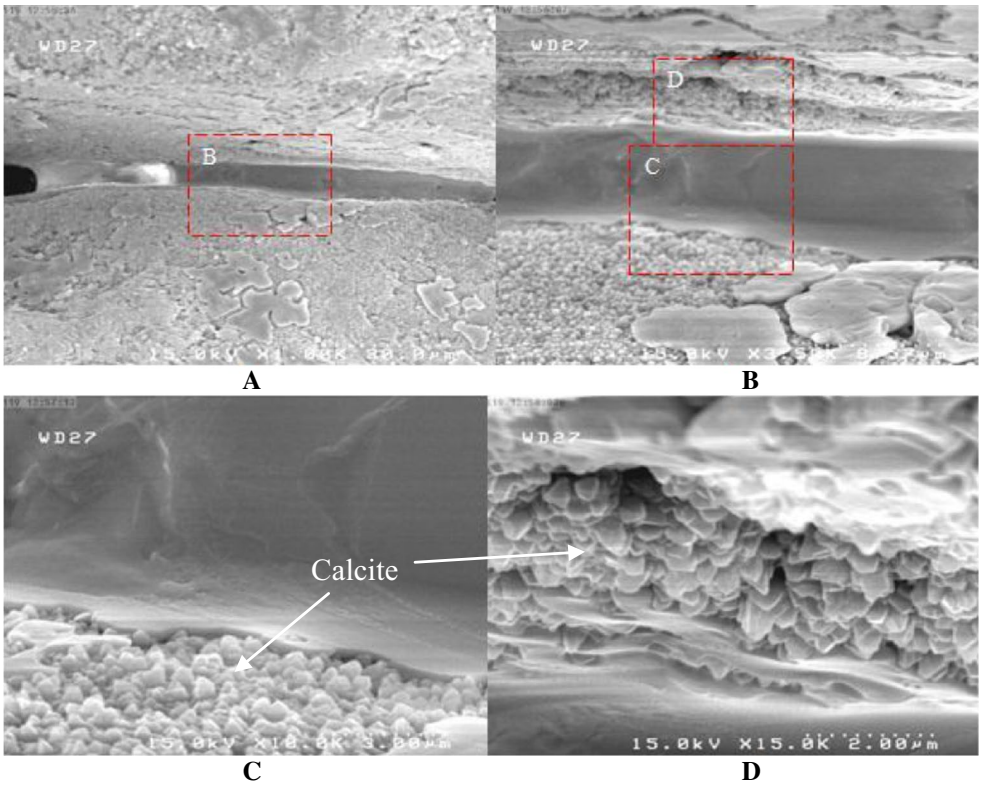

Fig. 6 SEM images from interface of soil particle, calcium carbonate cover a layer on the surface of two soil particles (top and bottom of image **A**), then connect them with a $CaCO_3$ bond

strength were achieved in treated samples. As mentioned in Table 4, the samples treated with the AC/1/Y-Ext* at 3 °C could not be prepared for test (Table 4).

Temperature reduction is more effective in the high urease activity bacteria. This lead bacterial activity of *S. pasteurii* to decrease temporary, and after a while its activity returned to normal amount (Fig. 2). This temporary reduction of activity rectifies initial clogging at the inlet point. Therefore, the reactant solutions diffuse more conveniently, and a more homogeneous distribution is attained. The mean UCS of the samples treated with SP/1/Y-Ext* and SP/1/Caso* at 3 °C was 230 and 85 kPa, respectively, whereas the mean $CaCO_3$ contents of them were 0.055 and 0.063 g/cm^3, respectively. Due to the presence of the carbonate ion in the SP/Caso* suspension, the premature precipitation and clogging were still occurred even at 3 °C state. So the relative increase in the mean UCS was not impressive in the sample treated with SP/Caso* at 3 °C state.

