TECHNICAL PAPER

MICROBIAL-INDUCED CARBONATE PRECIPITATION USING A SUSTAINABLE TREATMENT TECHNIQUE

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ABSTRACT

Biocementation is a green treatment technique which makes use of microbially induced carbonate precipitation (MICP) process to enhance the geotechnical features of sub-standard soils. The objective of this study was to conduct a biocement test in laboratory-scale using native urease-producing bacteria to improve the surface strength of poorly graded soil. Selected sand samples were pre-mixed with native bacterial culture and the cementation solution before being compacted into their respective columns. After completing the biocement process, all the sand columns were allowed to air-dry at room temperature ($26^{o}C$) for 14 days before the treated sands were removed from their respective moulds. Unconfined compression strength (UCS) test was performed on the moulds to determine their strengths, while quick acid test and calcium carbonate ($CaCO_3$) content measurement were conveyed to analyse the precipitated $CaCO_3$ minerals. The results showed that the native urease-producing bacteria could bind soil particles together. The proficiency of this treatment process to improve the strength of soil samples varied among the specimen samples, leading to a non-homogeneous distribution of $CaCO_3$ contents in the specimens. The UCS test showed that the sand treated with native isolate NB 28 had the highest strength (0.219 N/mm^2), sustaining a force of 1.020 kN, while the control strain (*Sporosarcina pasteurii* DSM 33) had the lowest strength (0.143 N/mm^2) with a sustaining force of 0.697 kN. The findings in this study suggest that the native urease-producing bacteria isolated from Sarawak limestone cave can be used as alternative MICP agents for the biocement application for sustainability in the construction industry.

Keywords: Microbially induced carbonate precipitation (MICP); biocement; urease-producing bacteria; surface strength; sustainability; calcium carbonate

INTRODUCTION

Construction Biotechnology is an emerging discipline experiencing a rapid exponential development in science and engineering (Stabnikov, Ivanov & Chu, 2015). It often comprises researchers and academics from microbiology, biotechnology, civil engineering and geotechnical engineering disciplines to promote sustainable practices in the construction industry. The convention of various scholars to produce biocement at low-cost has resulted in the development of relevant and useful cross-disciplinary research. Successful applications of microbially induced carbonate precipitation (MICP) have been demonstrated. Nevertheless, there are several hindrances affecting the scaling-up of these processes (Zhu & Dittrich, 2016) to large-scale or field-scale and managing of the by-products (DeJong et al., 2013). Some challenges involving MICP include the intrinsic obstacles of an uneven distribution of carbonate precipitation in tested sand columns and also clogging formation at regions around the injection points (Cheng & Shahin, 2016). Another challenge surrounding MICP involves the attempt to grow highly active urease-producing bacteria from environments with favouring conditions. Majority of bacteria used for MICP are purchased from various culture collection centres (Cuzman, Richter, Wittig & Tiano, 2015; Sharma & Ramkrishnan, 2016; Soon, Lee, Khun & Ling, 2014), while few studies (Dhami, Reddy & Mukherjee, 2013; Omoregie et al., 2016b; Seshabala & Mukkanti, 2013; Wei et al., 2015) have reported opting for the use of native strains for potential MICP applications. These bacteria are not abundant, considering their specific environmental conditions and biochemical reactions which inhibit their growth (Zhu & Dittrich, 2016). Hence, alternative bacteria with high enzyme production are suggested for the best option of engineering projects to ensure greater sustainability (e.g. the cost and environmental aspects). This study was conducted by performing a biocement test on poorly graded sand using native urease-producing bacteria to determine the calcium carbonate ($CaCO_3$) homogeneity in the biocement samples. It reports the use of an improved MICP treatment technique to minimise the uneven distribution of carbonate precipitated in the treated soil.

LITERATURE REVIEW

Microbially Induced Carbonate Precipitation (MICP)

MICP refers to the precipitation of $CaCO_3$ from overfilled solution in a microenvironment that occurs due to the presence of microbes and chemical activities (Bosak, 2011; Hamilton, 2003). The precipitation of $CaCO_3$ via ureolysis is a straightforward and an easily well-ordered mechanism with the ability to produce a high amount of $CaCO_3$ over a short duration (Dhami, Reddy & Mukherjee, 2014). During the MICP process, microorganisms are able to

make metabolic products that react with ions in the microenvironment which result in the production of $CaCO_3$ mineral (Anbu, Kang, Shin & So, 2016). This process can provide useful insights for sustainable practices in the construction industry.

