Integrating Biotechnology into Geotechnical Engineering: A Laboratory Exercise

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ABSTRACT Microbially induced carbonate precipitation (MICP) is a new and promising technique that uses biocementation technology via microbial activities to improve soil properties. This natural occurring biochemical process that utilises the metabolic pathways of bacteria to form calcium carbonate, has drawn the attention of scientists, engineers and entrepreneurs to explore various applicable prospects for industrial purposes. The aim of this study was to execute practical activities designed to enable students discover the availability of urease-producing bacteria from local environment and perform a small-scale biocement treatment. Enrichment culture technique and Christensen's medium were used to screen for urease-producing bacteria from soil samples. Conductivity method was then used to quantify the specific urease activities of the local isolates. A biocement treatment test via MICP process was used to investigate the suitability of using three methods to improve geotechnical properties of loose soils and determine their respective surface strengths. A total of 12 bacterial isolates were obtained from samples collected at Swinburne University of Technology Sarawak Campus. Among these, only eight bacterial isolates (designated as SUTS-1, SUTS-2, SUTS-3, SUTS-4, SUTS-5, SUTS-6, SUTS-7 and SUTS-8) were urease positive. The conductivity results, showed that bacterial isolate SUTS-6 had the highest specific urease activity (23.340 mM urea hydrolysed.min^{-1}. OD^{-1}) amongst all the bacterial isolates. This value is comparable to that of *Sporosarcina pasteurii* DS33 (23.755 mM urea hydrolysed.min^{-1}. OD^{-1}), a control strain used in this study. In addition, the biocement result showed that Group 1 (sand without premix) and Group 2 (sand premixed with bacterial culture) treatment produced more compactible biocemented soil samples when compared with those treated with Group 3 (sand premixed with 1 M urea and calcium chloride). However, the surface strength test revealed that Group 2 treatment method showed the highest strength (430.922 kPa), hence making it the most preferred treatment method.

KEYWORDS: Bacterial isolation; Urease activity; *Sporosarcina pasteurii*; Biocementation; Surface percolation

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INTRODUCTION

Urease enzyme produced by microorganisms play an essential role in soil strengthening and stabilisation, because it acts as a biocatalyst which induces the precipitation of $CaCO_3$, a cementing agent employed in construction industry. The prospect of utilising non-pathogenic microorganisms for bio-geotechnical engineering applications was first introduced by studying a novel permeability reduction process that utilized urease-producing bacteria (Ferris et al., 1996). This idea inspired numerous studies on microbially induced carbonate precipitation (MICP), an eco-friendly technology for soil strengthening that does not harm the environment (Kim & Youn 2016). During MICP process, urease catalyses the hydrolysis of urea to produce ammonium and carbonate ions, which then react with calcium ions to form $CaCO_3$ (Hammes & Verstraete 2002). During urease

activity, 1 mol of urea is hydrolyzed intracellularly to 1 mol of carbonate, which spontaneously hydrolyzes to form an additional 1 mol of ammonia and carbonic ions (Stocks-Fischer et al., 1999).

Biocementation is an alternative ground improvement technique which makes use of MICP process to improve the properties of soil in a way similar to ordinary cement (Ivanov and Chu 2008). Generally, loose sand particles are mixed with bacterial culture water which often contains growth media (i.e. yeast extract), urea and calcium ions (i.e. calcium chloride). Biocement treatment via MICP process allows the pores of loose soils with $CaCO_3$ minerals, thus resulting to water permeability reduction and enhanced strength. The process of precipitating $CaCO_3$ is very slow under normal conditions requiring long geological time, however, with MICP process, $CaCO_3$ precipitation can be induced in a shorter period of time (Dhami et al., 2013). Majority of ureolytic bacteria capable of inducing $CaCO_3$ are commonly isolated from soil samples. However, these bacteria are often not suitable for MICP applications due to factors such as low urease activity, minimal $CaCO_3$ precipitation and virulence or pathogenicity factors.

Bacterial strains such as *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*) and *Lysinibacillus sphaericus* (formerly *Bacillus sphaericus*) have been reported in MICP various studies to have high urease activity, capable of inducing high amount of $CaCO_3$ minerals and are non-pathogenic, hence making them a preferred choice for MICP applications. Numerous studies have reported utilising different type strains of the aforementioned bacteria from various microorganism culture collection centres such as National collection of industrial and marine bacteria, German collection of microorganisms and cell cultures, American type culture collection and Korean Collection of Type Culture for their respective MICP investigative studies (Harkes et al 2010, Lee et al 2015, Sidik et al 2014, Zhang et al 2015). Additionally, studies on the isolation of highly active non-pathogenic urease-producing bacterial species are very limited in the literature. It is thus essential to screen for more ureolytic bacteria from local samples which possess high urease capabilities with MICP prospects. The advantage of using local isolates rely on the fact that they are well adapted to native environments and are less likely to become harmful when they are under stressed conditions.

