

INFLUENCE OF CEMENTATION REAGENT CONCENTRATION ON
MICROBIAL-INDUCED CALCITE PRECIPITATION IN MALAYSIA

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ABSTRACT

With the high population growth rate, land with competent soil which can use for the construction purposes decreased. As the competent soil availability decreased, many soil improvement methods are used to improve the soil in term of strength and stability. One of the new sustainable methods, known as Microbial-Induced Calcite Precipitation (MICP), which utilizes the biological process to produce calcite in soil which can induce the biocementation and bioclogging process. These processes will improve the properties of soil in term of permeability, strength, and liquefaction resistance. The efficiency of MICP might be influenced by several factors such as type of sand, reaction time, reactant concentration, and bacteria concentration. In this research, two of the factors, concentration of cementation reagents and treatment durations, were being studied by using 2 different mixing methods (spraying method and pressurize method). The bacterial concentration used is 1.6×10^6 cfu/ml and the atmospheric temperature used is 30°c . The optimum cementation reagent concentration found to be 0.25 M with spraying method under the treatment duration of 24 hours which increase the soil strength up to 215.79 % (from 19 kPa to 41 kPa). For pressurize method, the best concentration of cementation reagent is 0.25 M under treatment duration of 48 hours which gave a soil strength improvement of 184.21 % (from 19 kPa to 35 kPa). Hence, with the cementation reagent of 0.25 M, spraying method is more effective than pressurize method.

ABSTRAK

Kadar pertumbuhan penduduk yang tinggi menyebabkan kekurangan kawasan tanah yang boleh digunakan untuk tujuan pembinaan. Untuk menyelesaikan masalah ini, kaedah-kaedah pembaikan tanah banyak digunakan. Kaedah-kaedah pembaikan tanah boleh meningkatkan kekuatan dan kestabilan tanah. Salah satu kaedah ialah MICP, ia menggunakan process biologi untuk menghasilkan kalsit dalam tanah. Kalsit boleh meningkatkan ciri-ciri kejuruteraan tanah seperti kekuatan, kebolehtelapan, dan pencecairan. Keberkesanan MICP akan dijejaskan oleh beberapa factor, seperti jenis pasir, masa tindak balas, kepekatan bahan tindak balas, dan kepekatan bakteria. Dalam kajian ini, kepekatan perekatan reagen dan tempoh rawatan akan dikaji dengan menggunakan 2 jenis kaedah memasukkan perekatan reagen (tekan dan sembur). Kepekatan bakteria yang digunakan ialah 1.6×10^6 cfu/ml dan suhu atmosfera ialah 30°C . Optimum kepekatan perekatan reagen didapati adalah 0.25 M dengan menyemburkan perekatan reagen dalam tempoh rawatan 24 jam (kekuatan tanah meningkat 215.79 %, daripada 19 kPa ke 41 kPa). Kaedah memasukkan perekatan reagen ini Dengan menggunakan tekanan, optimum kepekatan perekatan reagen adalah 0.25 M dalam tempoh rawatan 48 jam (kekuatan tanah meningkat 184.21 %, daripada 19 kPa to 35 kPa). Oleh demikian, dengan kepekatan perekatan reagen 0.25 M, perekatan reagen disemburkan dalam tanah memberikan kesan yang lebih baik.

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LIST OF SYMBOLS

ρ_b	-	Bulk density
ρ_d	-	Dry density
w_0	-	Original moisture content of air-dried soil
M	-	Mass of soil specimen
V	-	Volume of soil specimen
W_{bac}	-	Volume of bacteria
W_{opt}	-	Optimum moisture content

CHAPTER 1

INTRODUCTION

1.1 Background of Study

With the unexpected rate of population growth, construction field is in its aggressive state. More and more residential buildings have to be constructed and the same goes to the facilities required. The more construction works undergo, the more lands are being occupied. The lands with competent soil which fulfil the construction purposes keep on decreased. Mitigations are needed to prevent the problem of lack of construction sites. As the competent soil availability decreased, soil improvement methods are used to improve the soil in term of strength and stability so that the soil can meet the requirement of construction purposes.

One of the methods known as Microbial-Induced Calcite Precipitation (MICP), which emerged to enhance the soil strength. MICP is an economical and environmental-friendly method which utilizes the biological process to produce calcite in soil. The calcite precipitation can induce the biocementation and bioclogging process in soil. These processes will improve the properties of soil in term of permeability, strength, and liquefaction resistance. The efficiency of MICP might be influenced by several factors such as type of soil, reaction time, reactant concentration, and bacteria concentration (Velpuri, 2015).

1.2 Problem Statement

In Malaysia, the population of 30 million is growing at the rate of 1.5% annually (Index Mundi, 2014; Central Intelligence Agency, 2015). The growth of population increased the infrastructure demands. With the increasing of infrastructure demand, more and more land with competent soil being occupied. The continuing growth of population may cause lack of land with suitable soil for construction. Hence, the less competent soil must be treated and improved.