MICP for Sustainability

Sustainability can be regarded as products that are economically effective and maintainable (Doualle, Medini, Boucher & Laforest, 2015). The increasing public awareness of how buildings can affect human health and the environment has resulted in increasing interest for sustainable buildings (Contreras, Roth & Lewis, 2011). MICP method is a sustainable technique in soil stabilisation, which can alter and improve ground conditions (Lee, Soon, Tan & Hii, 2012). MICP technology can help to meet the related green construction requirements because the treatment results in minimal disturbance to soil environments (Soon et al., 2014). The use of biocement, produced via the MICP process seeks to improve the efficiency and moderation of using green materials for the improvement of soils. Studies by Filet, Gadret, Loygue, and Borel (2012) from Soletanche Bachy's group and van Paassen et al. (2009) have revealed that this technology propels useful prospects for industrial and field applications as alternative ground improvement techniques.

Biocement Challenges and Improved Treatment Techniques

The success of MICP treatment depends strongly on even distribution of $CaCO_3$ produced in soils (Naga, Xinbao, Hae-In & Woo-Suk, 2016). Nevertheless, during the injection process, some of the bacteria tend to attach to the surface of the soil grains or near the entrance of the treatment points, hence leading to a formation of clogs (Burbank, Weaver, Green, Williams & Crawford, 2011; DeJong, Mortensen, Martinez & Nelson, 2010). A recent study by Michael, Jason, Collin, Douglas, and Charles (2016) on the treatment of loose sand materials confined in a large-scale biocement tank specimen reported that $CaCO_3$ contents were mostly formed at a location near the treatment pathways. Investigations on an alternative approach to solving the unfavourable formations of clogging and uneven distributions of $CaCO_3$ content had been researched in order to further improve the required performance.

METHODOLOGY

Bacterial Selection

The bacteria selected in this study for the biocement treatment test were cultivated from the local limestone cave samples using a selective enrichment culture and enzyme assay methods (Omoregie et al., 2016a). The bacteria obtained from local sources denoted to be NB33, LPB21, NB28 and NB30 were identified to be *Sporosarcina pasteurii*. These isolates were routinely maintained on a growth media called nutrient agar which was supplemented with 6% urea substrate, serving as a source of energy and nitrogen. The Media was stored at $4^{o}C$ in the fridge, before being used for subsequent tests. The bacteria were cultivated in the same way from a bacterial culture not grown for more than one month (Cuzman et al., 2015).

Sand Preparation

The sand specimens used were all typical uniform sand. Sieve analysis which based on the article size distribution curve of the fine and coarse sand (Figure 1) was used to determine the particle size distribution, which is one of the primary components that govern the mechanical behaviour of soils. The sand is classified as poorly graded sand according to the Unified Soil Classification System (USCS) and British Standards, BS5930. The specimens had a coefficient of uniformity, cu = 1.6 and a coefficient of gradation, cc = 0.907 (D10 = 0.220 mm, D30 = 0.265 mm, D60 = 0.352 mm). The sand samples were selected by sieving for designated particle size ranges and the sands that could pass through sieve number 10 (2 mm) were used. The sands were then oven dried ($105^{o}C$) for 24 hours and allowed to cool ($\pm\,26^{o}C$).

Bacterial Culture and Cementation Solution

The bacteria were grown overnight in sterile flasks containing 125 mL growth media. The flasks containing the growth medium were then cultivated for 24 hours with agitation (130 rpm) in an incubation shaker (CERTOMAT$^{®}$ CT plus – Sartorius) under aerobic batch conditions at $30^{o}C$. The cementation solutions used to treat the sand were modified from Cheng and Cord-Ruwisch (2014) and Weaver et al. (2011). The constituents and concentration of the cementation solution were urea (1 M), calcium chloride (1 M), sodium acetate (0.17 M), ammonium chloride (0.0125 M) and nutrient broth (13 g/L).
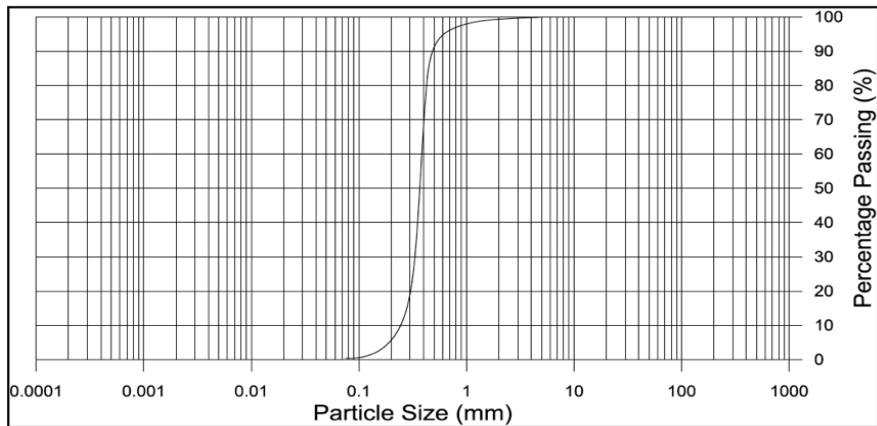
Figure 1: Grain Size Distribution Curve for the Sand Used