The aim of this present study was to perform a simple and inexpensive screening procedure for urease-producing bacteria isolated from Sarawak soil samples via enrichment culture technique, and to determine urease production and biocement capabilities of the bacterial isolates. This practicum was designed for students who were taking an industrial microbiology module of an undergraduate biotechnology degree in Swinburne University of Technology (Sarawak Campus), Kuching, Malaysia. The students were taught on the use of conductivity method to quantify urease activity of the isolated ureolytic bacteria and also perform *in vitro* biocement treatment on poorly-grade soil via different methods to enhance the strength and properties of loose sand.

MATERIALS AND METHODS

Biological material

Sporosarcina pasteurii (DSM33, type strain) was purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). This bacterial strain was used as a positive control for urease-production, conductivity measurement and biocement treatment experiments in this study. It was aseptically grown under aerobic batch conditions according to the DSMZ instruction and stored on Petri plates containing nutrient agar (28 $g.L^{-1}$, HiMedia, Laboratories Pvt. Ltd). After 24 hr of cultivation at 32°C, the bacteria were collected and stored in the fridge (4°C) until needed.

Sampling and enrichment culture

Soil samples were aseptically collected from Swinburne University of Technology Sarawak Campus, Kuching, Sarawak, Malaysia (1°31'32.99" N 110°21'14.99" E). The samples were collected at a depth of 5-25 cm using Sterileware™ sampling spatulas which were then kept in an ultraviolent radiation-sterilized polyethene zipper bag. The samples were then placed inside polystyrene ice box container before being transferred to the laboratory for further microbiological analysis. 1 g of soil sample was weighed and kept in sterile conical flasks (250 mL capacity) containing 50 mL tryptic soy broth (30 $g.L^{-1}$, Merck Millipore) supplemented with urea (40 $g.L^{-1}$, Bendosen *Laboratory Chemicals*), ammonium sulphate (10 $g.L^{-1}$, HiMedia Laboratories Pvt. Ltd) and sodium acetate (8.2 $g.L^{-1}$, HiMedia Laboratories Pvt. Ltd). The initial pH of the growth medium was adjusted to 8.0 using 0.1 M NaOH or 0.1 M HCl before sterilisation (Reyes et al 2009). All bacterial growth mediums, chemicals (except urea) and glassware used in this practicum were sterilised by autoclaving at 121°C, 103.42 kPa for 20 min using an autoclave machine (Hirayama-HVE-110). However, urea was sterile filtered through 0.45 µm syringe filters. All the media and chemicals used were of analytical grade. The conical flasks were then incubated (CERTOMAT® CT plus – Sartorius) aerobically 32°C for 72 hr with shaking condition (150 rpm).

Isolation, screening and morphological analysis

The enriched cultures were serially diluted (tenfold) and plated on tryptic soy agar (40 $g.L^{-1}$, Merck Millipore) supplemented with 6 % (w/v) urea. The agar petri plates were then incubated (MMM Incucell) aerobically at 30°C for 42 hr. Upon growth of the isolates, subsequent sub-culturing was performed until single bacterial colonies were obtained. Christensen's medium (9.0 $g.L^{-1}$, Oxoid Thermo Scientific Microbiology Sdn Bhd) was used to screen for urease positive bacteria based on urea hydrolysis. A loopful of the bacterial colony was heavily streaked on universal bottles containing 10 mL Christensen's medium and incubated at 37°C for 72 hr. The urease production test was studied through visual observation for colour changes. The bacterial isolate able to turn the Christensen's medium from pale yellow to pink during the incubation period was selected while others were discarded. Positive urease producers were selected and stored for long-term preservation by adopting procedures from Fortier & Moineau (2009). Morphological analysis was used for a more definitive identification of bacterial isolates. A loopful of individual isolates was serially subcultured onto Petri plates containing tryptic soy agar and incubated at 32°C for 24 hr. Colony appearance of the overnight sub-cultured isolates was recorded with reference to Bergey's Manual of Determinative Bacteriology (Holt et al 1994).

Conductivity measurement

Conductivity method is an easy and economical assay system often used to determine the enzymatic rate reaction of the bacterial-urea solution. The assay was performed by adopting procedures from Omoregie et al. (2017). The changes in conductivity were monitored for 5 min at 25°C ±1 and the respective conductivity values were measured by using conductivity meter (Walk LAB conductivity pro meter, Trans Instruments COMPRO). At the end of the assay, conductivity variation rate (mS cm−1 min−1) was acquired from the slope of the plotted graph, which was then multiplied by a dilution factor. Biomass concentration was determined by measuring the optical density (OD) of the bacterial suspension using a spectrophotometer (GENESYS™ 20, Thermo Fisher Scientific) at a wavelength of 600 nm. The results obtained were used to determine the specific urease activity of the bacterial culture (Whiffin 2004).

