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Published

2016

Conference Title

Borneo Research Education Conference 2016 Proceedings: Developing as a Researcher by Building Research Skills

Version

Version of Record (VoR)

4th Borneo Research Education Conference, BREC 2016, 17th-18th August 2016,
Universiti Teknologi MARA Sarawak, Malaysia

Cross-Disciplinary Research: Developing Biocement Applications Using Locally Isolated Ureolytic Bacteria

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Abstract

Construction microbial biotechnology is a new area of science and engineering that makes use of microbially induced calcite precipitation, a type of biocementation technique to improve the geotechnical properties of soil. The process of calcite precipitation is influenced by the production of microbial urease enzyme. Microbially induced calcite precipitation implementation as an established ground improvement method has been partially limited by the need for highly active urease producers and improved injection procedures. This paper discusses the collaborative efforts between the Science and Civil Engineering departments towards developing cross-disciplinary research Master programmes. The aim of the Master of Science research was to isolate highly active ureolytic bacteria from the limestone caves of Sarawak and to conduct comparative biocement test using the isolated ureolytic bacteria, alongside bacterial consortia, negative and positive controls. The best yielding isolates would then be further evaluated by a Master of Engineering student in large scale trials. In the Master of Science research activities, soil samples were collected from limestone caves of Sarawak, then enriched for highly active ureolytic bacteria before being assessed in small-scale biocementation experiments. Poorly graded soils were treated with locally isolated ureolytic bacteria and the surface strength of the treated soil samples were measured with a pocket penetrometer. The test showed that biocementation using the selected ureolytic bacteria can significantly improve the engineering properties of poorly graded soils. However, the efficiency of the microbially induced calcite precipitation process in improving the soil strength varied among the samples which were treated with different isolates, bacterial consortia, and the control strain. The findings in this study suggest that the locally isolated ureolytic bacteria have the potential to be used as alternative microbially induced calcite precipitation agents for biocement applications. This paper demonstrates developing a postgraduate research programme through the collaboration between the different disciplines of biotechnology and engineering.

Keywords: Urease; bacteria; microbially-induce calcite precipitation; biocement, cross-disciplinary research

1. Introduction

Cross-disciplinary research (CDR) is highly relevant as it tackles a wide range of issues, various institutions and faces of the industries (Donovan, O'Rourke, & Looney, 2015). However, CDR teams encounter some significant challenges such as communication and technical terminology. Hence, it is important to have a strong performing collaborative research team consisting of those who are keen in learning and understanding the various important virtues across disciplines (Bracken & Oughton, 2006; Brewer, 1999; Eigenbrode et al., 2007). CDR teams are required to understand their respective disciplinary perspective well enough to convey its meaning to others, develop an essential understanding of certain used terms across disciplines and be able to learn the various skills required to carry out certain research work or data analysis (Donovan et al., 2015; Jeffrey, 2003). Considering the intricacy of the problems the world faces, it is apparent that they require a cross-disciplinary framework and research aimed at combining ideas and skills to solve the complex challenges at hand (Mutz, Bornmann, & Daniel, 2015). Over two decades, innovations in research and technology have facilitated enormous improvements in the scientific research process. The involvement of experts from across disciplines has been able to improve and revolutionise the study of genomics, nanotechnology and system biology (Welsh, Jirotka, & Gavaghan, 2006). Previously, biology was merely a discipline of description and classification of organisms, with

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little complexity of biological systems involving mathematical descriptions (Finholt, 2003; Galison, 1992). However, this opinion is now rapidly changing with biologists getting involved in sequencing, determination of biological functions and genomics study (Welsh et al., 2006). Another noticeable CDR is in cancer (Urquhart, Grunfeld, Jackson, Sargeant, & Porter, 2013). Health service researchers have come up with ways of narrowing the knowledge-practice gap they encountered by involving multiple academic disciplines and non-academic individuals and integrating them into the research process in order to tackle real world patient care issue, aid in translating clinical results and help in improving community health care (Grol, 2001; McGlynn et al., 2003).