Treatment Test

Dry sands (294.73 g) were pre-mixed with a cementation solution and the overnight grown bacterial culture and placed into respective moulds (internal diameter of 75 mm and length of 49 mm). The sand samples were treated with the bacteria (Table 1) and cementation solutions using the percolation treatment method (i.e. unrestrained flushing of fluid from top to bottom). All columns were placed on the flat surface of polypropylene sheets; five holes were drilled on the surfaces of the polypropylene sheets to allow the effluents of the cementation solution to pass through. The polypropylene sheets containing drilled holes were then covered with Whatman filter papers to prevent any sand particles from being washed away during the treatments. A plastic container was placed below the polypropylene sheets to accumulate the effluents. The MICP treatment was performed by introducing 80 mL of bacterial culture and 80 mL of cementation solution into the sand specimens at an interval of 12 hours for a duration of 96 hours. Upon completion of the treatments, all the sand columns were cured at room temperature for 14 days before the treated sands were removed from their respective moulds.

Strength Measurement

Strength test on the biocemented samples was performed using Unconfined Compression Strength (UCS) test in reference to American Society for Testing and Materials (ASTM) C67-07a for conventional bricks and structural clay tile test (ASTM, 2007). The test was performed on an automatic mortar compression/flexural and concrete flexural machine (NL[®] Scientific Instruments Sdn. Bhd., NL 3027 X/002) with a maximum load of 300 kN. All the surfaces of the testing apparatus were cleaned and the sand specimens were placed on it. The

tests were performed until the sand column reached its failure and maximum stress level.

Quick Acid Test and Calcium Carbonate Content Measurement

The quick acid test adapted from Cordua (2010) was used to confirm the precipitates seen on the surface of the biocemented samples were $CaCO_3$. The samples were collected, weighed and kept inside sterile test tubes. Each of the test tubes was filled with 10 mL sterile deionized water. The test tubes containing the precipitates were then added with 2 mL of 10% diluted hydrochloric acid. The presence of $CaCO_3$ was visually determined by observing for bubble formation. The content of $CaCO_3$ in the biocemented samples was measured by using a method described by Weaver et al. (2011). Samples were collected from the apex, central and the lower surface of each treated sand after the strength test. The dry weight of each sample was taken, then washed with 2M hydrochloric acid, dried and weighed again after being washed with acid to determine the relative amount of $CaCO_3$ present. The samples were dried for 3 hours at 90°C in an oven before being weighed.

Statistical Analysis

The data were reported as mean with a standard deviation value for three replicates. The results were analysed using Excel spreadsheets available in Microsoft Excel (version 2016) and were subjected to student's *t*-test analysis, with statistical significance taken as p<0.05. GraphPad (Quick Calc) programme was used to analyse the student's *t*-test data.
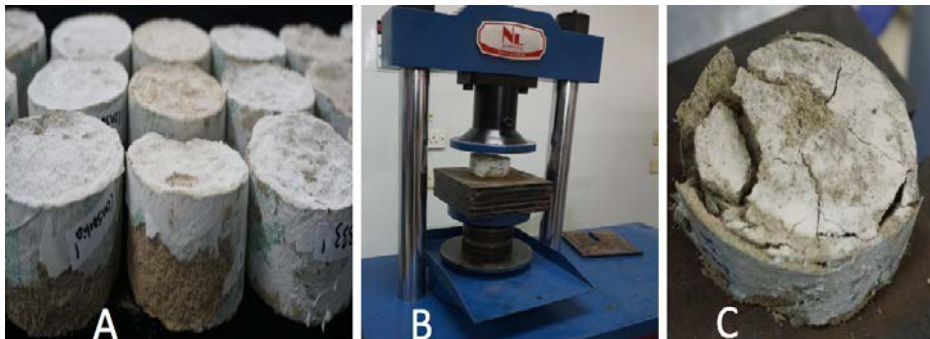
RESULTS AND DISCUSSION