Biocement treatment and strength test

The sand specimens used in this study were typical uniform sands, classified as poorly graded according to British Standards (BS5930), with particle size ranging from fine sand (0.08 mm) to fine

gravel (4.75 mm). The sand samples were considered to have disadvantageous engineering properties for most geotechnical engineering applications, hence making them suitable for biocement treatment test. Sand columns were prepared by packing sands into the paper rolls and then wrapping the columns (95 mm by height and 45 mm by inner diameter) with masking tape. Each column was packed with 130 g of unsterilized sand. All the columns were placed on treatment-setup adopted from Omoregie et al. (2017). Before the treatment, students were grouped and assigned to use three different treatment methods. Group one used sand containing no bacterial culture and cementation solution, group two used a sand premixed with only bacterial culture (20 mL), and group three used sand premixed with cementation solution (20 mL). For each treatment, 50 mL of overnight grown *Sporosarcina pasteurii* (DSM 33) culture and 50 mL cementation solution containing mixture of calcium chloride (0.5 M, Sigma-Aldrich Co. LLC), urea (0.5 M, Bendosen Laboratory Chemicals), and yeast extract (5 $g.L^{-1}$, Merck Millipore) were used and the treatment was performed for 72 hr with 24 hr interval to allow reaction to occur. The sand columns were kept inside a fume hood (LabCraft, BASIX 52) and left cure for 14 days under room temperature before removed from their columns. The surface strength of the treated sand columns were then measured using a pocket penetrometer (ELE International, 29-3729). The penetrometer used has a reading scale from 23.940 to 430.922 kPa.

Statistical analysis

The data were reported as mean with a standard deviation value for experiments performed in three replicates (conductivity and biocement treatment). The results were analysed using GraphPad Prism software (version 7).

RESULT AND DISCUSSION

Isolation and screening of ureolytic bacteria

In in this laboratory exercise, we sought to explore the availability of urease-producing bacteria from local soil samples collected from Swinburne University of Technology Sarawak Campus, Kuching, Sarawak, Malaysia. A total of 12 morphologically different isolates (Table 1) were selectively sub-cultured by the students and tested for their ability to produce urease enzyme on Christensen's medium. As shown in Figure 1, out of the 12 isolates only 8 were able to turn their respective media from yellow-orange colour to bright pink (fuchsia) colour within 48 hr of incubation. However, out of the remaining 4 isolates, 2 showed negative reactions (yellow) and 2 false positives (orange with slight pink) reactions were observed from the urease production test. Christensen's medium contains peptone and glucose which supports growths of a wider variety of urease-producing microorganisms. When urea is hydrolysed by the urease enzyme from the microorganism, ammonia is released and becomes accumulated in the medium which then increases the pH, making it alkaline (Zoheir et al 2013). False-positive results (Figure 1) may occur due to hydrolysis of proteins such as peptone in the medium and result to an increase in pH of the medium (Canteros et al 1996). Several studies have reported using Christensen's medium as a preferred qualitative urease assay for isolation of urease-producing microorganisms (Dhami et al 2013, Elmanama and Alhour 2013).

It was observed (morphologically) that all the isolates had circular shapes, had either an entire or curled margin, with size ranging from 10-40 mm. They also either had an opaque or translucent optical property with creamy colour (dull or shinny). Further tests which involve biochemical analysis such as Gram staining, endospore staining, motility, oxidase and catalase tests, and molecular identification via 16S rRNA gene sequencing were not performed during the course of the

practicum for the unknown bacterial isolates. However, it is recommended to perform such tests so that students can familiarise with the methods involved in characterising urease bacteria and most importantly know the identity of the bacterial isolates obtained from their respective samples. Upon completion of the urease production test, glycerol stock method was used for long-term storage of the bacterial isolates which were urease-positive by adopting a modified procedure from (Fortier & Moineau (2009). For the maintenance of the bacterial glycerol stock, 500 µL of overnight grown cultures were inoculated into 2.0 mL cryogenic vials containing sterilised 500 µL of 50% glycerol to obtain a final glycerol concentration of 25% (v/v). The stocks were mixed prudently and kept in the refrigerator at -80°C. For the case of reviving stored cells, sterile toothpick or inoculation loop was used to scrap off the splinters of solid ice and then streaked onto the tryptic soy agar.

Table 1. Morphological characteristics of locally isolated urease-producing bacteria

Isolate-Code	Shape	Size (mm)	Margin	Elevation	Texture	Appearance	Optical property
SUTS-1	circular	10	entire	flat	rough	dull and cream	opaque
SUTS-2	circular	15	entire	flat	rough	dull and cream	translucent
SUTS-3	circular	10	entire	flat	smooth	dull and cream	translucent
SUTS-4	circular	20	entire	flat	rough	shinny and cream	opaque
SUTS-5	circular	40	curled	flat	moist	shinny and cream	opaque
SUTS-6	circular	40	curled	raised	rough	shinny and cream	opaque
SUTS-7	circular	30	curled	flat	rough	shinny and cream	translucent
SUTS-8	circular	10	entire	convex	rough	shinny and cream	translucent

Figure 1: Urease test on Christensen's medium.

