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***ELECTRO-BIOGROUTING STABILIZATION OF
KAOLIN SOIL***

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By

HAMED ABDEH KEYKHA

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Dedicated to:

*My wife, **Elham**
and
The other half of me, my beloved mother
Gohar*



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

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By

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April 2014

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Biogrouting is a new soil improvement method based on microbiologically induced precipitation of calcium carbonate. Bacteria, which are able to convert urea into ammonium and carbonate, are injected into the soil, followed by a solution containing urea and calcium chloride. *Sporosarcina pasteurii* is one of the most significant bacteria that hydrolyze urea to carbon dioxide and ammonia by releasing enzyme urease. The calcium carbonate crystals form bridges between the sand grains, which increase the strength of the soil mass.

The application of electrical potential between two electrodes in soil has two effects; electroosmosis, the movement of interstitial water toward the cathode; and electromigration, the movement of ionic species, both soluble and particulate. Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. Electrokinetic stabilization is a ground improvement method in which stabilizing agents are induced into soil under direct current. The movement of stabilizing agents into the soil masses is governed by the principles of electrokinetics, while the mechanisms of stabilization can be explained by the principles of chemical stabilization. When ions are used as stabilizing agents, the ions migrate into soils through processes of electro migration and osmotic advection. These ions improve the soil strength by three mechanisms, namely ion replacement, mineralization and precipitation of species in the pore fluid. It is precipitation that provides the greatest contribution to increases in strength.

The aim of this study was to use the combination of biogrouting and electrokinetic techniques for injection of bacteria and agents into the kaolin soil to improve soil strength.

Firstly, surface electrical properties of two bacillus type strain *Sporosarcina pasteurii* and *Sporosarcina aquimarina*, which produce the intracellular urease enzymes were investigated. *S. pasteurii* is an aerobic soil bacterium while *S. aquimarina* is anaerobic

seawater. The results of zeta potential and electrophoresis mobility (EPM) of both bacteria demonstrated negative surface charge of bacteria in different values of pH. The zeta potential of *S. pasteurii* was more negative than *S. aquimarina*. The maximum negative surface charge of *S. pasteurii* was observed in the pH range of 8.5 to 9.5. The effect of different materials (i.e. NaCl, CaCl₂, NH₄⁺ and Urea) on ζ potential of *S. pasteurii* was studied. The presence of Na⁺, Ca⁺², and urea in the culture media of the bacteria decreased the negative surface charge.

Microbially induced carbonate precipitation (MICP) of both bacteria was achieved by varying the concentration of bacterial cells, urea and calcium chloride. The results showed that the *S. pasteurii* have more activity than *S. aquimarina* to precipitate CaCO₃. The effective laboratory concentrations for MICP were the bacterial cell concentration (B2= 7×10⁸ cells) with a blend of CaCl₂ (1M) and urea (1M) for *S. aquimarina* and CaCl₂ (2M) and urea (1M) for *S. pasteurii* in this study. The greatest amount of CaCO₃ was precipitated with concentration of bacteria about 3×10⁸ cells/ml.

Secondly, the transport of bacteria in soil specimen (i.e. kaolin) was detected using electrokinetic set up. The result of transportation of *S. pasteurii* in porous media of fine soil with low permeability demonstrated that the bacteria were moved directly from the cathode to the anode with rate about 6-8 cm/h. The rate of bacteria was increased with the rising current field. Therefore, the bacteria were able to move through soil by electrokinetic technique.

Thirdly, A new soil stabilization technique namely electro-biogrouting method (EBM) was developed to improve the soft soil with low permeability. The EBM setup was designed and fabricated at the University Putra Malaysia. The EBM set up consisted of soil container, two injection chambers, pH controllers, mariote bottles, voltmeter, DC power supply.

Two methods including the bacteria injection and bacterial products injection were proposed for soil improvement. In the bacterial injection, initially the calcium ions were moved across the specimen by electromigration from the anode to the cathode. Then, the urea, which is non-ionic and solvable, was transported by the electroosmotic flow from the anode to the cathode. Finally, the bacteria with negative surface charge were induced as particles by electrophoresis mobility from the anode to the cathode. The polarity reversal was applied to distribute the bacteria and agents for more uniform precipitation of CaCO₃ across the sample. As the bacteria expose the urea, release the urease enzyme and produce carbonate ions resulting of the CaCO₃ precipitation in porous media of the soil, and consequently increasing the shear strength.