MICP Treatment

The soil samples were first pre-mixed with bacterial culture (Table 1) for the bacterial attachment with soil before the MICP process. Poorly graded sands were used in this study as they demonstrate detrimental engineering behaviour for most geotechnical engineering applications (Gurbuz, Sari & Yuksekdag, 2015). At the end of the biocement treatment test, the loose sands with microbial culture harden and $CaCO_3$ precipitates were seen at the top layer of the specimens. After the columns were fully removed from the biocemented sands, any other parts of the columns which remained on the biocemented sands were then carefully taken out. The sands were then kept in an incubator at 37°C for 24 hours to minimise the influence of any remaining water in the biocemented sands before their mechanical properties were evaluated. The white precipitates on the top layers of the biocemented sand shown were also reported by Zhao et al.

(2014). The geometric binding of the urease-producing bacteria is important for biocementation via percolation method (Soon et al., 2014). Bacterial sizes which range between 0.3 to 3.5 μm (Al Qabany, Soga & Santamarina, 2011; Mitchell & Santamarina, 2005), are capable of moving easily within soil with particle size range of 0.05–2.0 mm (Maier, Pepper & Gerba, 2009). *Sporosarcina pasteurii* cell has an average size of 2.8 μm (Tobler, Cuthbert & Phoenix, 2014), which is advantageous for the ureolytic bacteria's movement in the sand and successful MICP result. Visual observation of the MICP treatment showed improved compatibility of the loose soil due to the formation of $CaCO_3$ within the soil matrix (Figure 2A). Compatibility of soil between the soil grain and bacterial size allows easy bacteria transportation and enzyme activity (Mitchell & Santamarina, 2005; Naga et al., 2016).

Table 1: Selected Urease-producing Bacteria Prior to Biocement Test

Isolate	Biomass (OD_{600})	Colony Forming Unit ($CFU.mL^{-1}$)	Urease Activity (mM urea hydrolysed.min^{-1}. OD^{-1})
LPB21	0.79	4.8×10^7	16.6
NB30	0.52	4.0×10^7	17.26
NB33	0.69	1.5×10^7	20.96
NB28	0.76	4.1×10^7	23.49
control	0.64	5.0×10^7	13.65
consortia	0.56	4.7×10^7	12.51

Figure 2: UCS Test Performed on the Biocemented Sand Sample After Successful MICP Treatment. (a) Biocemented Sand Samples [left]; (b) Biocemented Sand Sample Before Being Crushed Using an Automatic Mortar Compression/Flexural and Concrete Flexural Machine [middle]; and (c) Biocemented Sand Sample After Being Crushed [right].

Unconfined Compression Strength (UCS)

The UCS result indicates that native urease-producing bacteria have the possibility of improving poorly graded soils via surface percolation method. The results from Table 2 indicate that the biocemented sands with the highest test

were treated with isolate NB28 (0.219 N/mm^2), sustaining a force of 1.020 kN, while soils treated with the lowest strength was treated with the control strain (0.143 N/mm^2) with a sustaining force of 0.697 kN. The biocement test was primarily designed to test the ability of the locally isolated urease-producing bacteria in treating loose soils by filling their pores and testing the surface strength of the samples without being in their respective columns. However, the UCS test was later performed to get an estimated unconfined compression strength and to understand the state of force needed for the samples to reach their respective failed points. Surface percolation method is suitable for soil treatment because it does not disturb the structure of soil and reduces costs required for machinery and labour. However, the lack of homogeneity of CaCO$_3$ content which leads to uneven UCS results could be due to biochemical reaction during permeation (Cheng & Cord-Ruwisch, 2014).

Table 2: Unconfined Compressive Strength of the Treated Sands

Bacteria ID	Condition of cemented sand	Force (kN)	Pressure (N/mm^2)
- control	-	-	-
+ control	+	0.647	0.143
LPB21	+	0.697	0.152
NB33	+	0.833	0.176
NB30	+	0.647	0.143
NB28	+	1.020	0.219
consortia	+	0.623	0.147

(-) the column was not cemented; it was extremely soft and unable to be measured.
(+) the cemented column was broken when the maximum strength was applied.

