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Assessment of different treatment methods by microbial-induced calcite precipitation for clayey soil improvement

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ABSTRACT

Microbial-induced calcite precipitation (MICP) utilises the metabolic pathway of ureolytic bacteria to form calcium carbonate precipitation throughout the soil matrix, leading to increased soil strength and stiffness. MICP has proved to be an efficient technique for treatment of clean sand; however, there is lack of knowledge for MICP treatment of clayey soils. This paper assesses three different MICP methods including injection, premixing and diffusion, for treatment of clayey sand containing up to 20% clay content. The results indicate that the injection method is applicable only of treating sand columns that contain < 5% clay content, with an exponential relationship between the unconfined compressive strength (UCS) and calcite content similar to that of treated pure sand samples. The premixing method, on the other hand, allowed a homogeneous placement of the bacteria cells in the sand columns and the subsequent injection of cementation solution under low pressure of 100 kPa enabled an adequate bio-cementation along specimens that contain up to 10% clay content, resulting in up to 150% increase in the UCS values. The results also show that sand columns with 20% clay content can be treated using the diffusion method, leading to increased UCS values of up to 200% depending on the treatment duration. The overall conclusion of this study is that bio-cementation of clayey soils is a tremendous challenge and requires further investigation on the ureolytic bacteria placement and supply of cementation solution at large scale before field application.

RÉSUMÉ

La précipitation de la calcite par les microbes (MICP) utilise la voie métabolique des bactéries uréolytiques pour former un précipité de carbonate de calcium à travers la matrice du sol, conduisant à une augmentation de la résistance et de la rigidité du sol. La technique MICP s'est avérée efficace pour le traitement d'un sable propre. Cependant, les connaissances sur le traitement des sols argileux par cette technique sont limitées. Cet article étudie trois méthodes différentes de traitement MICP (l'injection, le pré-mélange, et la diffusion) et ce, pour les sables argileux qui contiennent jusqu'à 20% d'argile. Les résultats indiquent que le procédé par injection est applicable seulement à des colonnes de sable contenant moins de 5% de teneur en argile, avec une relation exponentielle entre la résistance à la compression non confinée (UCS) et la teneur en calcite comme c'est le cas pour les échantillons de sable pur traités. D'autre part, le procédé de pré-mélange a permis une mise en place homogène des cellules bactériennes dans les colonnes de sable. L'injection de solution de cimentation sous basse pression de 100 kPa qui s'en suit, permet une bio-cimentation adéquate le long des échantillons qui contiennent jusqu'à 10% d'argile, aboutissant à une augmentation de 150% des valeurs UCS. Les résultats montrent également que les colonnes de sable avec 20% de teneur en argile peuvent être traitées selon la méthode de diffusion, conduisant à une augmentation des valeurs UCS jusqu'à 200% en fonction de la durée du traitement. La conclusion générale de cette étude est que la bio-cimentation des sols argileux est un énorme défi et nécessite une recherche plus approfondie sur la mise en place des bactéries uréolytiques et l'approvisionnement d'une solution de cimentation à grande échelle avant l'application in situ.

1 INTRODUCTION

The current rapid growth of population causes a rise in the demands for new infrastructures, which are limited by the presence of poor soil conditions. In order to utilise a weak soils, it needs to be either improved or replaced by a more suitable soil deposit. Existing technologies for ground improvement include cementation (e.g., grouting), densification (e.g., mechanical compaction), drainage (e.g., vertical drains) and thermal stabilisation (Burbank et al. 2011). Although many of these ground improvement techniques have proven to be successful in many situations, they suffer from some problems. For example, chemical grouting, which is currently the most widely used

ground improvement method, utilizing grouts such as epoxy, acrylamide, silicates and polyurethane raise the issues of cost, health and safety. The most commonly used chemical grout nowadays (i.e., Portland cement) is one of the major sources of green-house gas emission causing global warming. Furthermore, the effective treatment distance of chemical grouting is only 1–2 m from the injection point due to the limitation of the mixing equipment (DeJong et al. 2010) and such soil mixing method is not applicable to treat large ground volumes underneath existing constructions (Karol 2003). Therefore, there is a need for alternative soil improvement methods that can be more sustainable, environmentally-friendly and cost-effective.