Conductivity and urease measurement

Conductivity (mS.cm^{-1}) method was used to determine the enzymatic rate of reaction of the bacterial cultures. This method employs the use of conductivity meter, a device that is robust, easy to operate and an inexpensive (Al-Thawadi 2008). In the absence of calcium ions, conductivity measurement is a suitable method to measure urease activity, because it reads the reactions between two charged ions; ammonium (NH_4^+, positively charged) and carbonates (CO_3^{2-}, negatively charged) in the bacteria-urea solution (Cuzman et al 2015). The ability of the local isolates to hydrolyse urea were quantified as shown in Figure 2 and compared with that of the control strain (*Sporosarcina pasteurii*). The conductivity variation rate for the local bacterial isolates and control strain were obtained from slope gradient of the conductivity (mS.cm^{-1}) against time (hr). The conductivity variation rate for Figure 2 for bacterial isolates SUTS-1, SUTS-2, SUTS-3, SUTS-4, SUTS-5, SUTS-6, SUTS-7, SUTS-8 and control strain were 0.053, 0.061, 0.104, 0.087,0.133, 0.172, 0.064, 0.099 and 0.198 mS.cm^{-1}.min-1, respectively. When compared to the local isolates, SUTS-6 had the highest conductivity variation rate, while SUTS-1 had the lowest conductivity variation rate. It was noticed none of the isolates had a higher urea hydrolysis rate when compared to the control strain. The conductivity variation rate for ureolytic bacteria reported in the literature ranged from 0.063 to 0.230 mS.cm^{-1}.min^{-1} (Chu et al 2012, Cuzman et al 2015, Whiffin 2004, Zoheir et al 2013), which are similar to the values obtained in this present study. The conductivity variation rate (mS.cm^{-1}.min^{-1}) of each bacterial isolates obtained from Figure 1 were converted to specific urease activity by taking the biomass readings at the end the incubation. Results of specific urease activities as seen in Figure 3 showed that, all the isolates had lower values when compared with the control strain (23.755 mM urea hydrolysed.min^{-1}.OD^{-1}) except for SUTS-6 (23.340 mM urea hydrolysed.min^{-1}.OD^{-1}). On the other hand, SUTS-1 and SUTS-2 had the lowest specific urease activities with 7.309 mM urea hydrolysed.min^{-1}.OD^{-1} and 7.162 mM urea hydrolysed.min^{-1}.OD^{-1}, respectively. Reports from the literature have shown that urease activities ranges between 2.2 to 20 mM urea hydrolysed.min^{-1} for ureolytic bacteria (Harkes et al 2010, Whiffin 2004). The capability of bacterial isolates to be able to produce urease and induce $CaCO_3$ have been widely studied and reported, however most are not suitable for MICP applications due to their pathogenicity level.

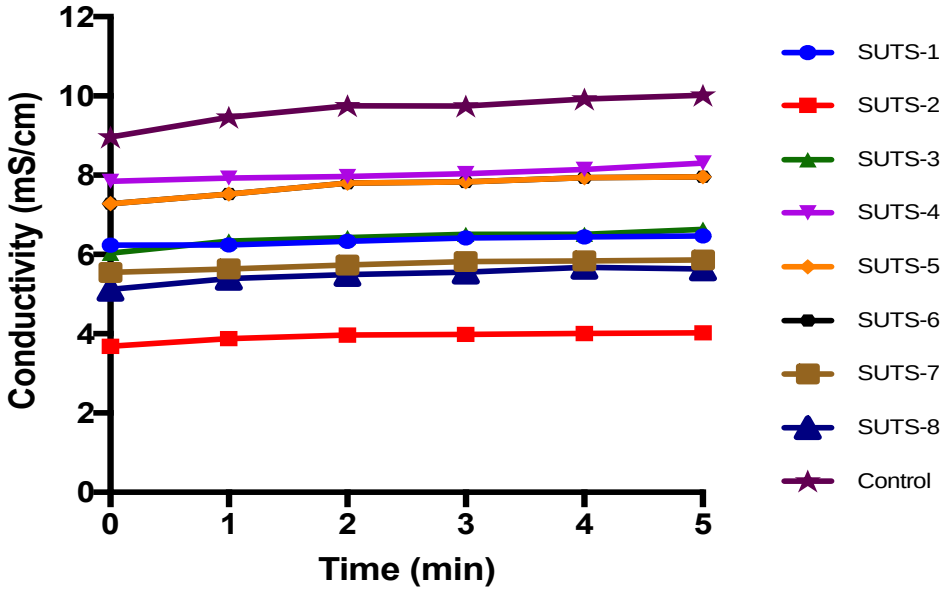

Figure 2: Conductivity measurement showing the hydrolysis of urea by ureolytic bacteria.

