

**AERATED STATIC PILE (ASP)- ENHANCED
BIOAUGMENTATION OF OIL SLUDGE
CONTAMINATED SOIL BY LOCALLY ISOLATED
BENEFICIAL MICROORGANISM (LIBeM) USING
DIFFERENT DELIVERY TECHNIQUES**



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**FACULTY OF SCIENCE & NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
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**THESIS SUBMITTED IN FULFILLMENT FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY**

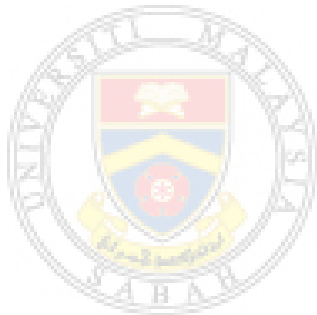
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DECLARATION

I hereby declare that the material in this thesis is of my own effort except for the quotations, excerpts, equations, references and summaries which have been duly acknowledged and cited clearly it's sources.

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CERTIFICATION

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SOIL BY LOCALLY ISOLATED
BENEFICIALMICROORGANISM (LIBeM) USING
DIFFERENT DELIVERY TECHNIQUES**
DEGREE : **DOCTOR OF PHILOSOPHY (ENVIRONMENTAL
SCIENCE)**
VIVA DATE : **13TH AUGUST 2018**

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ACKNOWLEDGEMENT

First of foremost, I praise Allah S.W.T. for his blessing that enabled me to complete my doctoral dissertation successfully. Thank you Allah.

I would like to express my gratitude towards my supervisor, Assoc. Prof. Dr. Piakong Mohd Tuah for his constant dedication and guidance throughout my research. This feat was possible only because of the unconditional support provided by him. He is a person with an amicable and positive disposition. I consider it is a great opportunity to do my doctoral programme under his guidance and to learn from his research expertise. Thank you so much Dr for your help and support.

I feel indebted with the scholarship given from Ministry of Higher Education (MOHE) Malaysia (MyPhD), UMSGreat (GUG0035-SG-P-1/2016) and also, the financial support from Mr. Kok Fook Seng (ZNK Consult Sdn. Bhd, Selangor) is greatly acknowledged. I also wish to acknowledge Mr. Fitzgerald Spencer James and Labuan Shipyard Engineering for giving me opportunity to treat their oil sludge under SW311.

Besides that, I want to thank my faculty dean, examiners, staffs and lecturers who have supported me all the time. Some faculty members has very kind enough to extend their help at various phase of this research, whenever I approached them, I do hereby acknowledge all of them especially my supportive lab assistants who had always assist me in handling my lab work (En. Timani B. Kumin, Pn. Nur Hidayah Thaddeus Abdullah, Pn. Azimah Jaafar, En. Shahrizal Mohd, En. Azmidy AG. Tengah, En. Syaufie Lamjin and En. Neldin Jeoffery) from Faculty of Science & Natural Resources & Center for Reseach & Innovation, UMS.

Not to forget, my colleagues who never failed to cheer me throughout the years I have spent in Universiti Malaysia Sabah to complete my thesis by research and also I wish to extend my appreciation my fellow researchers: Victor James, Erma Hani Baharudzaman, Laurencia Debbie Bernard, Fera Cleopas, Muhammad Haniff Mohd Taba, Christopher George, Nor Zilah Jailani, Rukiah Chan and Aslinah

Mohd Japirin who had given me a great support and momentum during hard times throughout my study.

My special thank you to my beloved father, Encik Zahari Yaacob and mother, Puan Zainab Termizi and my siblings, Zarizal, Nur Zaila, Nur Zaima and Mohd Zaidi for their love, understanding, perseverance and constant prayers. My family is my inspiration to not just to finish my Ph.D but also to complete it with great success, without them my journey might be next to impossible.