The microbial-induced calcite precipitation (MICP) is a promising technique that is recently emerged as an environmentally-friendly ground improvement technique in which non-pathogenic ureolytic bacteria are injected into the soils to react with an injected calcium-rich cementation solution to form precipitated calcite (calcium carbonate or CaCO_3). The formation of calcite in the soil binds the soil particles together, leading to increased soil strength and stiffness. MICP is a process in which the CaCO_3 precipitation is controlled by the hydrolysis of urea catalysed by urease active bacteria (De Muynck et al. 2010). This process can be described as follows:

- Urea is hydrolysed by microbial urease to form ammonium and carbonate ions: $\text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_3^{2-}$
- The produced carbonate ions react with calcium ions and produce calcium carbonate: $\text{CO}_3^{2-} + \text{Ca}^{2+} \rightarrow \text{CaCO}_3$

Theoretically, the rate of calcite precipitation can be governed by many factors such as the concentration of dissolved inorganic carbon, amount of pH, concentration of calcium ions and presence of nucleation sites in the soil specimen (Al Qabany et al. 2012).

Most available studies on MICP that have been conducted so far have focused on treatment of sands, and there are very few studies available in relation to MICP for clayey soils. For example, Soon et al. (2014) have found that bio-cementation may improve the shear strength of soils that contain up to 20% fines by 69%, using the premixing method associated with a high flow pressure of 1.1 bar of the cementation reagent. Nevertheless, the amount of calcite precipitation and required number of flushes of the cementation solutions needed to gain the soil strength has not been identified.

The main aim of this paper is to investigate the use of MICP for improving the engineering properties of clayey sand, using three treatment approaches including injection, premixing and diffusion. This study also aims to gain improved understanding of bio-cementation for clayey soils through a series of tests that will investigate several parameters including the urease activity, crystal content, permeability and strength of bio-cemented samples. Scanning Electron Microscopy (SEM) will also be conducted to identify the microstructure of formed crystals, which could then be correlated to the engineering properties of bio-treated soils.

2 MATERIALS AND METHODS

2.1 Bacterial Culture and Cementation Solution

The urease active strain used in the current study was MCP-11 (*Bacillus sphaericus*, DSM 23526, Germany) (Al-Thawadi et al. 2012). The MCP-11 strain was cultivated under sterile aerobic batch conditions in a medium consisting of 20 g/L of yeast extract, 0.17 M ammonia sulphate and 0.1 mM NiCl_2 and pH of 9.25. After 24 hours of cultivation under 28°C , the bacteria culture was collected and stored at 4°C prior to use. The optical density of the harvested culture varied between 1.5–2,

and the urease activity was approximately 10 U/ml (1U = 1 μmole urea hydrolysed per min). The cementation solution consisted of 1 M of calcium chloride (111 g/L) and 1 M of urea (60 g/L).

2.2 Sand Column Setup and Sample Preparation

Pure silica sand of size 425 μm (Cook Industrial Minerals Pty. Ltd., Western Australia) was used in this study. The particle-size distribution curve of the sand used is shown in Figure 1. The coefficient of uniformity was 1.65, whereas the coefficient of curvature was 0.84. The sand was classified as poorly graded sand according to the Unified Soil Classification System (USCS). Kaolin clay (Prestige Kaolin Forming Clays) was used because it is chemically stable and was added to the sand as fines. Different percentages of clay content were mixed with the sand to represent 5%, 10% and 20% by weight of the soil samples. PVC pipes with an internal diameter of 45 mm and length of 180 mm were used for the soil columns. The soil samples were packed into the PVC columns to achieve the maximum dry densities at the corresponding optimum moisture contents, as shown in Table 1.