This paper discusses a CDR involving the civil engineering department and science department at Swinburne University of Technology (Sarawak Campus) led by Assoc. Prof. Dr Dominic Ek Leong Ong (Director, Swinburne Sarawak Research Centre for Sustainable Technologies) and Assoc. Prof. Dr Peter Morin Nissom (Associate Dean, Science). The research team comprised of M.Sc. and M.Eng. research candidates (fig. 1) focuses on improving the mechanical properties of soil for construction and environmental purposes by making use of microbial activities and products to enhance the strength and stiffness properties of soil through microbial activities and products.

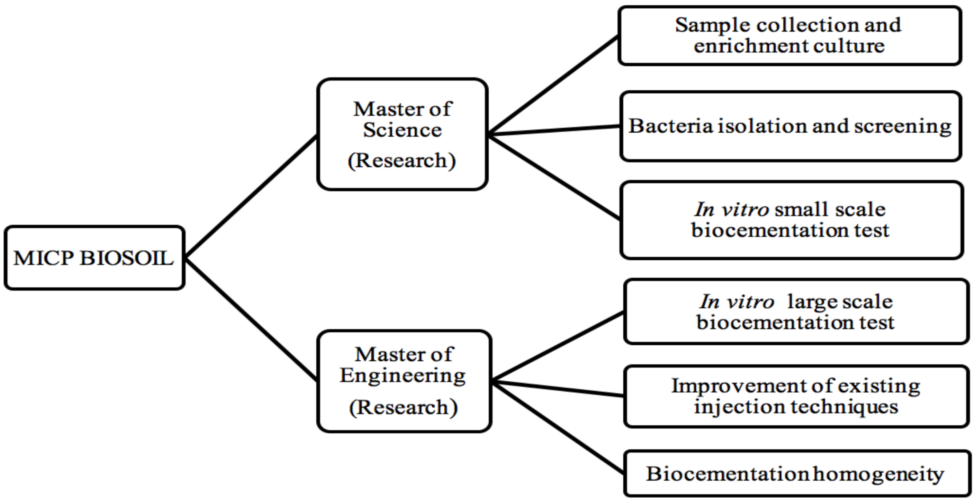

Fig. 1. A schematic framework showing part of the collaborative research aims between the Science and Civil Engineering departments in Swinburne University of Technology (Sarawak Campus) towards developing cross-disciplinary research Master programmes.

Despite Malaysia's abundance of limestone regions situated in places such as Langkawi Island, Kedah-Perlis, Kinta Valley, Perak, Selangor, Gua Musang, and Kelantan as reported by Bakhshipouri et al., (2009), there are limited reported studies on the exploitation of microbial diversity for MICP applications from these regions and also in Sarawak. This research gap initiated the relevance of screening for urease producing bacteria from cave regions in Sarawak. The aim of the M.Sc. research was to isolate highly active ureolytic bacteria from the limestone caves of Sarawak and to conduct comparative biocement test using the isolated ureolytic bacteria, alongside bacterial consortia, as well as negative and positive controls. The best yielding isolates would then be further evaluated by a M.Eng. student in large scale trials. In the M.Sc. research activities, soil samples were collected from limestone caves of Sarawak, then enriched for highly active ureolytic bacteria, before being assessed in small-scale biocementation experiments. The significance of this research involves making use of the enrichment culture technique to cultivate non-pathogenic ureolytic bacteria from limestone cave with potential capabilities to serve as microbially induced calcite precipitation (MICP) agents that are an alternative to already existing ureolytic bacteria commonly used by civil and geotechnical engineers for the production of sustainable and environmentally friendly biocement.

2. Literature Review

Microbially induce calcite precipitation (MICP) by urea hydrolysis has been a subject of high relevance in recent years (De Muynck, Verbeken, De Belie, & Verstraete, 2010; Ivanov & Chu, 2008). The possibility of using microorganisms to improve the mechanical properties of soils by undisturbed in situ or soil reinforcement technology based on MICP has enabled a cross-disciplinary research at the confluence of microbiology, geochemistry, civil engineering and geotechnical engineering (Cheng & Cord-Ruwisch, 2012; DeJong, Mortensen, Martinez, & Nelson, 2010). This cross-disciplinary research falls into a new area of science and engineering known as construction microbial technology which focuses on making use of microbially-mediated process and an environmentally-friendly technique to produce biocements and biogrouts which are useful for industrial construction (Ivanov, Chu, & Stabnikov, 2015). The discipline of microbial biotechnology employs the practical knowledge of highly active urease-producing bacteria cementation process to bind loose sand particles together (Ivanov et al., 2015).