The ureolytic bacterial isolate SUTS-6 shows the prospect of being utilised in biocementation treatments for solving geotechnical and civil engineering problems by enhancing the geotechnical properties of loose soil. However, since the isolate's identity and $CaCO_3$ precipitation ability have

not been performed, for biosafety purpose, this isolate was not used in this current study. Hence it would be preferable if this bacteria's characteristics and pathogenicity level be tested before being utilised for any engineering applications. It is also noteworthy that isolates SUTS-5 and SUTS-3 showed reasonable amount if specific urease activity, 14.390 and 18.526 mM urea hydrolysed.min^{-1}.OD^{-}, respectively. It is possible that urease production can be improved and be at a pace comparable with that of the control strain and SUTS-6 if cultivated in optimised conditions.

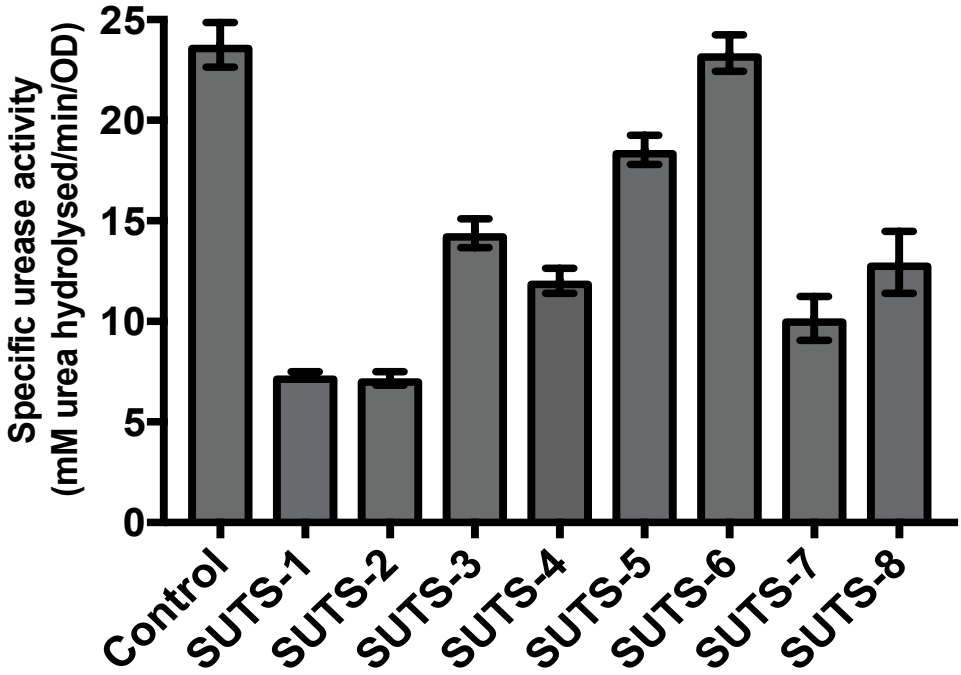

Figure 3: Specific urease activities of locally isolated bacteria compared with *Sporosarcina pasteurii* (DSM 33) as control.

Biocementation and strength test

Biocementation via MICP process was used to treat the poorly graded soil samples selected for this study. Prior to loading of the soils into their respective columns (Figure 4), they were autoclaved to eliminate the presence of any microorganism. This was performed as suggested by Burbank et al. (2011). To rule out the possibility of having false precipitations such as chemically induced calcite precipitation on any of the samples, *Sporosarcina pasteurii* (DSM 33) was employed as a positive control for biocement treatment test. This strain has been categorized by the US Department of Health and Human Services (1999) as a Risk Group 1 (RG1), low individual and community risk (Biosafety Level 1) based on United State of America's public health service guideline and biosafety guidelines, due to the bacteria's unlikeliness of causing human disease or animal disease of veterinary importance (Emmert 2013). In order to immobilise bacteria in the sand columns for use in subsequent biocement treatment, three separate methods were used as shown in Table 2.

Table 2. Different biocement treatment methods

Group	Treatment method
1	sand without premix
2	sand premixed with bacterial culture
3	sand premixed with 1 M urea and calcium chloride

An overnight bacterial culture with cementation solution (1M urea and 1M $CaCl_2$) were used to treat the loose sands carefully placed in their respective columns. Results in Figure 5 showed that treatment using Group 1 and 2 produced better results when compared with Group 3. The samples from these two treatment methods resulted in uniformly cylindrical shaped biocement columns . These results were consistent in all replicates for Group 1 and 2. This showed that, with repeated treatment methods, compacted biocemented samples having proper and uniform shape would be obtained. Results from Group 3 showed inconsistency in the cemented samples with disintegrated cylindrical shapes. It was also observed that among all samples treated with the three Groups, only samples from Group 1 showed no large pores, however large pours were visible from samples treated with Group 2 and 3. In addition, white calcite precipitates were vividly visible from samples treated with Group 3. This could be due to uneven distribution of calcite within the sand matrix.