In order to improve the soil, many methods can be used such as chemical grouting, stone column, and wet mixing method (“Geotechnical Design Manual,” 2013). However, not all the ground improvement techniques are sustainable. Cement is the material that pervasively used in ground improvement. In the process of producing cement, large amount of carbon dioxide will be released which can give an adverse effect to the environment such as greenhouse effect. Other than using cement, one of the common technique to improve the ground is chemical grouting. This technique may toxic to the groundwater and underground environment (“Geotechnical Design Manual,” 2013). In term of economic aspect, chemical grouting is generally expensive and not economical.

MICP, a low cost and sustainable method is a new technology using to enhance the soil strength. One of the factors that affect to the efficiency of MICP is concentration of cementation reagents. However, the effect of cementation reagents concentration to soil strength improvement have not been investigated yet.

1.3 Research Objectives

This research is to assess the influence of various concentrations of cementation reagents on the calcite precipitation in biomediated soil improvement. The following shows the sub objectives of this research:

- i) To determine the engineering properties of the residual soil to be used in the study.
- ii) To evaluate the strength of the soil with urease active microorganisms under different concentrations of cementation reagents, different ways of adding cementation reagent, and different durations of adding cementation reagent.
- iii) To find out the most suitable concentration, durations and ways of adding cementation reagent for effective improvement of the residual soil under study.

1.4 Scope of Study

This study is focussed on the soil improvement method which named as Microbial-Induced Calcite Precipitation (MICP). The method being used to induce calcite precipitation is ureolysis. There are few limitations in this study:

- Only concentration of cementation reagents, duration of adding cementation reagents, way to adding cementation reagents will be focussed
- Bacteria used is *Sporosarcina pasteurii*.
- Soil used is residual soil which obtained from P18 Universiti Teknologi Malaysia.
- All the laboratory testing will only be done in Universiti Teknologi Malaysia
- pH value and temperature are constant.
- Only strength of treated residual soil will be tested.
- The soil specimens with bacteria are all under optimum moisture content condition.

1.5 Significance of Proposed Research

In this research, the optimum concentration of cementation reagent, the optimum duration of mixing cementation reagent, and the better way of adding

cementation reagent into residual soil were found. These factors may influence the effectiveness of the MICP process. According to the result, the most effective soil improvement can be achieved and may lead to the better result of strength improvement of residual soil.

1.6 Gantt Chart

Table 1.1 : Gantt chart for semester 1

Task \ Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Brainstorming and planning														
Choosing research title														
Find and study relevant journal														
Consult to lecturer														
Chapter 1														
Chapter 2														
Chapter 3														

REFERENCES

- Alhour, M. A. (2013). *Isolation, Characterization and Application of Calcite Producing Bacteria from Urea Rich Soils*. Islamic University.
- Al-Thawadi, S. (2008). *High Strength In-Situ Biocementation of Soil by Calcite Precipitating Locally Isolated Ureolytic Bacteria*. Perth, Western Australia, Murdoch University.
- Al-Thawadi, S. (2011). Ureolytic Bacteria and Calcium Carbonate Formation as A Mechanism of Strength Enhancement of Sand. *Journal of Advanced Science and Engineering Research*, vol 1, no 1, 98-114.
- Ariyanti, D., Handayani, N. B., & Hadiyanto. (2011). An Overview of Biocement Production from Microalgae. *International Journal of Science and Engineering*, vol 2, no 2, pp. 30-33.
- Baskar, S., Baskar, R., Mauclaire, L., & McKenzie, J. A. (2006). Microbially Induced Calcite Precipitation in Culture Experiments: Possible Origin for Stalactites in Sahastradhara Caves, Dehradun, India. *Current Science*, vol 90, pp. 1-7.
- Biello, D. (2008). Cement from CO₂: A Concrete Cure for Global Warming. *Scientific American*. Retrieved from <http://www.scientificamerican.com/article/cement-from-carbon-dioxide/>.
- Bharathi, N. & Meyyappan, R. M. (2014). Calcium Carbonate Producing Yeast from Soil Enhance Chemical Resistance on Cement Concrete Specimen. *International Journal of ChemTech Research*, vol 7, no 1, pp. 435-439.

