Treatment conditions used in microbial induced calcite precipitation for sandy soil improvement

Soyson Arpajirakul[1], S. Likitlersuang[2,*] and W. Pungrasmi[1,3]

[1] Department of Environmental Engineering, Faculty of Engineering, Chulalongkorn University, Phayathai Road, Wang-Mai, Pathumwan, Bangkok 10330, Thailand.
[2] Centre of Excellence in Geotechnical and Geoenvironmental Engineering, Department of Civil Engineering, Faculty of Engineering, Chulalongkorn University, Phayathai Road, Wang-Mai, Pathumwan, Bangkok 10330, Thailand. (*corresponding author)
[3] Research network of NANOTEC-CU on Environmental

ABSTRACT

This work presents a study of treatment conditions for ureolytic bacteria in sand media aiming to promote the use of Microbial Induce Calcium carbonate Precipitation (MICP) technology in practice. The activity test of urease produced from *Sporosarcina pasteurii* ATCC 6453 was performed to define the optimum urea-$CaCl_2$ concentration and injection rate used in sand. The X-ray fluorescence (XRF) and X-ray diffraction (XRD) were tested to analyse the chemical compositions of sand. The results showed that the optimum treatment condition of 0.75 M urea-$CaCl_2$ with an injection rate of 7.5 mM/h can produce the highest clogging of the treated sand specimen. In addition, the chemical composition results obtained from the XRF and XRD analyses confirm that an existence of $CaCO_3$ precipitation induced by bacteria was found in the treated sand specimen.

Keywords: Ground improvement; Urease; Ureolytic bacteria; Microbial Induced Calcium Carbonate Precipitation; Optimum treatment condition; Strength and stiffness

1 INTRODUCTION

In the recent years, several researches on ground improvement have tried to find a new and/or enhance the existing techniques to efficiently improve the engineering properties of the soil mass. One of the alternative processes with environmental-friendly is biological approaches. Multicellular organisms, ranging from plant roots down to microbes, can change soils through various mechanical, hydrological and biochemical processes.

Research in biogeotechnical engineering to date has investigated many of potential processes for modifying engineering properties of soil. Microbial induced calcite precipitation (MICP) is one of promising techniques to improve strength, stiffness (Akyol et al. 2016) and permeability of soil (Dhami et al. 2016). In MICP, the creation of calcium carbonate (calcite) occurs as a consequence of microbial metabolic activity. Calcite can be created through different processes including urea hydrolysis by alkaliphilic urease – producing microorganisms (i.e., ureolytic bacteria). Urease catalyses the hydrolysis of urea into 2 moles of ammonium ion, which raise the pH, and 1 mole of carbonate ion (Eq. (1)). In the presence of calcium source from the supplied calcium compound results in precipitation of calcium carbonate at the bacterial cell surface (Eq. (2)) (Sharma and R. 2016). The efficiency of MICP for soil improvement is mainly influenced by soil type and the treatment conditions which various concentrations of urea and calcium ion in local system can affect the ability of bacteria to induce calcium carbonate precipitation between soil grains (Al Qabany et al. 2012).

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

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3\,(s) \quad (2)$$

This research aims to determine an appropriate treatment condition of MICP for improving the engineering properties of sandy soil. *Sporosarcina pasteurii*, formerly known as *Bacillus pasteurii*, is an ureolytic bacteria used in the study. Urea hydrolysis is tested to verify the activity of urease produced from bacteria. To investigate the optimum treatment conditions on the ability of MICP to improve soil samples, the various urea-$CaCl_2$ concentrations of cementation media and injection rates are selected to study.

2 EXPERIMENTAL AND METHODS

2.1 Bacterial culture media

For the cultivation and maintenance of pure culture of *Sporosarcina pasteurii* [American Type Culture Collection (ATCC) 6453]. NH_4-YE media (ATCC 1376) was prepared, includes 20 g/l of yeast extract, 10 g/l of $(NH_4)_2SO_4$, 20 g/l of agar (for solid media) and 0.13 M Tris buffer in pH 9.0. The bacterium was transferred to NH_4-YE solid media and incubated at 30°C for 48 to 72 h. After plate growth, bacterial

colonies were transferred to NH_4-YE broth and incubated under shaking condition at 30°C for 12 to 24 h to an optical density of 600 nm (OD_{600}) of 0.8–1.2 (10^6 cfu⁄ml). Bacterial cells were harvested by centrifugation at 8,000 rpm for 20 min at 4°C, remove the supernatant before wash cell pellets at the bottom of the test tube with saline solution and store at 4°C prior to use in all experiments.