Calcium carbonate ($CaCO_3$) is a biomineral widely secreted by most microorganisms (Barabesi et al., 2007; Sarayu, Iyer, & Murthy, 2014). Calcium carbonate mineralisation can be found in natural formations such as corals, ant hills or caves (Dhami, Reddy, & Mukherjee, 2013). Microbially induced calcite precipitation (MICP) is a process that refers to calcite precipitation from a supersaturated solution in a microenvironment that occurs due to the occurrence of microbial and biochemical activities (Anbu, Kang, Shin, & So, 2016; Bosak, 2011; Hamilton, 2003; Knorre & Krumbein, 2000; Rivadeneyra, Parraga, Delgado, Ramos-Cormenzana, & Delgado, 2004). MICP utilises the biologically induced pathway of biomineralisation (Whiffin, 2004; Whiffin, van Paassen, & Harkes, 2007). During the MICP process, microorganisms are able to produce metabolic products (CO_3^{2-}) that react with ions (Ca^{2+}) in the microenvironment which results in consequent minerals precipitated (Anbu et al., 2016).

The ability of microorganisms to induce biomineralisation, both in natural and laboratory conditions, are influenced by the type of microbes involved in salinity (Navdeep. Kaur. Dhami, Reddy, & Mukherjee, 2012) and compositions of nutrients available in the microenvironments (Knorre & Krumbein, 2000; Rivadeneyra et al., 2004). $CaCO_3$ precipitation by bacteria through urea hydrolysis is the most straightforward and easily controlled mechanism of MICP with the ability to induce a high amount of $CaCO_3$ in a short duration of time (Dhami, Reddy, & Mukherjee, 2014).

MICP process is an effective and environmentally friendly technology which can be applied to solve various environmental problems such as soil instability. Biocement or biosandstone serves as a novel method used for cementing loose sands to produce structural materials and improving the stability of the soil (Achal, 2015). In concrete, cracking is common due to relatively low tensile strength (De-Belie and De-Muynck, 2008). Cracking on concrete surfaces also results in the enhanced deterioration of embedded steel through the easy ingress of moisture and ions that react with the reinforcements in concrete which then, due to the expansive stress, leads to spalling (Gavimath et al., 2012). A conventional approach used in repairing cracks involves injecting epoxy resin or cement grout into the concrete. However, this approach results in various thermal expansion along with environmental and health hazards (De-Belie and De-Muynck, 2008). Several research groups have investigated the possibility of using MICP as an alternative effective repair method for cracks in concrete via bioremediation.

3. Methodology

3.1. Sampling permit and sample collection

Upon the authorization by the Sarawak Forest Department and Sarawak Biodiversity Centre (permit numbers *NCCD.907.4.4[JLD.11]-37* and *SBC-RA-0102-DO),* permission was given to collect samples from nature reserves in Sarawak and to conduct biological research. A field sampling took place at Fairy Cave Nature Reserve and Wind Cave Nature Reserve respectively and samples were taken from these sampling sites. The caves are located in Bau, Kuching Division, Sarawak, East Malaysia, on the island of Borneo. The samples collected were stored in the refrigerator set the temperature of 4^oC until the enrichment and isolation procedures were fully completed.

3.2. Enrichment culture techniques

Enrichment culturing of the cave samples was performed to target desired microorganisms by adding 1 g of each collected sample into 50 mL of growth media (250 mL shaking flasks). The cultures were then shaken under aerobic batch conditions at 30^oC for 120 hr at 130 rpm.

3.3. Isolation and screening of ureolytic bacteria

Upon completion of enrichment culturing, serial dilution technique was used on isolated microorganisms capable of surviving on 6% urea substrate in plates containing nutrient agar media. The plates were then incubated under aerobic conditions at 32^OC for 42 hr until bacteria colonies were seen on the plates. Christensen's medium, also called urea agar base, was used to screen for urease producing bacteria. The bacterial isolate, capable of turning the Christensen's medium from pale yellow to pink during the incubation period, were selected while others were discarded.