Figure 4. Sand columns wrapped with masking tapes were placed on a plastic tray before being treated with *Sporosarcina pasteurii* and cementation solution via surface percolation method.

Figure 5. Biocemented sand samples after being treated with different methods via MICP process. (A) sand without premix; (B) sand premixed with bacterial culture and (C) sand premixed with 1 M urea and calcium chloride.

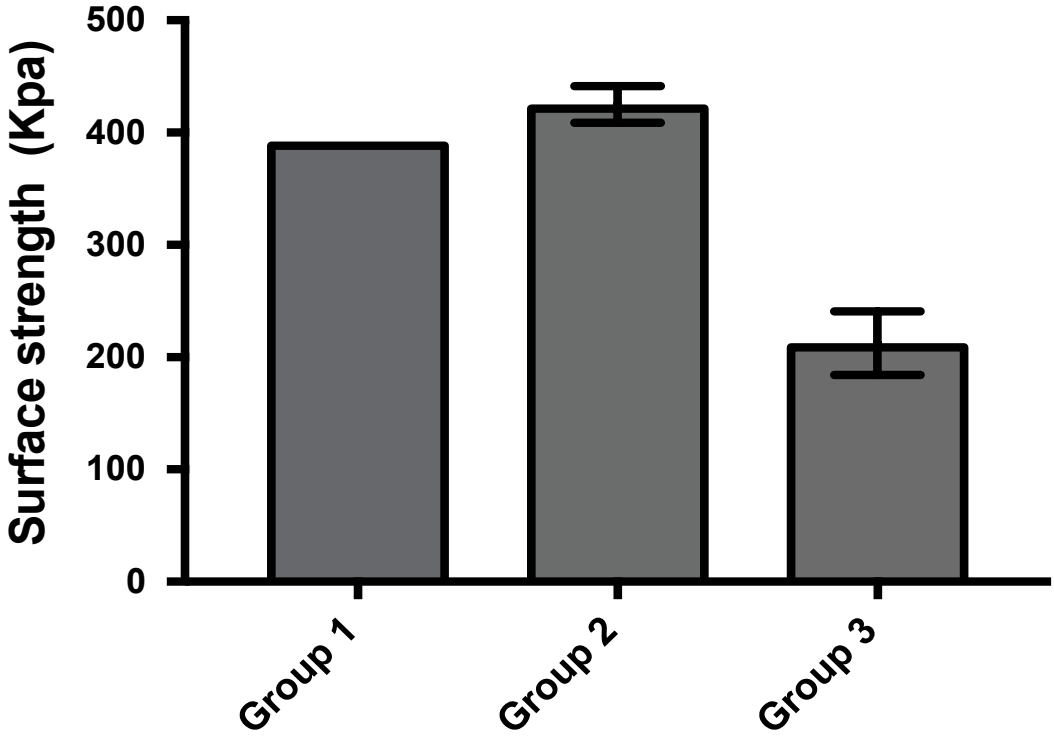

Figure 6. Surface strength of the treated sand samples from different treatment groups. Group 1 (sand samples without premix); Group 2 (sand premixed with bacterial culture) and Group 2 (sand premixed with 1 M urea and calcium chloride).

The reason to presence of more calcite formation at the top layers of the treated sand columns, is mainly due to the fact that *Sporosarcina pasteurii* is a facultative anaerobic bacterium, which grows at a higher rate in an oxygen rich environment and consequently leading to higher rates of calcite precipitates around the top surface areas (Whiffin et al 2007). In addition, the influence of biocementation is dependent on the ability of the bacteria to move freely throughout the pore spaces of the sand and on sufficient particle-particle contact per unit volumes at which cementation will occur. Hence, biocementation will most likely work best on soils with larger pore sizes. The surface strengths using penetrometer were measured for all the biocemented sand samples after curing for two weeks. Results shown in Figure 6 proved that the use of MICP process resulted to strengthening of the sand samples. The surface strength results were 393.266, 430.922 and 212. 477 KPa for samples treated with Group 1, Group 2 and Group 3, respectively.

The results present in Figure 6, suggested that Group 2 treatment method resulted in the highest surface strength (430.922 kPa). Some studies have shown that addition of more bacterial cultures and cementation solution result to an increase in strength due to production of more calcite precipitates (DeJong et al 2010, van Paassen et al 2010). Hence, longer duration of biocement treatment with more volume could yield stronger samples. Thus, it is imperative to maintain sufficient amount of repeated addition of bacterial culture to the sand columns so as to prevent possible accumulation of metabolic waste which could result in a decrease of urease activity, cell death and poor precipitation (Stocks-Fischer et al., 1999). However, it will be necessary to determine the best treatment duration and volume to obtain maximum calcite content and strength.