According to ATSM (ASTM D2166-00) standards, to test for unconfined compression strength of cohesive soil, specimen sizes are required to have a minimum diameter of 30 mm with a length of one-tenth of the specimen diameter or 72 mm with a length of one-sixth of the specimen diameter. Nevertheless, the diameter and length of column samples used in this experiment were 75 mm and 49 mm, respectively. Hence, it did not follow the standard set by ATSM. The results of the strength test on biocemented sands of the isolates and bacterial consortia suggested that there were noticeable significant differences between the strength for biocemented sands treated with isolates LPB21 (M = 0.152; SD = 0.006), NB33 (M = 0.176; SD = 0.025) and NB28 (M = 0.219; SD = 0.013) against the control strain (M = 0.143; SD = 0.006).

The white precipitates which were deposited on the top layer of sand columns were presumed to be CaCO$_3$ precipitates. Some amount of the excess precipitates were taken and kept in sterile test tubes as shown in Figure 3 (A). After the addition of 10% hydrochloric acid solution, the continual formation of bubbles was visually observed. The addition of acid onto the CaCO$_3$ resulted in bubbles

of carbon dioxide gas to be released as indicated in Figure 3 (B). This bubble formation signals the presence of $CaCO_3$. To confirm the presence of $CaCO_3$, a quick acid test was performed by adding a few drops of hydrochloric acid on $CaCO_3$ mineral. The reactions allowed the bubble formation and a vigorous effervescence which lasted for some minutes or seconds (Cordua, 2010).
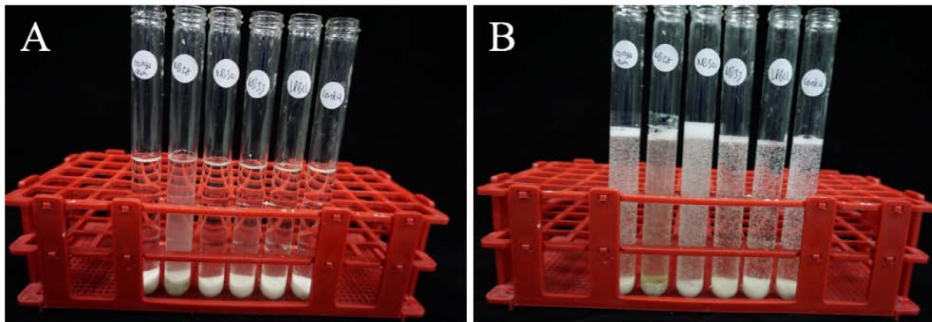
Figure 3: Confirming $CaCO_3$ Precipitates. The Calcium $CaCO_3$ Precipitate Found on the Surfaces of the Biocement Moulds were Tested Using Quick Acid Test. (A) Before Addition of Hydrochloric Acid [left]. (B) After Addition of Hydrochloric Acid [right].

Calcium Carbonate Content Determination

The content of the $CaCO_3$ precipitated in the sand specimens were determined by using acid wash method. The average $CaCO_3$ content of the biocemented sands was determined from samples collected at the top, middle and bottom sections. The results showed that most of the $CaCO_3$ contents precipitated at the top layer of the specimens (Figure 4). However, there was no homogeneity of the $CaCO_3$ contents within any layer of the biocemented sand samples.
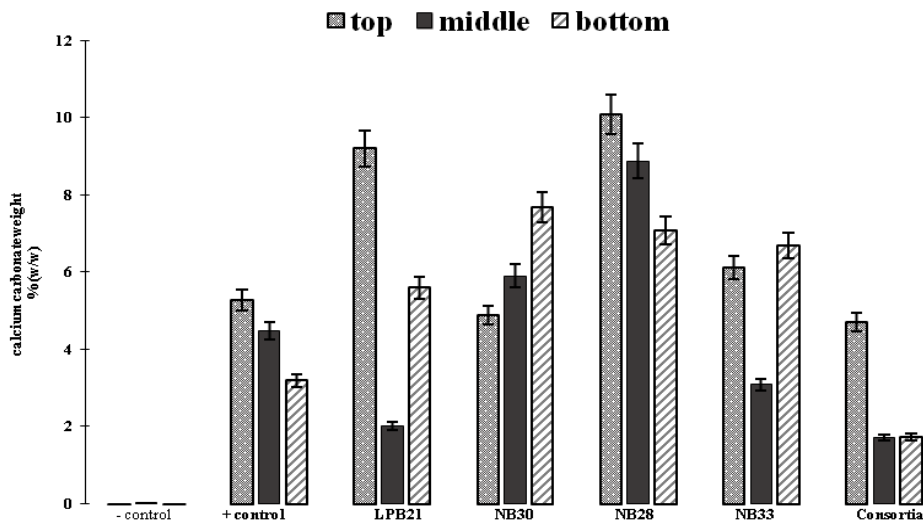
Figure4: Comparison of the Relative Quantity of Calcium Carbonate in the Biocemented Sands. The calcium carbonate contents were dried for 3 hours at 90°C in an oven before being weighed.