Nur Zaida Zahari

18th Aug 2018

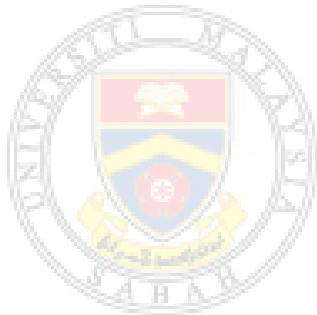


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ABSTRACT

Oil sludge has been classified by the United State Environmental Protection Agency (USEPA) as a hazardous organic complex. The use of selective oil degrading strains in remediating oil sludge has become a promising technique that could generate economy and it is a green technology to clean the environment. The main aim of this study is to determine and compare the ability locally isolated beneficial microorganism (LIBeM) in single and consortia for bioaugmentation of oil sludge contaminated soil at various concentration levels. A laboratory scale experiment was conducted using Aerated Static Pile (ASP)-bioreactor system made up of acrylic materials as a treatment plot. The ASP unit system has two main components with three silicones tubing parallel for aeration connected to air pump (Model RESUN LP100 Low Noise Air Pump) to supply oxygen continuously to the soil treatment zone. Eleven sets of ASP-bioreactor system containing 10 kg of soil mixed with 5%, 10%, 15% and 20% (v/v) of oil sludge were prepared as (LIBeM-LIQ) soil treated with single strain; S1= *C. tropicalis* RETL-Cr1, S2 = *C. violaceum* MAB-Cr1, S3 = *P. aeruginosa* BAS-Cr1, S4 = *S. paucimobilis* ReTOS-Cr1, S5 = *S. maltophilia* RAS-Cr1 and Consortia (LIBeM-LIQ); soil treated with consortia ; C1 = ReTOS-Cr1+ BAS-Cr + RAS-Cr1, C2 = RETL-Cr1+ MAB-Cr1+RAS-Cr1, C3= RETL-Cr1+ RAS-Cr1+BAS-Cr1, C4= RETL-Cr1+ MAB-Cr1+BAS-Cr1, C5 = ReTOS-Cr1+ BAS-Cr + RAS-Cr1+ RETL-Cr1+MAB-Cr1 and NA = natural attenuation as control plot. The experiments were incubated for 84 days and the TPH reduction was observed along with physiochemical parameters such as pH, soil moisture content, temperature and microbial population (CFU/mL). The results showed that among the single (LIBeM-LIQ), treatment S3 inoculation with *P. aeruginosa* BAS-Cr1 at 20% (v/v) concentration of oil sludge recorded the highest TPH degradation with 89.3%. Meanwhile for microbial consortia, treatment C4 inoculation with *C. tropicalis*-RETL-Cr1+ *C. violaceum*-MAB-Cr1 + *P. aeruginosa*-BAS-Cr1 showed the best performance TPH degradation with 94% within 84 days incubation periods. On the contrary, natural attenuation does not show significance TPH reduction with only 27% efficiency. The optimum condition of TPH degradation in soil was observed at temperature of 30⁰C, with soil pH at 7.0 and water holding capacity with 20%. The microbial population of 1x10⁷ – 1x10⁸ CFU/mL was observed along the treatment respectively. The biodegradation of oil sludge were improved with the new invention for best selected consortia LIBeM (C4= *C. tropicalis*-RETL-Cr1+ *C. violaceum*-MAB-Cr1 + *P. aeruginosa*-BAS-Cr1) with microbial formulation in powder form (LIBeM-POW) which is more stability and capsule form (LIBeM-CAP) which is more convenient method for long term preservation purpose. Bioaugmentation of LIBeM-POW at 20% v/v oil sludge show great improvement in TPH degradation with 92% within the short period recorded (56 days) as compared to LIBeM-CAP with 86%. This finding justified that LIBeM-POW was proven to be the most efficient delivery technique as compared to LIBeM-LIQ and LIBeM-CAP. The kinetic evaluation on biodegradation rate fitted the first order kinetics and proved that treatment augmented with consortia 4 at 10% (v/v) oil sludge represent high biodegradation rate with 0.0375 day⁻¹ and shortest half-life times of 18.5 days. This finding is 8.5-