In the second method, the calcium injected into the anode chamber and transported from the anode to cathode by electromigration. Then, the mixed bacteria and urea solution were added to the cathode chamber. The bacteria released enzyme to hydrolyze urea, and the carbonate (CO₃⁻²) was produced in the cathode chamber. Then, the carbonate which has a negative charge was moved directly from the cathode to the anode into the soil. Finally, the calcium carbonate was precipitated in the soil, and consequently increasing the shear strength of the soil.

After curing for seven days, the results showed that the undrained shear strength of the soil increased from 6 kPa to 65 and 60 kPa for the first and second injection method respectively. Washing acid technique and scanning electron microscope (SEM) confirmed the presence of CaCO_3 precipitation across the soil sample. The study was important in that it confirmed that the Electro- Biogrouting Method (EBM) is a novel application method of soil stabilization with precipitation of CaCO_3 .



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
mementuhi keperluan untuk ijazah Doktor Falsafah

ELECTRO-BIOGROUTING PENSTABILAN KAOLIN TANAH

Oleh

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Turapan-bio kaedah terbaru pemulihan tanah berasaskan pendorongan secara mikrobiologi dengan titisan kalsium karbonat. Bakteria yang boleh mengurai urea kepada ammonia dan karbonat, disuntik kedalam tanah, diikuti dengan cairan yang mengandungi urea dan kalsium klorida. *Sporosarcina pasteurii* adalah salah satu bakteria paling penting yang menghidrolisiskan urea kepada karbon dioksida dan ammonia dengan membebaskan enzim urea. Kristal kalsium karbonat membentuk jambatan antara butiran pasir, yang mana meningkatkan kekuatan jisim tanah.

Aplikasi potensi elektrik antara dua elektrod dalam tanah mempunyai dua kesan; osmosis elektrik, pergerakan celahan air mengarah ke katod; dan pemindahan elektrik, pergerakan spesies berion jenis cecair dan pepejal. Potensi zeta adalah keupayaan membezakan antara medium penyerakan dan lapisan pegun cecair yang terdapat pada zarah terurai. Penstabilan elektrokinetik adalah kaedah pemulihan tanah dimana agen penstabilan didorong kedalam tanah dibawah arus terus. Pergerakan agen penstabilan ke dalam tanah dirangkumi oleh prinsip elektrokinetik, manakala mekanisme penstabilan dapat diterangkan dengan prinsip penstabilan kimia. Apabila ion digunakan sebagai agen penstabilan, ion tersebut berpindah kedalam tanah melalui proses pemindahan elektro dan air lintang osmosis. Ion-ion ini meningkatkan kekuatan tanah dengan tiga mekanisme, iaitu pengantian ion, pengalihan dan pemendakan spesies kedalam liang cecair. Pemendakan memberikan sumbangan besar bagi meningkatkan kekuatan.

Tujuan kajian ini adalah untuk menggunakan gabungan turapan bio dan teknik elektrokinetik untuk menyuntik bakteria dan agen kedalam tanah lembut berketelapan rendah bagi meningkatkan kekuatan tanah secara homogen.

Pertamanya, dua bakteria jenis terikan pada permukaan elektrik iaitu *Sporosarcina pasteurii* dan *Sporosarcina aquimarina* yang menghasilkan enzim urea intrasel dikaji. *S.pasteurii* adalah bakteria tanah aerobik manakala *S.aquimarina* adalah air laut anaerobik. Keputusan potensi zeta dan mobiliti elektroforesis (EPM) pada kedua-dua

bakteria menunjukkan caj negatif permukaan bakteria pada nilai pH yang berbeza-beza. Potensi zeta *S.pasteurii* lebih negatif daripada *S.aquimarina*. Caj negatif maksimum pada permukaan *S.pasteurii* didapati mempunyai nilai pH dalam lingkungan antara 8.5 kepada 9.5. Kesan dari pelbagai bahan (contoh: NaCl, CaCl₂, NH₄⁺ dan Urea) keatas potensi zeta *S. pasteurii* dikaji. Kehadiran Na⁺, Ca⁺², dan urea dalam media kultur bakteria menurunkan caj negatif permukaan.