In Table 3, among all the biocemented sands, the highest average $CaCO_3$ content for the top, middle and bottom were determined to be 10.08% (NB28), 7.14% (NB28) and 7.19% (NB33), respectively. These results (Table 3) suggested similar $CaCO_3$ contents between the middle and bottom layers of biocemented sands treated with some of the microbial cultures. Furthermore, it also indicated that there was reasonable precipitation uniformity from middle to bottom layers of these sand samples. The reason there was predominantly more calcite formation at the top layers of the sand samples is mainly that *Sporosarcina pasteurii* is a facultative anaerobic bacterium, which grows at a higher rate in the environment containing oxygen and consequently leading to higher rates of calcites precipitated around the top surface areas (Whiffin, van Paassen & Harkes, 2007).

Table 3: Summary of Calcium Carbonate Content

Isolate ID	Top	Middle	Bottom
- control	0.00	0.00	0.00
+ control	5.28	3.95	3.19
LPB21	9.20	2.01	5.59
NB33	5.86	4.65	7.19
NB30	6.12	3.09	6.69
NB28	10.08	7.14	7.09
consortia	4.70	1.72	1.73

MICP can be highly regarded as a construction sustainability due to its low energy requirement and prospect for recycling (Achal & Mukherjee, 2015). However, the cost and safety aspects of MICP for construction purposes are of concern. A shortcoming of MICP is the production of ammonia and nitrate that are formed during the ureolysis-driven process. The release of these gases can be toxic and detrimental to health and soil microorganisms, especially when released at high concentration (van Paassen et al., 2010). If inhaled, this gas can cause serious respiratory complications (Gueye et al., 2001). Omoregie et al. (2016a) recommended the use of facial masks when handling these ureolytic bacteria, especially when they start releasing ammonia gas.

MICP is presently rather expensive when compared to other treatment methods (Mujah, Shahin & Cheng, 2016), which is highly influenced by the price of nutrients for bacterial production. The ingredients of the growth medium for bacterial production are a major cost factor, ranging between 10 to 60% of the total production cost (Whiffin, 2004). To reduce the cost of MICP for field applications, some studies have suggested utilizing dairy industrial waste such as corn steep liquor or lactose mother liquor to serve as alternative growth nutrients for bacterial production (Achal, Mukherjee, Basu & Reddy, 2009; Cuzman et al., 2015; Phillips et al., 2013). Cheng and Cord-Ruwisch (2013) proposed

cultivating urease-producing bacteria strains from local environments as an alternative to standard bacterial strains. This helps in reducing the buying cost of bacteria from various microbial collection centres.

CONCLUSION

As an act of service to the environment, this study was conducted to examine the potential of using native urease-producing bacteria to treat poorly graded sand via biocementation and to determine the homogeneity of $CaCO_3$ produced in treated sand samples. The results revealed that biocement using percolation method was successful in improving the mechanical properties of the sands. The unconfined compression strength test results showed the bacteria had comparative strengths to that of the representative strain. Among all the urease-producing bacteria used, isolate NB28 produced the highest UCS test result. The findings from the $CaCO_3$ content for all the samples treated with microbes showed the distribution of the $CaCO_3$ contents were not uniform. More studies need to be carried out on optimum urea-$CaCl_2$ solution for the local strains to determine the uniformity of $CaCO_3$ contents. Future work involving these four isolates may involve large-scale bacterial production using computerised bioreactor. The large scale production of bacteria can be utilised for MICP treatment involving field application. The use of alternative growth medium as a carbon source for large-scale production of bacteria can be studied to minimise the cost of purchasing nutrient source. A study on how this alternative medium enhances the production of bacterial growth, urease activity and $CaCO_3$ precipitation can be conducted. A comparison between the lab grade urea substrate and industrial grade urea or alternative nitrogen sources can also be carried out for future work. This will also be essential for field applications and reduction of cost for MICP treatments towards better sustainability.

ACKNOWLEDGEMENT

Cawangan Sarawak) journal teams are much appreciated. The authors are responsible for the technical accuracy and validity, and the inputs from various literature referred are highly valued.

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