3.4. Biocement small scale test

Poorly graded selected sands were pre-mixed with selected bacteria culture, calcium chloride (1M) and urea (1M) solution before being compacted into their respective columns. The sand columns were treated with the bacteria and cementation solutions by percolation (i.e. unrestrained flushing of fluid from top to bottom). 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 hr for a duration of 96 hrs. Upon completion of the treatments, all the sand columns were cured at room temperature for the duration of 14 days before the treated sand was removed from their respective moulds.

3.5. Surface strength test of treated soil samples

The surface strength measurements of the treated soil samples were performed by using a pocket penetrometer which had a reading scale ranging from 0 to 700 psi (0 to 4.826 MPa). The pocket penetrometers were used to measure the surface strength by pushing the tip of the penetrometer into the soil to a depth of approximately 0.25 inches. Three selected surface regions were tested on each of the cemented sand. The readings of the loaded weight were recorded when the samples were completely penetrated (breakage).

3.6. Statistical analysis using Graphpad Prism Software

The data were shown as mean ±SE (standard deviation) for three replicates. The results were subjected to the students' *t*-test analysis, with the statistical significance taken as $p<0.05$. The GraphPad (Quick Calc) programme was used to analyse the data.

4. Findings

A total of thirty-one ureolytic bacteria were isolated from Fairy Cave Reserve and Wind Cave Reserve. Enrichment culture technique is widely used to isolate bacteria in clinical, biotechnological and environmental studies because it brings about competition among microbiota for available nutrients and against growth inhibitors by favouring specific bacterial type strains or subgroups (Gorski, 2012; Muniesa, Blanch, Lucena, & Jofre, 2005). Christensen's medium serves as a quick method to primarily screen for ureolytic bacteria (Burbank, Weaver, Williams, & Crawford, 2012). The locally isolated bacteria were successfully screened for their ability to produce urease enzyme on Christensen's medium.

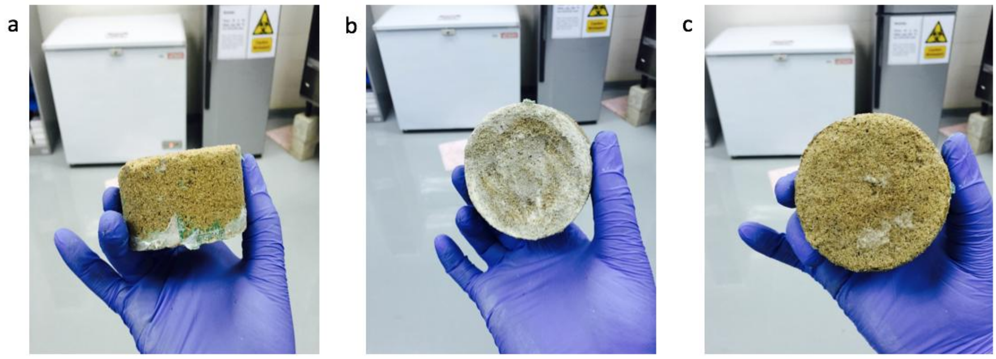

Fig. 2. Treated sand sample held after a curing period and columns were successfully removed.
(a) side view [left]; (b) top view [middle]; (c) bottom view [right].

In accordance with British Standards (BS5930), the sand specimen used in this study was classified as a poorly graded medium having particle sizes ranging from fine sand to fine gravel. The sand specimens were prepared and selected by using only sand capable of passing through the No. 10 sieve (the grain size passing the 2 mm sieve). This adapts the work of Rebata-Landa (2007) whereby the optimum particles size required to enhance a successful biocement product is between 0.05 mm to 0.40 mm. Upon the completion of the biocement treatment test using the selected isolated ureolytic bacteria, alongside the bacterial consortia, as well as negative and positive controls, the results showed that biocementation using the selected ureolytic bacteria can significantly improve the engineering properties of poorly graded soils (fig. 2). However, the efficiency of the MICP process in improving the soil strength varied among the samples which were treated with different isolates, bacteria consortia, and positive control strain. The results also showed there was higher cementation level at positions close to the injection points and more calcite contents were obtained from the top layers of the biocemented sand. Based on the surface strength tested using the penetrometer and statistical analysis using an independent *t*-test (table 1), it was observed that there was no significant difference in the surface strength between all of the local isolates and bacterial consortia, except one local isolate when compared with the control strain.