Educational implication and student learning experience

This paper describes a laboratory practicum designed to expose undergraduate students undertaking an industrial microbiology module of a biotechnology program to the methods behind screening for urease-producing bacteria and their industrial relevance in geotechnical and civil

engineering applications. Enrichment culture technique was used to target urease-producing bacteria which were employed in biocementation of poorly graded soils via surface percolation. This enabled students gain both biotechnological and engineering laboratory skills. Tropical rainforest regions such as Malaysia have abundant availability of loose soils (i.e. sands, peat soils or soft clay soils) which pose challenges to engineers during early stage of construction due to poor ground conditions. Some of these soils often experience further soil softening due to extreme and prolonged downpours, which can be problematic for engineers (Soon et al 2013). Constructions in these types of regions would require proper soil stabilisation efforts to prevent soil liquefaction (Perlea 2000). The utilisation of biocementation technique to resolve such problem exposes undergraduate students to real industrial problem-solving skills. For future perspective, it would be interesting to integrate science and civil engineering students in this laboratory exercise for proper cross-disciplinary discipline experience. We recommend that students and tutors read comprehensive texts such as, Construction Biotechnology: Biogeochemistry, Microbiology and Biotechnology of Construction Materials and Processes (Stabnikov et al 2015) or Biotechnologies and Biomimetics for Civil Engineering (Pacheco-Torgal et al 2015), to have vehement background knowledge about MICP technology and its applications. Anonymous feedbacks were obtained from undergraduate students at the end of the laboratory exercise. In general, students feedbacks were positive, as they found the module interesting, especially the biocement exercise. One student wrote, ''It was a fascinating experience because we got to learn how to make biocement products by using living microorganisms", while another student commented "we learnt how to screen for urease-producing bacteria from locally sourced environmental samples, quantify urease enzyme inexpensively and some basic engineering biocementation skills". From this feedback it was deduced that the students were very impressed with the cross-disciplinary practicum exercise.

CONCLUSION

The results obtained from this research confirms the presence of ureolytic bacteria in soil samples, indicating their ubiquitous characteristics in local environment. Using enrichment culture technique, 12 isolates were isolated with 8 showing urease positive prospects. Conductivity method was used to measure the urease activity from the indigenous ureolytic bacteria. The result showed that only one out of the 12 isolates had specific urease activity compared to the control strain (*Sporosarcina pasteurii*). Out of the three different biocement treatment methods used to treat poorly-graded soils, sand samples premixed with bacterial culture had the highest strength test. Hence, should be often considered when performing biocement applications. Further studies which could be performed involves SEM-EDX analysis in order to analyse the morphological and composition of biocement deposits in the sand pores, unconfined compressive strength which could be used to study the shell strength and failure pattern of biocemented samples. Conclusively, it would be interesting to introduce this laboratory exercise in practical classes, so students from science and engineering disciplines could have cross-disciplinary research skills.

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REFERENCES

[1] Al-Thawadi SM (2008) High strength in-situ biocementation of soil by calcite precipitating locally isolated ureolytic bacteria. *School of Biological Sciences and Biotechnology*, PhD thesis, Murdoch University, Perth, Australia.

[2] Burbank MB, Weaver TJ, Green TL, Williams B and Crawford RL (2011) Precipitation of calcite by indigenous microorganisms to strengthen liquefiable soils. *Geomicrobiology Journal* 28(4): 301–312.

[3] Canteros CE, Rodero L, Rivas MC and Davel G (1996) A rapid urease test for presumptive identification of Cryptococcus neoformans. *Mycopathologia* 136(1): 21–23.

[4] Chu J, Stabnikov V and Ivanov V (2012) Microbially Induced Calcium Carbonate Precipitation on Surface or in the Bulk of Soil. *Geomicrobiology Journal* 29(6): 544–549.

[5] Cuzman OA, Richter K, Wittig L and Tiano P (2015) Alternative nutrient sources for biotechnological use of *Sporosarcina pasteurii*. *World Journal of Microbiology and Biotechnology*. Springer Netherlands 31(6): 897–906.

[6] DeJong JT, Mortensen BM, Martinez BC and Nelson DC (2010) Bio-mediated soil improvement. *Ecological Engineering* 36(2): 197–210.

[7] Dhami NK, Reddy MS and Mukherjee A (2013) *Bacillus megaterium* mediated mineralization of calcium carbonate as biogenic surface treatment of green building materials. *World Journal of Microbiology and Biotechnology* 29(12): 2397–2406.

[8] Elmanama AA and Alhour MT (2013) Isolation, Characterization and Application of Calcite Producing Bacteria from Urea Rich Soils. *Journal of Advanced Science and Engineering Research* 3(4): 388–399.