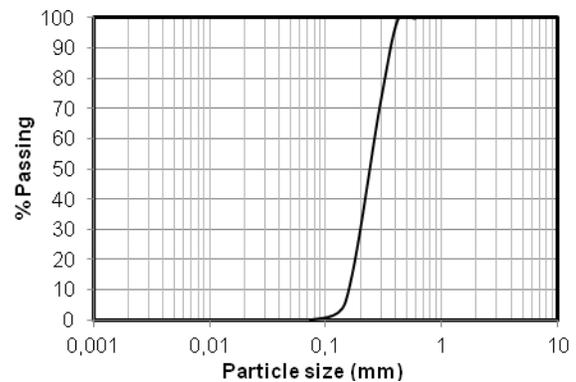


Figure 1. Particle size distribution of silica sand used

Table 1. Summary of maximum dry density and optimum moisture content

Specimen (clay content)	Maximum Dry Density (kN/m^3)	Optimum Moisture Content (%)
0%	16.3	14.1
5%	17.3	13.9
10%	18.3	12.0
20%	20.2	9.6

2.3 Bio-cementation Treatment Methods

Three different bacteria treatment methods were carried out in the current study including injection, premixing and diffusion. The three methods are described in some detail below.

Injection method: the bacteria introduction was achieved by down-flushing half void volume of bacterial culture followed by half void volume of cementation solution into the pre-packed sand column (Cheng et al. 2013). Then, the sand column was kept at the room temperature ($25 \pm 1^\circ\text{C}$) for 24 hours of incubation to allow the immobilisation of bacterial cells in the specimen. After

the 24 hours incubation period, one void of cementation solution was injected into the sand column followed by 24 hours of reaction period at the room temperature. Repeated injection of cementation solution and additional injection of bacteria culture was applied to achieve strong bio-cemented sample.

Premixing method: the bacteria supply was achieved by premixing a certain amount of concentrated bacterial culture (5 times concentrated with about 50 U/mL urease activity) with soil to the reach the optimum moisture content. After being packed in the PVC, the soil sample column was kept at room temperature for 24 hours for bacterial cells immobilisation. Then, one void volume of cementation solution was injected into the sand column followed by 24 hours of reaction period. Repeated injection of cementation solution was carried out to achieve strong bio-cemented sample.

Diffusion method: the approach used for the bacteria supply was the same as for the premixing method explained above. The soil samples were immersed with the immobilised bacteria (horizontally placed) in cementation solution (5 L), and left to cure for 7, 14 and 21 days, respectively. The ends of the sand column were wrapped with geofabric, which would allow cementation solution to diffuse through but prevent the soil particles from being washed out.

2.4 Monitoring Experiments

2.4.1 Unconfined Compression Test

To quantify the strength imparted into the MICP-treated silica sand under different saturation conditions, unconfined compressive strength (UCS) tests, in accordance with ASTM D2166 (ASTM 2006), were conducted on cemented specimens of 45 mm in diameter and selected diameter-to-height ratio of 1:1.5 and 1:2. The axial load was applied at a constant rate of 1.0 mm/min.

2.4.2 CaCO₃ Content Test

Calcite content test was conducted by adding 2 mL of 2 M HCl solution into 0.5-2 g of dry samples. The volume of carbon dioxide gas produced was measured using U-tube manometer under standard conditions (25°C, 1 atm). A standard curve was made with laboratory grade CaCO₃ powder.

2.4.3 Scanning Electron Microscopy

To characterize the shapes and locations of the precipitated CaCO₃ and investigate the bonding behavior between the grain hosts and cement agent, microscopy analysis was conducted on the cemented soil samples, which were taken from the center of the cemented sand columns. Before conducting the microscopy investigation, all samples were flushed with tap water and dried at 60 °C for 24 hours. The microscopy investigation was carried out via scanning electron microscopy (SEM) using a PHILIPS XL20 scanning electron microscope (Eindhoven, the Netherlands).

3 DISCUSSION OF RESULTS

3.1 Injection Method

From the results obtained using the injection method as a stop-flow mode, it was possible to produce high strength of bio-cemented soils soil samples having clay content of up to 5% (Figure 2). It can be seen that the UCS of MICP treated specimens exponentially increases with the increase in CaCO₃ content. The slight increase in UCS of the 5% clay sample compared to the pure silica sand sample may be attributed to the increase in the soil cohesion produced as a result of the clay content (Akayuli et al. 2013). It may also be attributed to the increase in contacting surface area provided by the clay fine particles, which may facilitate the bridging formation between the sand particles via CaCO₃ crystals.