Table 1. *t*-test results comparing the strength (psi) differences between the biocemented sands. (N=3; df=2).

Bacteria	M	SD	SE	*P*-value	*t*	*P* <*
Positive control	563.33	42.10	nil	nil	nil	nil
Local isolate-1	626.67	99.81	57.097	0.3829	1.1092	-
Local isolate-2	582.33	67.35	21.221	0.4651	0.8953	-

Local isolate-3	700.00	0.00	24.306	0.0302	5.6228	+
Local isolate-4	573.33	6.51	26.312	0.7405	0.3801	-
Bacterial consortia	533.33	116.93	76.374	0.7324	0.3928	-

(N) number of sample size; (df) degree of freedom; (M) mean; (SE) standard error; (SD) standard deviation; (*P*-value) calculated probability; (*t*) test statistic; (+) significant; (-) not significant ;(*<) *P*-value is significant at 0.05 level.

During the course of this CDR which involved the science and engineering postgraduate students, there were certain issues and limitations that were encountered. The use of some technical terminologies such as "cultivation", sub-culture", "autoclaving", "reviving" and "isolation" often used by microbiologists and, those such as "curing time", "porosity", "shear strength" and "void" used by civil engineers were an issue as it resulted in misunderstandings and misinterpretations at the beginning of this collaborative CDR. In order to solve this misconception, certain technical terminologies were discussed and explained until properly understood. It was quite the challenge for the civil engineering students to learn about some microbiological skills such as the culturing and handling of the microorganisms. It is suggested that a short course on basic microbiology or biotechnology involving microbial growth and behaviour would be helpful.

In order to keep a high maintenance of the CDR aim, meetings were conducted (once or twice a month) and presentations were carried out by the postgraduate research students. The frequent presentations by both the science and engineering research students helped to enhance their presentation skills because they needed to be able to present their research findings to an audience not limited to their discipline. This aimed at helping both the science and engineering departments in understanding the significance of this collaborative work. Cross contamination during bacteria cultivation was regarded as a risk and a limitation in this CDR. However, with training and proper conduction of good laboratory practices such as wearing hand gloves, performing laboratory work in a clean and disinfected work bench helped in reducing the risk of cross-contamination. The collaborative CDR led by Assoc. Prof. Dr Dominic Ek Leong Ong and Assoc. Prof. Dr Peter Morin Nissom in Swinburne University of Technology (Sarawak Campus) was the first collaborative research involving both civil engineering and science departments which successfully lead to the developments and completion of M.Sc. and M.Eng. research thesis.

Conclusion

The cross-disciplinary research discussed in this study was used to develop an M.Sc. postgraduate thesis which aimed to isolate highly active ureolytic bacteria from the limestone caves of Sarawak and to conduct comparative biocement test using the isolated ureolytic bacteria, alongside bacterial consortia, as well as negative and positive controls. The results showed that enrichment culture technique was useful in targeting microorganisms capable of degrading urea and these ureolytic bacteria could produce urease enzyme. The biocement treatment and surface strength tests showed that the selected highly active local ureolytic bacteria could serve as alternative MICP agents for biocementation applications. It is suggested that more research across disciplines should be carried out and designed to develop postgraduate programmes and for the acquisition of new skills.

Acknowledgements

The authors express their gratitude to the Civil Engineering and Chemical Engineering Departments, Swinburne University of Technology, Sarawak Campus, Malaysia, for their financial assistance (SSRG) used to support this research. The authors also extend their gratitude to Nurnajwani Senian, Ye Li Phua and Ghazaleh Khoshdelnezamiha from the civil engineering department for playing a significant role in the MICP Biosoil project by providing the essential guide needed during the biocement test, the surface strength test and for the sample collection. A special thanks goes to the Sarawak Biodiversity Centre (SBC) and Sarawak Forestry Department (SFD) for their permission to collect samples from Fairy Cave Nature Reserve and Wind Cave Nature Reserve.

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