fold higher and 76 days differences as compared to natural attenuation. The biodegradation kinetic confirmed that LIBeM-POW has higher biodegradation rate constant with 0.0435 day^{-1} and shortest biodegradation half-life times with 15.9 days which indicates 1.2-fold higher and 4 days differences as compared to LIBeM-CAP. The SEM image also confirmed the attachment of augmented cells in the soil and the images were observed overlap since the concentrated cells in powder form have been distributed surrounded the entire of soil surface. The excellent control of bioprocess parameters along with Technical Procedures Protocol (TPP) gives major advantages of bioreactor based bioremediation which effectively produce high percentage reduction of oil sludge in the soil. This study has resulting a novel invention of ASP- bioreactor system enhanced bioaugmentation of oil sludge contaminated soil by locally isolated beneficial microorganisms using different delivery techniques. Thus it has a great potential as an environmental friendly, cost effectiveness and sustainable treatment of oil sludge or other related organic compounds produced by oil and gas industries.



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ABSTRAK

AERATIK STATIK PILE (ASP) - MENINGKATKAN BIOAUGMENTASI PENCEMARAN TANAH DARI ENAPCEMAR MINYAK MENGGUNAKAN KELEBIHAN MIKROORGANISMA ISOLASI TEMPATAN (LIBeM) DENGAN TEKNIK PENGHANTARAN YANG BERBEZA

Enapcemar minyak telah dikelaskan oleh Agensi Perlindungan Alam Sekitar Amerika Syarikat (USEPA) sebagai kompleks organik berbahaya. Penggunaan strain minyak degradasi terpilih dalam merawat enapcemar minyak telah menjadi teknik yang menjanjikan ekonomi dan merupakan teknologi hijau untuk membersihkan alam sekitar. Tujuan utama kajian ini adalah untuk menentukan dan membandingkan kebolehan strain tunggal dan konsortia mikroorganisma berfaedah tempatan yang terisolasi (LIBeM) dalam bioaugmentasi tanah tercemar enapcemar minyak pada pelbagai tahap kepekatan. Percubaan skala makmal telah dijalankan menggunakan sistem Aeratik Statik Pile (ASP) -bioreaktor yang terdiri daripada bahan akrilik sebagai plot rawatan. Sistem unit ASP mempunyai dua komponen utama dengan tiga tiub silikon selari untuk pengudaraan dan disambungkan ke pam udara (Model RESUN LP100 Low Noise Air Pump) untuk membekalkan oksigen secara berterusan ke tanah. Sebelas set sistem bioreaktor ASP yang mengandungi 10 kg tanah dengan campuran 5%, 10%, 15% dan 20% (v / v) enapcemar minyak telah disediakan sebagai tanah Rawatan Tunggal (LIBeM-LIQ) yang dirawat dengan; S1 = *C. tropicalis* RETL-Cr1, S2 = *C. violaceum* MAB-Cr1, S3 = *P. aeruginosa* BAS-Cr1, S4 = *S. paucimobilis* ReTOS-Cr1, S5 = *S. maltophilia* RAS-Cr1 dan konsortia (LIBeM); tanah yang dirawat dengan; C1 = ReTOS-Cr1 + BAS-Cr + RAS-Cr1, C2 = RETL-Cr1 + MAB-Cr1 + RAS-Cr1, C3 = RETL-Cr1 + RAS-Cr1 + Cr1, C5 = ReTOS-Cr1 + BAS-Cr + RAS-Cr1 + RETL-Cr1 + MAB-Cr1 dan NA = penyingkiran semulajadi sebagai plot kawalan. Eksperimen diinkubasi selama 84 hari dan pengurangan TPH diperhatikan bersama-sama dengan parameter fisiokimia seperti pH, kandungan lembapan tanah, suhu dan populasi mikrob (CFU/mL). Hasilnya menunjukkan bahawa dalam kalangan tunggal (LIBeM-LIQ), inokulasi S3 rawatan dengan *P. aeruginosa* BAS-Cr1 pada kepekatan 20% (v/v) enapcemar minyak mencatatkan kemerosotan TPH tertinggi dengan 89.3%. Sementara itu untuk konsortia mikrob, inokulasi C4 rawatan dengan *C. tropicalis*-RETL-Cr1+ *C. violaceum*-MAB-Cr1 + *P. aeruginosa*-BAS-Cr1 menunjukkan kemerosotan prestasi TPH yang terbaik dengan 94%. Sebaliknya, penyingkiran semulajadi tidak menunjukkan pengurangan TPH yang signifikan dengan hanya 27%. Keadaan optimum TPH degradasi dalam tanah diperhatikan pada 30°C, dengan pH tanah pada 7.0 dan kapasiti pegangan air dengan 20%. Populasi mikrob 1×10^7 - 1×10^8 CFU/mL diperhatikan sepanjang rawatan dijalankan. Biodegradasi enapcemar minyak telah diperbaiki dengan konsortia terpilih (C4 = *C. tropicalis*-RETL-Cr1+ *C. violaceum*-MAB-Cr1 + *P. aeruginosa*-BAS-Cr1) dengan formulasi mikrob LIBeM dalam bentuk serbuk (LIBeM-POW) yang lebih stabil dan