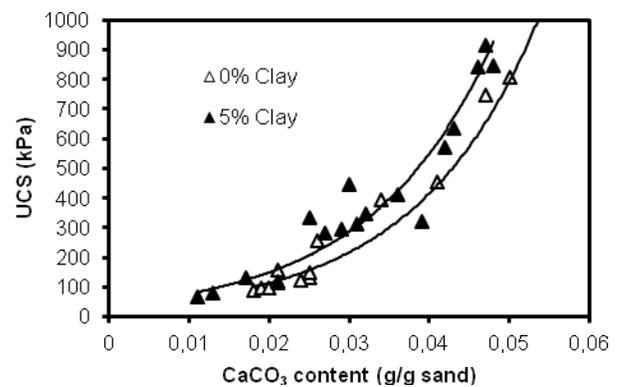


Figure 2. Relationship between CaCO₃ (calcite) content (g/g sand) and UCS using injection method

In Figure 3, the injection end blocking (excessive cementation) and minor CaCO₃ precipitation (about 0.004 g/g sand) inside the column was observed at 10% clay content after 3 times treatment. Further treatment became difficult to carry out due to the serious clogging. The phenomenon of clogging possibly attributed to the high amount of urea hydrolysed and hence the CaCO₃ formed at the injection end, which is determined by the presence of urease activity and duration over which the cementation solution is exposed to the clogging area. The 10% clay content sand samples, which have smaller pores acted as a filter to the bacteria resulted in accumulation of bacterial cells (also urease activity) around the injection end. The accumulated urease activity associated with the low infiltration rate of cementation solution (hydraulic conductivity: 0.11×10^{-6} m/s) resulted in excessive bio-cementation occurring at the injection end. The low infiltration rate of cementation solution can be increased by applying a higher flow pressure of the cementation solution (100 kPa was used in the current study). It can be concluded from the above results that bio-cementation treatment using the injection method may not be applicable to soils that contain 20% clay due to an immediate bio-clogging at the injection end (bacterial cells blocked the pores).



Figure 3. CaCO₃ content distribution along the sand column containing 10% clay

3.2 Premixing Method

In the injection method described above, the injection end clogging and lack of cementation inside the high content clay samples are likely due to the reaction of cementation solution during the infiltration leading to less reagents moving to deeper areas (Whiffin et al. 2007). Apart from this issue, the urease activity distribution also plays a significant role on the injection clogging and it is expected that more reagents are converted and precipitate as CaCO₃ crystals in the areas where the ureolytic bacteria are accumulated. Consequently, the bacteria accumulation around the injection end of the high content clay soil samples can be avoided by the premixing method of the bacterial culture with the soil, allowing more homogeneous placement of ureolytic bacteria and urease activity. The results indicate that the premixing method may treat soils containing clay of up to 10% (Figure 4). The UCS of such clay content specimens was increased to 250 kPa, which is about 2.5 times higher compared to the benchmark soil sample (i.e., bacteria-free sample treated with the same cementation solution). The CaCO₃ content up to 0.019 g/g sand was also detected around the outlet of the sand columns, indicating a successful cementation over the entire sand column.

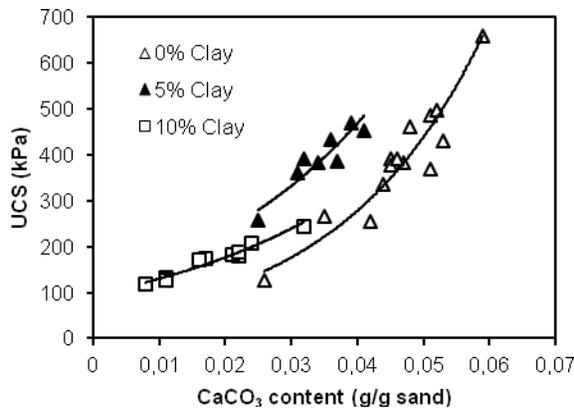


Figure 4. Relationship between CaCO₃ (calcite) content (g/g sand) and UCS using premixing method

