BIODEGRADATION OF CRUDE OIL AND OIL SPILL PLUME MODELING OF LOCALLY ISOLATED BENEFICIAL MICROORGANISMS

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries and references, which have been duly acknowledged.

18th September 2017

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Laurencia Debbie Benard

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CERTIFICATION

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Assoc. Prof. Dr. Piakong Mohd Tuah

Signature

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ABSTRACT

A biodegradation study was performed to assess the biodegradation of crude oil by five locally isolated beneficial microorganisms (LIBeM) which were *Candida tropicalis* RETL-Cr1, *Pseudomonas aeruginosa* BAS-Cr1, *Chromobacterium valaceum* MAB-Cr1, *Stenotrophomonas maltophilia* RAS-Cr1 and *Aeromonas hydrophila* RETL-Cr3, as single and consortia cultures. Sabah Light Crude Oil was used as sole carbon source for all sets of experiments. The study was carried out using shake-flask culture at optimum temperature (30°C to 37°C), agitated at 200rpm. Microbial growth profile was monitored by measuring the optical density ($O.D._{600}$) on interval time. Biodegradation rate and efficiency were quantified by comparing the initial and final crude oil concentration, whereas the degradation of selected aliphatic hydrocarbons was quantified by comparing the initial and final area in chromatogram. The biodegradation ratios were monitored using Gas Chromatography-Mass Spectrometry (GC-MS). Present finding showed that all single and consortia culture able to grow in 5%, 10% and 15% (v/v) crude oil. The growth of all single species and consortia culture in crude oil proceed even after 70 days of the study. In 5% (v/v) crude oil, consortia culture has the highest biodegradation efficiency with overall biodegradation efficiency is 96.11% and the overall biodegradation rate after 70 days is 7.84 g/L/d. In 10% (v/v) crude oil, consortia culture has the highest overall biodegradation efficiency and rate which are 96.04% and 9.55 g/L/d respectively. In 15% (v/v), consortia culture also has the highest overall biodegradation efficiency of 94.02% and overall biodegradation rate of 10.75 g/L/d. The reduction of hydrocarbons by all single species and consortia culture are varies. In 5% (v/v) crude oil, single culture *C. tropicalis* RETL-Cr1 showed almost 100% degradation of n-alkane (heptadecane). In 10% (v/v) crude oil, consortia culture degraded 98% of n-alkane (eicosane). Meanwhile, on 15% (v/v) crude oil, consortia culture degraded 96% n-alkane (heptadecane) after 70 treatment days. Ultimately, the biodegradation ratio shows that biodegradation of crude oil had taken place during the specified biodegradation study. It can be concluded that the potency of microbe as hydrocarbon degrader in descending order is as follows: Consortia (all five species) > *C. tropicalis* RETL-Cr1 > *A. hydrophila* RETL-Cr3 > *C. valaceum* MAB-Cr1 > *S. maltophilia* RAS-Cr1 > *P.*

aeruginosa BAS-Cr1. Two spill simulations incorporating biodegradation rate constant of consortia culture were run using MIKE 21/3 OS model. The spills flow to North East and South West direction due to different metocean conditions. The heavy oil was visually affected by the biodegradation compared to semi-volatile and volatile oil with the arrangement $k_1 > k_2 > k_3 > k_{ANBR}$ for all simulations. Therefore, the hypothetical spills represent significant effect of biodegradation to total oil mass after 14 days.