[9] Emmert EAB (2013) Biosafety Guidelines for Handling Microorganisms in the Teaching Laboratory: Development and Rationale †. *Journal of Microbiology & Biology Education* 14(1): 78–83.

[10] Ferris FG, Stehmeier LG, Kantzas A and Mourits FM (1996) Bacteriogenic mineral plugging. *Journal of Canadian Petroleum Technology* 35(8): 56–61.

[11] Fortier L-C and Moineau S (2009) Phage production and maintenance of stocks, including expected stock lifetimes. In: Clokie MRJ, Kropinski AM and Clokie MRJ (eds) *Bacteriophages*. Springer, 203–219.

[12] Hammes F and Verstraete W (2002) Key roles of pH and calcium metabolism in microbial carbonate precipitation. *Reviews in Environmental Science and Biotechnology* 1(1): 3–7.

[13] Harkes MP, van Paassen LA, Booster JL, Whiffin VS and van Loosdrecht MCM (2010) Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement. *Ecological Engineering* 36(2): 112–117.

[14] Holt JH, Krieg NR, Sneath PH a., Staley JT and Williams ST (1994) Bergey's manual of determinative bacteriology ninth edition. *European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society* 13(6): 560.

[15] Ivanov V and Chu J (2008) Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ. *Reviews in Environmental Science and Biotechnology* 7(2): 139–153.

[16] Kim G and Youn H (2016) Microbially induced calcite precipitation employing environmental isolates. *Materials* 9(6).

[17] Lee JC, Lee CJ, Chun WY, Kim WJ and Chung CW (2015) Effect of microorganism *Sporosarcina pasteurii* on the hydration of cement paste. *Journal of Microbiology and Biotechnology* 25(8): 1328–1338.

[18] Omoregie AI, Khoshdelnezamiha G, Senian N, Ong DEL and Nissom PM (2017) Experimental optimisation of various cultural conditions on urease activity for isolated *Sporosarcina pasteurii* strains and evaluation of their biocement potentials. *Ecological Engineering* 109: 65–75.

[19] van Paassen LA, Ghose R, van der Linden TJM, van der Star WRL and van Loosdrecht MCM (2010) Quantifying Biomediated Ground Improvement by Ureolysis: Large-Scale Biogrout Experiment. *Journal of Geotechnical and Geoenvironmental Engineering* 136(12): 1721–1728.

[20] Pacheco-Torgal F, Labrincha JA, Diamanti M V., Yu CP and Lee HK (2015) *Biotechnologies and biomimetics for civil engineering*. *Biotechnologies and Biomimetics for Civil Engineering*.

[21] Perlea VG (2000) Liquefaction of Cohesive Soils. *Soil Dynamics and Liquefaction 2000*, 58–76.

[22] Reyes RG, Lou L, Lopez M a, Kumakura K and Kalaw SP (2009) Coprinus comatus, a newly domesticated wild nutriceutical mushroom in the Philippines. *Journal of Agricultural Technology* 5(2): 299–316.

[23] Sidik WS, Canakci H, Kilic IH and Celik F (2014) Applicability of biocementation for organic soil and its effect on permeability. *Geomechanics and Engineering* 7(6): 649–663.

[24] Soon NW, Lee LM, Khun TC and Ling HS (2013) Improvements in engineering properties of soils through microbial-induced calcite precipitation. *KSCE Journal of Civil Engineering* 17(4): 718–728.

[25] Stabnikov V, Ivanov V and Chu J (2015) Construction Biotechnology: a new area of biotechnological research and applications. *World Journal of Microbiology and Biotechnology*. Springer Netherlands 31(9): 1303–1314.

[26] Stocks-Fischer S, Galinat JK and Bang SS (1999) Microbiological precipitation of CaCO3. *Soil Biology and Biochemistry* 31(11): 1563–1571.

[27] US Department of Health and Human Services (1999) Biosafety in Microbiological and Biomedical Laboratories. *Public Health Service.* 5th Edition, 1–250.

[28] Whiffin VS (2004) Microbial CaCO3 Precipitation for the Production of Biocement. PhD thesis, Murdoch University, Perth, Australia.

[29] Whiffin VS, van Paassen LA and Harkes MP (2007) Microbial carbonate precipitation as a soil improvement technique. *Geomicrobiology Journal* 24(5): 417–423.

[30] Zhang Y, Guo HX and Cheng XH (2015) Role of calcium sources in the strength and microstructure of microbial mortar. *Construction and Building Materials*. Elsevier Ltd 77: 160–167.

[31] Zoheir AE, Hammad IA and Talkhan FN (2013) Urease activity and induction of calcium carbonate precipitation by *Sporosarcina pasteurii* NCIMB 8841. *Journal of Applied Sciences Research* 9(3): 1525–1533.