It has been recognized inhibitory tactics that prevent undue activity of the bacteria, facilitates normal diffusion of the stabilizing fluids before the onset of MICP (Al-Thawadi 2008), contributing to a more homogenous mass. Obviously to achieve a certain degree of cementation, attainment of higher degree of homogeneity becomes more critical for single-phase treatment processes.

The less active bacteria hydrolyze a specified amount of urea during a longer time which causes the reactant solutions to be wasted in the samples treated by lower activity bacteria. The experimental observations shown too that the cementation degree of the samples treated with *A. crystallopoietes* bacteria were the lowest amount among the presented result. The UCS and $CaCO_3$ contents of the sample treated by *A. crystallopoietes* can be increased if the resting time of the reactant solutions is increased too. Based on the results was not presented here, increasing the resting time to 48 h led the UCS and $CaCO_3$ contents of the treated soil to increase up to 600 kPa and 0.12 g/cm^3.

The decrease in the temperature of the reactant solutions postpones the beginning of the precipitation, in other words the temperature reduction imposes a retardation to the precipitation process. As the temperature goes up, the urease activity and sedimentation formation rate increase (Figs. 2, 3).

Similarly, as the chilled reactant solutions permeate through the soil pores, its temperature is balanced to the soil temperature, and consequently its urease activity is increased. So clogging at the inlet point was faded, and a more homogenous mass could be attained (Figs. 4, 5). Consequently, the injection of the reactant solutions was perfumed with less pressure. In addition, the reactant solutions waste which is observed in the lower activity bacteria (*A. crystallopoietes* at 3 °C) is faded too.

Generally, a uniform distribution of the available $CaCO_3$ entails the maximum achievable "macro" strength of the

mass. It is worth noticing that localized amassing of $CaCO_3$ produces zoned strength, while the strength registered in compression test denotes the strength of the weakest pockets of cemented sands that form the failure mechanism.

SEM and XRD analysis

Figure 6 shows the SEM[11] images taken from the contact surface of two soil particles and sediment around it. (Sedimentation was produced using *S. pasteurii* bacteria cultivated in Y-Ext * culture media at 3 °C.) The figures indicate that firstly a layer of sediment covers the soil particles, then the gap between two particles is filled with sediment, like the results presented by DeJong et al. (2010).

As shown in Fig. 6, the dominant formed sediment in soil is rhomboidal shape that indicates the fact that the sediment is calcite type (Berdonosov et al. 2005). The XRD[12] analysis was performed on precipitated $CaCO_3$. The XRD analysis also confirmed that Calcite was the dominant mineral phase.

The XRD analysis and SEM image also proved that the $CaCO_3$ sediment was produced with *S. pasteurii* bacteria suspension at 3 °C was mainly calcite type. It should be noted that calcite was the most stable polymorph of the calcium carbonate (Falini et al. 1996).

Conclusion

In this research, it was endeavored to simplify the MICP procedure by doing away with multiple injection or recirculation of the agents. In order to subdue the above-mentioned problems, the reactant solutions were mixed prior to the introduction into the soil.

Lowering of initial temperature of the suspension and the reaction agents causes temporary urease activity reduction which circumvents inlet clogging due to premature activity. The urease activity of *S. pasteurii* in Caso* and Y-Ext* at room temperature was 42.6 and 19 mM urea/min, respectively, whereas no bacterial activity was recorded in Y-Ext* at 3 °C temperature. After a while, activity of cooled suspension was retrieved. Therefore, the reactant solutions were diffused more homogenous, and a better degree of cementation was attained. Based on the presented results, in the similar conditions, the uniaxial compressive strength of improvised samples was increased from around 70 kPa to about 230 kPa when initial temperature of reactant agent was reduced.

It seems that by this method a fully controlled process with lower costs for soil strength by controlling the temperature as an inhibitor factor along with considering the effect of flow speed can be reached. Temperature reduction potentially can also develop the injection distance of reactant solutions which should be studied in the further research.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests relevant to this article.

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[12] This test was done by Iranian Material and Energy Research Center.

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