To promote calcium carbonate precipitation, cementation media suggested by Al Qabany et al. (2012) was prepared, includes 3 g/l of Nutrient broth, 10 g/l of NH_4Cl and 2.12 g/l of $Na(HCO)_3$ as a nutrient source and pH stabilisation. The liquid media also contained urea as an energy and ammonium source for bacteria, and $CaCl_2$ as a calcium source. To determine the optimum treatment condition, different equimolar urea-$CaCl_2$ concentrations were varied at 0.25, 0.5, 0.75 and 1.0 M.

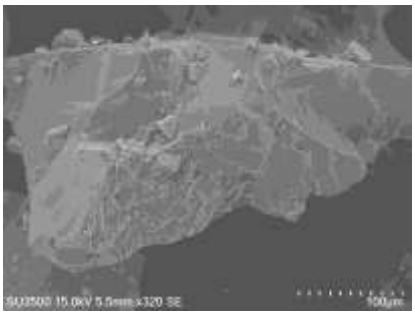
2.2 Urease activity

The urease activity was examined by transferring cell pellets of *S. pasteurii* to the cementation media with urea concentration of 0.2 M and shaking at 100 rpm, 30°C for 3 h. The control samples were tested by incubating the liquid media without bacteria at the same condition. The estimation of urea remaining using colorimetric urea analysis method (Knorst et al. 1997) was performed. 2 ml of the liquid media was mixed with 0.5 ml of the mixture solution (4% w/v *p*-dimethylbenzaldehyde and 4% v/v sulphuric acid in absolute ethanol) and incubated at room temperature for 10 min. The absorbance of the yellow colour solution was measured at 422 nm against urea standard solutions between 0.05 and 0.2 M and determined the concentrations of urea remaining by reference to the calibration curve.

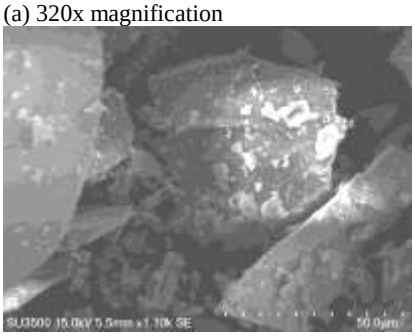
2.3 Preparation of soil sample

A fine-grained poorly graded silica sand (SP) was used in this study. Physical and engineering properties of untreated sand were determined. The specific gravity (G_s) is 2.67. A standard Proctor test (ASTM D698-70) provides a maximum dry unit weight ($\gamma_{d\ max}$) of 1.46 g/cm^3 with an optimum water content (W_{opt}) of 21%. Figure 1 presents the microstructure of irregularly shaped sand particles analysed by Scanning electron microscopy (SEM). The natural sand was passed through a #20-mesh sieve and oven dried to remove moisture at 105±5°C for 24 h. For bacterial sand samples, the sand and cell pellets were pre-mixed together in order to achieve homogeneous distribution. The control sand samples were prepared by mixing the sand with liquid media alone. All samples were compacted into a PVC mould (with height of 7.6 cm and diameter of 3.8 cm) at 80% of maximum dry density (Fig. 2).

For investigation of the optimum treatment conditions, the experiment was carried out with the cementation media, with urea-$CaCl_2$ concentrations of 0.25, 0.5, 0.75 and 1.0 M using injection rates as described in Table 1. The liquid media was supplied into four sets of both bacterial and control sand columns at room temperature (25 to 30°C) for 7 days. The flow rate of the effluent from each column was measured every day to observe the effect of treatment conditions on MICP efficiency. Noted that in the preliminary study, when the urea-$CaCl_2$ was applied once a day the upper part of sand sample was partially clogged by $CaCO_3$ precipitation resulted in the non-uniformity of the treatment. The injection methods presented in Table 1 could allow $CaCO_3$ precipitation occurring throughout the sand sample.



(a) 320x magnification



(b) 1100x magnification

Fig. 1. Scanning electron micrograph of untreated sand particles

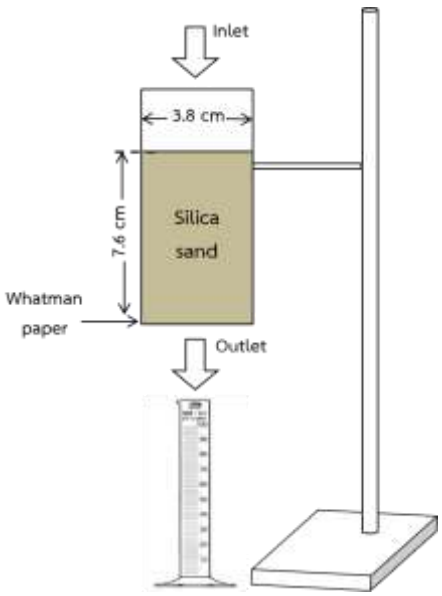


Fig. 2. Schematic diagram of the column setup.