Pemendakan mikrob karbonat (MICP) pada kedua-dua bakteria diperoleh dengan cara mempelbagaikan kepekatan sel bakteria, urea dan kalsium klorida. Keputusan eksperimen menunjukkan *S. pasteurii* mempunyai lebih banyak aktiviti berbanding dengan *S.aquimarina* untuk menyerap CaCO₃. Bagi kedua-dua bakteria, larutan urea kisar (1M) dan CaCl₂ (1M) dengan kadar 1:1 adalah sukatan optimum untuk MICP. Sejumlah besar CaCO₃ dimendakkan bersama bakteria dengan kepekatan sebanyak 3×10⁸ sel/ml.

Keduanya, pemindahan bakteria dalam specimen tanah (contoh: kaolinite) di kesan menggunakan elektrokinetik. Keputusan pemindahan *S. pasteurii* dalam liang tanah lembut berketelapan rendah menunjukkan bakteria bergerak terus dari katod ke anod dengan kadar 6-8 cm/h. Kadar bakteria meningkat dengan kenaikan arus. Ini membolehkan bakteria bergerak melalui tanah dengan teknik elektrokinetik.

Ketiganya, elektro-turapan bio (EBM) adalah teknik penstabilan tanah yang baru, electro-turapan bio yang dihasilkan untuk menambahbaik tanah lembut dengan pengaliran hidraulik rendah. EBM direka dan dicipta di University Putra Malaysia. EBM merangkumi bekas tanah, dua kebuk suntikan, pengawal pH, botol mariote, voltmeter dan bekalan tenaga elektrik DC.

Dua kaedah termasuk suntikan bakteria dan suntikan produk bakteria dikemukakan untuk penambahbaikkan tanah. Dalam suntikan bakteria, pada awalnya ion kalsium bergerak melalui specimen dengan pemindahan elektro dari anod ke katod. Kemudian, urea yang mana bukan ionik dan boleh larut, dipindahkan melalui aliran elektrosmosis dari anod ke katod. Akhirnya, bakteria dengan caj permukaan negatif disuntik sebagai partikel oleh pergerakan elektroforesis dari anod ke katod. Keterbalikkan kutub diaplikasikan untuk memperoleh taburan agen yang homogen dalam pemprosesan. Apabila bakteria terdedah kepada urea, ia membebaskan enzim urea dan menghasilkan ion karbon yang menyebabkan pemendakan CaCO₃ dalam liang media tanah dan sekali gus meningkatkan kekuatan ricih.

Dalam keadah kedua, kalsium disuntik kedalam kebuk anod dan dipindahkan dari anod ke katod dengan pemindahan electro. Kemudian, campuran bakteria dan larutan urea dicampurkan kedalam kebuk katod. Dengan kadar segera, bakteria membebaskan enzim kepada urea hidrolis, dan bicarbonate (CO₃⁻²) yang terhasil di kebuk katod. Karbonat yang mempunyai caj negatif bergerak terus dari katod ke anod dalam tanah. Akhir sekali, kalsium karbonat dimendakan kedalam tanah dan seterusnya ia meningkatkan kekuatan ricih tanah. Teknik pembasuhan asid dan pengimbasan elektron mikroskop (SEM) mengesahkan kehadiran pemendakan CaCO₃ sepanjang sampel tanah. Kajian ini adalah penting kerana mengesahkan kaedah Elektro-Turapan bio (EBM) adalah aplikasi novel dalam penstabilan tanah dengan pemendakan CaCO₃.

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APPROVAL

I certify that a Thesis Examination Committee has met on 25th April 2014 to conduct the final examination of Hamed Abdeh Keykha on his thesis entitled “Electro-biogrouting stabilization of kaolin soil” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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