Although similar to the injection method the MICP treatment using the premixing method may not be applicable to soils that contain 20% clay content due to the injection end clogging; however, the reason that causes the clogging in the premixing method is different from that of the injection method. In the premixing method, the clogging was induced by the excessive CaCO₃ precipitation after the second treatment of the cementation solution, whereas the clogging encountered in the injection method was due to the pore blocking by the injected bacterial cells.

3.3 Diffusion Method

In this method, successful cementation was only achieved around the end of the soil columns (about 5 cm in depth) for all clayey soil samples. Figure 5 shows that the UCS and CaCO₃ increase with the increase in the curing time. The maximum UCS values of MICP treated soils containing 10% and 20% clay samples were about 280 and 400 kPa after 21 days of curing period, which are about 2.5 and 1.6 times, respectively, of the benchmark soil samples (i.e., bacteria-free sample submerged in cementation solution). After 21 days of curing period, the average CaCO₃ content in the samples containing less clay is much higher than that of the samples having higher clay content. This is attributed to the higher mass diffusion speed in the lower content clayey samples, which has greater porosity. In the current study, the mass diffusion speed of CaCl₂ and urea is determined by the effective diffusivity of each component in the soil porous media. It is well known that the mass diffusion speed increases with an increase in effective diffusivity, which increases with the increase in porosity. Thereby, high porosity facilitates the cementation solution to diffuse into soil specimens and promote the MICP process (Zhao et al. 2014).

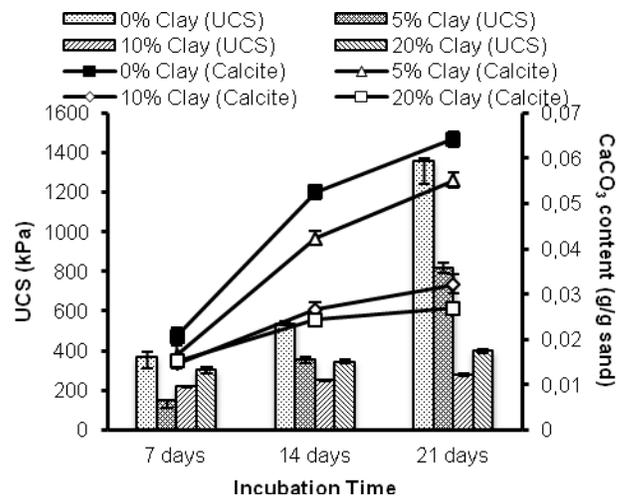


Figure 5. Average CaCO₃ (calcite) content (g/g sand) and UCS of bio-cemented samples using diffusion method

Further investigation for the evolution of the CaCO₃ content along the soil specimens during the curing time reveals that the MICP started to occur from the end of the

sand columns, and then gradually developed into the column due to the diffusion of the cementation solution (Figure 6). Therefore, the precipitation rate of CaCO_3 inside the specimens is determined by the mass diffusion speed of the cementation solution in porous media. The development of CaCO_3 precipitation in the middle of the sand column is about 0.05 g/g sand after 21 days of curing (Figure 6a). As the pure sand has the highest porosity (about 34%) compared to the clay-contained samples, the CaCO_3 precipitation rate is much faster (about 7 times) compared to that of specimens containing 10% clay content (Figure 6b). It can be concluded that soil porosity has a great influence on MICP treatment using the diffusion method.