ABSTRAK

BIODEGRADASI MINYAK MENTAH DAN PERMODELAN KEPULAN TUMPAHAN MINYAK OLEH MIKROORGANISMA PENCILAN TEMPATAN BERMANFAAT

Kajian bioaugmentasi terhadap penguraian minyak mentah dan permodelan kepulan tumpahan minyak oleh lima mikroorganisma pencilan tempatan bermanfaat yang berpotensi (LIBeM) telah dilaksanakan oleh Candida tropicalis RETL-Cr1, Pseudomonas aeruginosa BAS-Cr1, Chromobacterium valaceum MAB-Cr1, Stenotrophomonas maltophilia RAS-Cr1 dan Aeromonas hydrophila RETL-Cr3, secara kultur tunggal dan campuran. Minyak mentah ringan (yang ditemui dalam perairan Malaysia) telah digunakan sebagai sumber utama karbon dalam semua eksperimen. Kajian ini telah dijalankan dengan menggunakan kaedah penggoncangan kelalang pada suhu yang optimum (30°C hingga 37°C), agitasi 200rpm. Profil atau keluk pertumbuhan mikrob dipantau dengan menukur rapat optic (OD_{600}) selang kadar masa tertentu. Kadar dan kecekapan biodegradasi dinilai melalui perbandingan kepekatan minyak mentah pada awal dan akhir degradasi, manakala penguraian hidrokarbon alifatik yang terpilih dinilai melalui perbandingan kawasan kromatogram pada awal dan akhir degradasi. Nisbah biodegradasi ditentukan dengan menggunakan kromatografi gas-spektrometri jisim (GC-MS). Kajian ini menunjukkan bahawa semua spesis dalam kultur tunggal dan campuran dapat berkembang dengan baik dalam semua kepekatan minyak mentah (5%, 10% dan 15% (j/j) dan pertumbuhan masih berterusan walaupun selepas 70 hari kajian. Dalam minyak mentah 5% (j/j), kultur campuran menunjukkan kecekapan dan kadar biodegradasi tertinggi iaitu 96.11% manakala kadar biodegradasi keseluruhan selepas 70 hari ialah 7.84 g/L/h. Dalam minyak mentah berkepekatan 10% (j/j), kultur campuran mempunyai kecekapan dan kadar biodegradasi keseluruhan tertinggi iaitu 96.04% dan 9.55 g/L/h. Dalam 15% (j/j) kepekatan minyak mentah, sekali lagi kultur campuran menunjukkan kecekapan biodegradasi tertinggi iaitu 94.02% dan kadar biodegradasi keseluruhan iaitu 10.75 g/L/h. Pengurangan hidrokarbon terpilih oleh semua kultur tunggal dan campuran adalah berbeza.

Dalam minyak mentah kepekatan 5% (j/j), kultur tunggal C. tropicalis RETL-Cr1 berupaya mengurai hampir 100% n-alkana (heptadecane) dalam minyak mentah. Dalam minyak mentah kepekatan 10% (j/j), kultur campuran berupaya mengurai 98% n-alkana (eicosane). Manakala, dalam kepekatan minyak mentah (15% j/j), sekali lagi kultur campuran menunjukkan penguraian 96% n-alkana (heptadecane) selepas 70 hari. Selain itu, nisbah biodegradasi menunjukkan biodegradasi telah berlaku sepanjang tempoh degradasi. Ini dapat disimpulkan bahawa semua kultur tunggal dan campuran mempunyai kecekapan untuk dipilih sebagai pengurai hidrokarbon dalam urutan menurun ialah seperti berikut: Kultur campuran (kesemua lima spesis tunggal) > C. tropicalis RETL-Cr1 > A. hydrophila RETL-Cr3 > C. valaceum MAB-Cr1 > S. maltophilia RAS-Cr1 > P. aeruginosa BAS-Cr1. Dua simulasi tumpahan yang menggabungkan kadar tetap biodegradasi kultur campuran telah dijalankan menggunakan model MIKE 21/OS. Aliran tumpahan menuju ke arah Timur Laut dan Barat Daya disebabkan oleh keadaan laut yang berbeza. Secara visual, minyak berat kelihatan terjejas oleh biodegradasi berbanding dengan minyak separuh berubah-ubah dan minyak tidak menentu dengan susunan $k_1 > k_2 > k_3 > k_{ANBR}$ *bagi semua simulasi. Oleh itu, tumpahan hipotesis menunjukkan kesan penting biodegradasi kepada jumlah jisim minyak selepas 14 hari.*

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LIST OF ABBREVIATIONS

LIBeM	-	Locally Isolated Beneficial Microorganisms
EQA	-	Environmental Quality Act
ATSDR	-	Agency of Toxic Substances and Disease Registrar
SOP	-	Standard of Procedures
U.S. EPA	-	United States Environmental Protection Agency
GC-MS	-	Gas Chromatography-Mass Spectrometry
PAHs	-	Polycyclic aromatic hydrocarbons
API	-	American Petroleum Institute
NSW	-	Natural Seawater
NA	-	Nutrient agar
RB	-	Ramsay broth
RA	-	Ramsay agar
OS	-	Oil Spill
ANBR	-	Anticipated Natural Biodegradation Rate
NE	-	North East
SW	-	South West

LIST OF SYMBOLS

%	-	Percentage
μm	-	Micrometer
°	-	Degree
°C	-	Degree Celsius
>	-	Larger than
>	-	Smaller than
±	-	Plus-minus
μL	-	Microliter
g	-	Gram
g/L	-	Gram per liter
g/L/d	-	Gram per liter per day
L	-	Liter
mg/L	-	Milligram per liter
mL	-	Milliliter
mM	-	Millimolar
nm	-	Nanometer
OD	-	Optical Density
$O.D._{600}$	-	Optical density at wavelength of 600 nm
v/v	-	Volume per volume
% per day	-	Percentage per day
-ve	-	Negative
+ve	-	Positive
(*g)	-	Gas production
k	-	Constant
SD	-	Standard Deviation
Kg	-	Kilogram
day^{-1}	-	per day

LIST OF FORMULAS