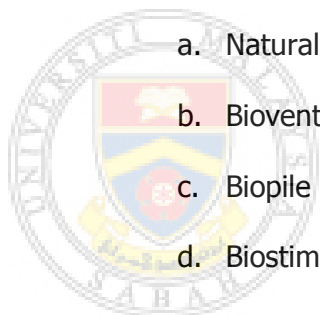
bentuk kapsul (LIBeM-CAP) yang merupakan kaedah yang lebih mudah untuk tujuan pemeliharaan jangka panjang. Bioaugmentasi LIBeM-POW pada enapcemar minyak 20% v/v menunjukkan peningkatan yang besar dengan kemerosotan TPH sebanyak 92% pada tempoh singkat direkodkan iaitu (56 hari) berbanding LIBeM-CAP dengan penurunan TPH 86%. Penemuan ini membuktikan bahawa LIBeM-POW terbukti menjadi teknik rawatan yang paling berkesan berbanding dengan LIBeM-LIQ dan LIBeM-CAP. Penilaian kinetik degradasi memenuhi kinetik urutan pertama dan membuktikan rawatan dengan konsortia 4 (*C. tropicalis*-RETL-Cr1+ *C. violaceum*-MAB-Cr1 + *P. aeruginosa*-BAS-Cr1) pada enapcemar 10% (v/v) mewakili kadar biodegradasi tertinggi dengan 0.0375 day^{-1} dengan masa separuh hayat yang singkat 18.5 hari. Penemuan ini adalah 8.5 kali ganda dan 76 hari berbeza dengan rawatan penyingkiran semulajadi. Kinetik biodegradasi mengesahkan bahawa LIBeM-POW mempunyai kadar biodegradasi yang tertinggi dengan 0.0435 day^{-1} dan masa separuh hayat biodegradasi yang rendah dengan 15.9 hari. Ini menunjukkan perbezaan sebanyak 1.2 kali ganda dan 4 hari berbeza berbanding LIBeM-CAP. Imej SEM juga mengesahkan lampiran sel tambahan di dalam tanah dan imej tersebut diperhatikan dalam keadaan bertindih kerana sel-sel pekat dalam bentuk serbuk telah menyelubungi seluruh permukaan tanah. Kawalan yang sangat baik terhadap parameter bioproses bersama dengan Teknikal Prosedur Protokol (TPP) memberikan kelebihan utama bioremediasi berasaskan bioreaktor yang berkesan bagi menghasilkan pengurangan TPH yang tinggi bagi enapcemar minyak di dalam tanah. Kajian ini menghasilkan sistem Aeratik Statik Pile (ASP) –bioreaktor yang meningkatkan bioaugmentasi tanah tercemar enapcemar minyak oleh mikroorganisma berfaedah tempatan yang terisolasi menggunakan teknik penyampaian yang berbeza. Oleh itu ia mempunyai potensi besar sebagai rawatan yang mesra alam, kos efektif dan mampan untuk enapcemar minyak atau sebatian organik lain yang dihasilkan oleh industri minyak dan gas.

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