Table 1. Injection rates used in the study

Urea-$CaCl_2$ concentration (mol/l)	Input volume per time (ml/h)	Injection rate (mmol/h)
0.25	10	2.5
0.5	10	5.0
0.75	10	7.5
1.0	10	10.0

2.4 Chemical characterisation

All samples were oven dried at 105±5°C for 24 h. X-ray fluorescence (XRF) and X-ray diffraction (XRD) were employed to analyse the chemical compositions, compounds and mineral deposits formed of treated sand samples comparing with natural untreated sand. The results can verify the formation of calcite crystals induced by bacterial.

3 RESULTS AND DISCUSSION

3.1 Urease activity

Urease activity was investigated in order to indicate an occurrence of urea hydrolysis. The calibration curve of urea standard solutions showed an equation for determining urea remaining in the cementation media (Eq. (3) and Fig. 3).

$$y = 7.93x \quad (3)$$

where x = urea concentration of sample (M) and y = absorbance value from the measurement at 422 nm.

It was observed that the amount of urea in the liquid media significantly decreased with time in the presence of *Sporosarcina pasteurii*. Urea were consumed approximately 92.84% of the initial concentration of urea after 3 hours of incubation. On the other hand, the quantity of urea remaining in control samples were consistent (Fig. 4). This indicates that urea hydrolysis occurred as a result of urease activity which could lead to the precipitation of calcium carbonates.

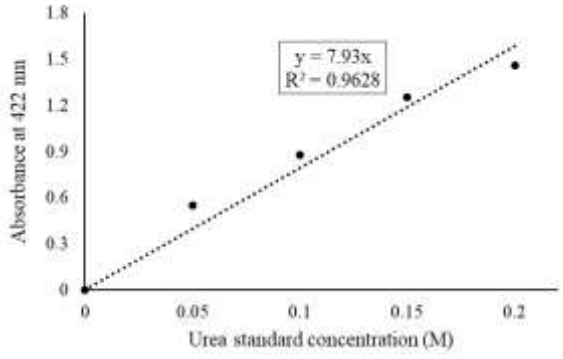


Fig. 3. The calibration curve for urea determination

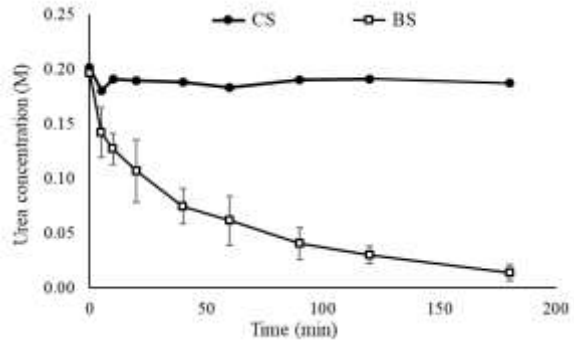


Fig. 4. Urea concentration in the control samples media (CS) and bacterial samples media (BS) for urease activity investigation

3.2 Optimum treatment conditions for sand

The flow rate of sand columns treated with various urea-$CaCl_2$ concentrations of cementation media were measured to assess the efficiency of different treatment conditions on MICP. The result showed the reduction in flow rate of all sand columns mixed with *Sporosarcina pasteurii* as a consequence of calcite precipitation (Fig. 5). In sand columns treated with 0.25, 0.5, 0.75 and 1.0 M urea-$CaCl_2$, the flow rate decreased from 11.0 to 10.8, 10.7 to 7.8, 10.8 to 4.9 and 10.8 to 8.0 ml/min, respectively (with 28.6, 33.6, 54.5 and 26.1% reduction). It is obvious that the treatment condition of 0.75 M urea-$CaCl_2$ with an injection rate of 7.5 mM/h is the appropriate condition used in this study.