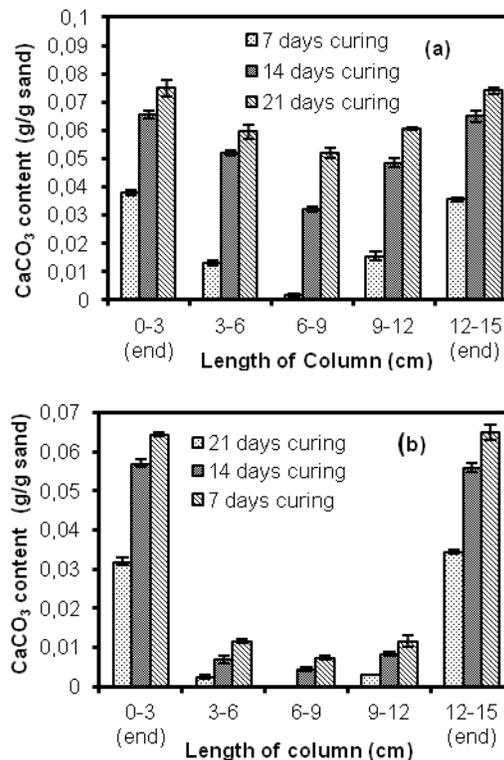


Figure 6. Revolution of CaCO_3 content along the sand column over curing time of diffusion method for: (a) pure sand; and (b) sand containing 10% clay

4 DISCUSSIONS

4.1 Bio-cementation of clayey soil

The current study reveals clearly that clogging during MICP application is a serious problem for clayey soils, leading to insufficient depth of cementation. The possible factors that are conducive to the clogging are:

1. The accumulation of high amount of ureolytic bacteria at the injection end, which is due to filtering of the bacterial cells by the fine particles; and
2. Excessive CaCO_3 precipitation at the injection end, which is due to the slow infiltration rate of the cementation solution.

During the injection method, the bacterial cells are filtered out by the fine particles and accumulated around the injection end, which further reduced the local porosity and limit the infiltration rate of the subsequent cementation solution. Under low pressure, the slow infiltration of cementation solution results in a long retention time, which leads to excessive urea conversion in the injection end, hence, enhancement of local MICP cementation. This leaves subsequently insufficient calcium and urea ions to reach the deeper level of the specimens (Cheng and Cord-Ruwisch 2014; Whiffin et al. 2007). The precipitated CaCO_3 in turn lowers the porosity and permeability, and reduces the infiltration rate further (self-enhancement). Therefore, the development of clogging is much faster in clayey soils compared to pure sands.

The premixing method on the other hand can avoid the heterogeneity of bacterial distribution. The homogenous placement of bacteria in high content clayey samples (10%) would allow more reagents to infiltrate further in the specimens. Accordingly, the clogging would be partly avoided and the attainable cementation distance is the whole length of the specimen. However, the heterogeneous CaCO_3 content distribution can be still observed in the 10% clayey specimens, where the CaCO_3 around the injection end is about 2 times higher than that around the outlet of the sand column. This issue may be solved by applying a faster solution flow rate under higher pressure, which would move more cementation reagents further into the column (Whiffin et al. 2007). Due to the extremely low permeability and infiltration rate, the injection end clogging developed are tremendously fast in the 20% clayey samples due to the self-enhancement phenomenon. A continuous decrease in the infiltration rate was observed even during the first injection of the cementation solution in the 20% clayey samples. In the clogging areas, the decreased permeability led to an increase in the hydraulic retention time of the cementation solution during the infiltration, resulting in more reagents being converted as crystals precipitated in the clogging areas.

It is of interest to note that the CaCO_3 produced by the injection method is more effective compared to that produced via the premixing method (see Figures 3 and 4). The premixing method requires adequate bacteria (concentrated bacterial culture) to be mixed with soil, as additional supply of bacteria during the treatment is not feasible. The concentrated bacteria provide much higher in-situ urease activity (i.e., about 50 U/mL) than that normally obtained in the injection method (i.e., about 10 U/mL). The larger amount of bacterial cell and faster urease activity provide more nucleation sites and higher oversaturation, resulting in quicker CaCO_3 precipitation in a smaller size. It can be seen from Figure 7 (left column) that the crystals produced using the premixing method are relatively small in size (about 2-5 μm in diameter) and fully cover the surface of the sand grains where most the crystals cannot contribute to the strength development. On the other hand, the samples treated using the injection method produced larger size crystals of about 20-30 μm in diameter (see Figure 7, right column), which can benefit

the gap filling between the adjacent sand grains which efficiently contribute to the strength development.