- Boling, J., W. (2015). *Bioprecipitation of Calcite by Sporosarcina Pasteurii: Developing Efficient Methodologies for Microbially Indurated Rammed Earth*. University of Kansas.
- Central Intelligence Agency. (2015). *The World Factbook*. Retrieved from <https://www.cia.gov/library/publications/the-world-factbook/fields/2002.html>.
- Chiet, K. T. P., Kassim, K. A., & Siaw, Y. C. (2015). Biocementation Potential of Tropical Residual Soil Infused with Facultative Anaerobic Bacteria. *Applied Mechanics and Materials*, vol 773-774, pp. 1412-1416.
- Chaurasia, L., Verma, R. K., & Bisht, V. (2014). Microbial Carbonate Precipitation by Urease Producing Bacteria in Cementitious Materials. *International Journal of Advanced Biotechnology and Research (IJBR)*, vol 5, pp. 671-679.
- Chu, J., Ivanov, V., He, J., Naeimi, M., Li, B., & Stabnikov, V. (2011). Development of Microbial Geotechnology in Singapore, 4070–4078.
- Dejong, J. T., & Martinez, B. C. (2010). Bio-mediated soil improvement Bio-mediated soil improvement, (FEBRUARY). <http://doi.org/10.1016/j.ecoleng.2008.12.029>
- Geotechnical Design Manual. (2013) (pp. 1–112). New York. Retrieved from https://www.dot.ny.gov/divisions/engineering/technical-services/geotechnical-engineering-bureau/geotech-eng-repository/GDM_Ch-14_GroundImprovement.pdf
- Gonsalves, G. (2011). *Bioconcrete: A Sustainable Substitute for Concrete*. Polytechnic University of Catalonia (UPC).
- Hammes, F., Seka, A., Van Hege, K., Van De Wiele, T., Vanderdeelen, J., Siciliano, S. D., Verstraete, W. (2003b). Calcium Removal from Industrial Wastewater by Biocatalytic CaCO₃ precipitation. *Journal of Chemical Technology and Biotechnology*, vol 78, no 6, pp. 670-677.

- Index Mundi. (2014). *Malaysia Population*. Retrieved from <http://www.indexmundi.com/malaysia/population.html>.
- Ivanov, V., Chu, J., & Stabnikov, V. (2012). 'Iron- and Calcium-Based Biogrouts for Porous Soils', *Proceeding of The Institution of Civil Engineers*. pp. 36-41.
- Ivanov, V. & Chu, J. (2008). Applications of Microorganisms to Geotechnical Engineering for Bioclogging and Biocementation of Soil in Situ. *Reviews in Environmental Science and Biotechnology*, pp 139–153. Retrieved from <http://doi.org/10.1007/s11157-007-9126-3>
- Lee, L. M., Ng, W. S., Tan, C. K., & Hii, S. L. (2012). Bio-Mediated Soil Improvement under Various Concentrations of Cementation Reagent. Retrieved from <http://doi.org/10.4028/www.scientific.net/AMM.204-208.326>
- Montoya, B. M. (2012). *Bio-Mediated Soil Improvement and The Effect of Cementation on The Behavior, Improvement, and Performance of Sand*. University of California.
- Nekolny, D. and Chaloupka, J. (2000). Protein Catabolism in Growing *Bacillus Megaterium* during Adaptation to Salt Stress. *FEMS Microbial. Lett.* vol 184, pp. 173-177.
- Ng, W., Lee, M., & Hii, S. (2012). An Overview of the Factors Affecting Microbial-Induced Calcite Precipitation and its Potential Application in Soil Improvement. *International Journal of Civil, Environmental, Structural, Construction and Architectural Engineering*, vol 6, no. 2, pp. 188–194. Retrieved from <http://waset.org/journals/waset/v62/v62-131.pdf>
- Parks, S. L. (2009). *Kinetics Of Calcite Precipitation By Ureolytic Bacteria Under Aerobic and Anaerobic Conditions*. Monata State University.
- Pedreira, R. R. (2012). Bio-Cementation of Sandy Soils for Improving Their Hydro-Mechanical Characteristics, pp. 1–14.

- Shahrokhi-Shahraki, R., Zomorodian, S. M. A., Niazi, A., & O'Kelly, B. C. (2014). Improving Sand with Microbial-Induced Carbonate Precipitation, *Proceeding of The Institution of Civil Engineers*. pp. 1–14.
- Sharma, A. & Ramkrishnan, R. (2016). Study on Effect of Microbial Induced Calcite Precipitates on Strength of Fine Grained Soils. *Perspectives in Science*. <http://doi.org/10.1016/j.pisc.2016.03.017>
- Tang, J., Yu, Z., Ruan, Y., & Lin, X. (2016). Development of Microbially Induced Calcium Carbonate Precipitation Technology in Soil Improvement, vol 3, pp. 26–29.
- Van Langerak, E.P. A., Hamelers, H. V. M., Lettinga, G. (1997). Influent Calcium Removal by Crystallization Reusing Anaerobic Effluent Alkalinity. *Water Science and Technology*, vol 36, pp. 341-348.
- Velpuri, N. V. P. K. (2015). *Factors Influencing The Microbial Calcium Carbonate Precipitation*. The University of Texas of Arlington.