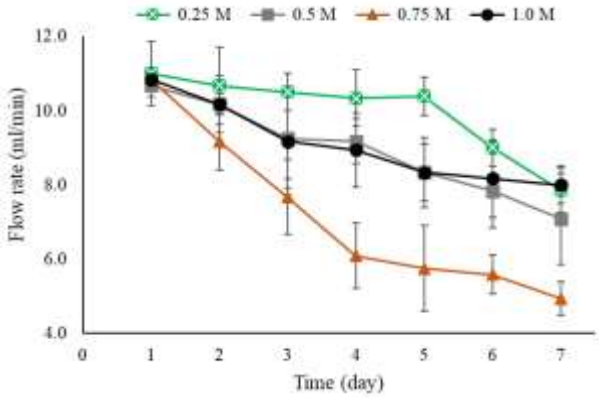


Fig. 5. Flow rate of the sand columns treated with different concentrations of urea-$CaCl_2$ in cementation media

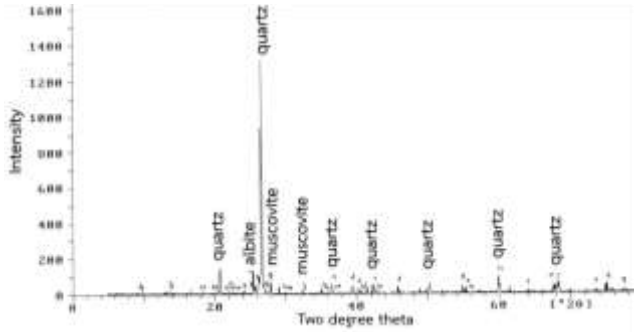
However, at 1.0 M urea-$CaCl_2$ which higher than the optimum concentration and injection rate, the reduction in flow rate is similar to 0.25 M urea-$CaCl_2$ sand columns. This finding is consistent with the results from previous study by Carmona et al. (2016) reported that excessively high concentration could retard the precipitation of calcite due to the large quantities of calcium ions results in high salinity condition, which tend to inhibit the activity of urease, subsequently the MICP process is less effective.

3.3 Chemical composition analysis

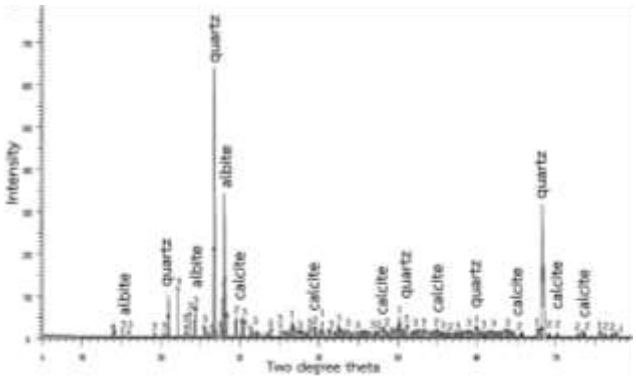
As depicted in Table 2, the result of chemical compositions determined by XRF analysis showed the greater amount of calcium content in treated sand improved with 0.75 M of urea-$CaCl_2$ comparing with natural untreated sand. In addition, the results of XRD analysis obtained revealed that the mineral constituents of treated sand sample consists fundamentally of quartz and calcite ($CaCO_3$) crystals (Fig. 6), whereas the untreated sand presents quartz along with some other components such as albite and muscovite, which confirms that the precipitation of calcium carbonate had taken place due to bacterial activity.

Table 2. Chemical composition of sand samples analysed with X-ray fluorescence (XRF)

Compound	Chemical formula	% w/w	
		Untreated	Treated
Sodium oxide	Na_2O	0.85	1.00
Magnesium oxide	MgO	-	0.05
Aluminum oxide	Al_2O_3	3.24	2.86
Silica	SiO_2	94.40	72.70
Phosphorus pentoxide	P_2O_5	0.01	0.05
Sulfur trioxide	SO_3	0.02	0.04
Chlorine	Cl	-	4.50
Potassium oxide	K_2O	1.09	1.06
Calcium oxide	CaO	0.22	11.80
Ferrous oxide	Fe_2O_3	0.10	0.11



(a) untreated sand sample



(b) treated sand sample

Fig. 7. Mineral constituents of sand samples analysed with X-Ray diffraction (XRD)

4 CONCLUSION AND RECOMMENDATION

In this study, urease activity and the effect of urea-$CaCl_2$ concentrations on the flow rate in bacterial sand columns were investigated to determine the suitable treatment condition for the improvement of soil by MICP technique. The results showed that the urea concentration decreased in the presence of *S. pasteurii* which indicate the ability of bacteria to produce urease, hydrolyse urea and induce the precipitation of calcium carbonate. The efficiency of MICP was influenced by treatment condition. The 0.75 M urea-$CaCl_2$ with an injection rate of 7.5 mM/h was the optimum condition which caused the highest clogging of the bacterial sand column. The existent of calcite ($CaCO_3$) crystals obtained from chemical composition analysis confirms the calcium carbonate precipitation induced by bacteria.

It is recommended that the research should extend to other soil types such as silty and clayey soils. In the future, other engineering properties such as strength and permeability of treated sample shall be performed.

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