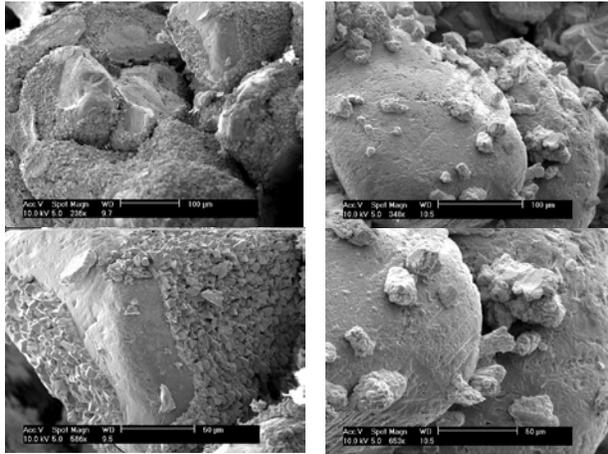


Figure 7. SEM of CaCO_3 crystals formed using premixing method (left) and injection method (right)

The advantage of the diffusion method is the continuous supply of cementation solution. However, due to the slow mass diffusion, the drawback of slow precipitation of CaCO_3 inside specimen only allows the bio-cementation occurring within a short distance and prevents its application in a large scale. However, the diffusion method can be used to strengthen marine clay soil using bio-encapsulation, as shown in Figure 8. As described by Ivanov et al. (2015), the wet marine clay premixed with dry bacterial biomass is made into spherical aggregates, which are immersed subsequently in a solution containing CaCl_2 and urea. After 48 hours of curing time, bio-encapsulation can increase the UCS of clay aggregates with a size of 5 mm from almost zero to more than 2 MPa due to the strong CaCO_3 shell formation. This may link to a new approach of clayey soil stabilization.

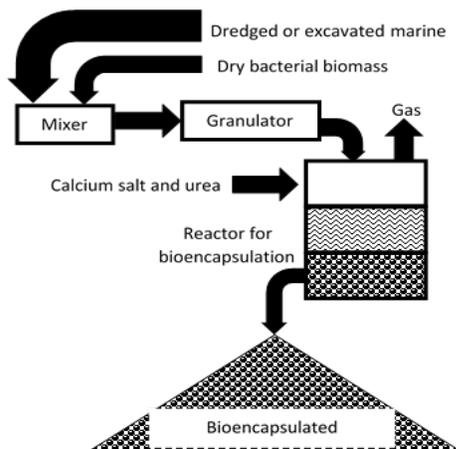


Figure 8. Schematic diagram of strengthening marine clay aggregates using bio-encapsulation. The facility includes a mixer, an extruder of clay aggregates, a reactor for bio-encapsulation, and a pile of bio-encapsulated marine clay (Ivanov et al. 2015)

5 CONCLUSIONS

This paper assesses three different MICP treatment methods including injection, premixing and diffusion, for sand soil containing up to 20% clay. The injection method was only applicable to soil samples with clay content less than 5%. The premixing method allowed a homogeneous placement of bacterial cells in the clayey soil columns (clay content up to 10%), allowing subsequent injection of cementation solution under low pressure of 100 kPa which enabled an adequate bio-cementation along the specimens, resulting in up to 150% increase in the UCS values. The diffusion method provided a continuous supply of cementation solution, which resulted in sufficient cementation of clayey soil specimens of up to 5 cm in depth, including 20% clay content. Although the UCS of 20% clayey soil increased by up to 70% after 21 days of curing time, the CaCO_3 content inside the specimens was significantly less than that obtained at the ends of the sand columns. In other words, the slow mass diffusion in the porous materials having clay content of 20% only allows MICP occurring over a short distance. The overall conclusion of this study is that bio-cementation of clayey soil still faces a tremendous challenge and requires further investigation on the homogeneous ureolytic bacteria placement and fast supply of cementation solution at large scale before field application.

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