

ISOLATION AND IDENTIFICATION OF POLYMER
SOLUBLE POLYMER GROUNDWATER CONTAMINATED WITH
COARSE BEDROCK

THE UNIVERSITY OF MALAYA

INSTITUTE OF CHEMISTRY
UNIVERSITY OF MALAYA

2006

C/n: 4576



LP 6 FST 3 2006



1100046009

Isolation and identification of free-living amoebae from groundwater contaminated with crude petroleum / Che Ku Dahlan Che Ku Daud.

PERPUSTAKAAN

KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA
21030 KUALA TERENGGANU

1100046009		

Lihat sebelah

HAK MILIK
PERPUSTAKAAN KUSTEM

ISOLATION AND IDENTIFICATION OF FREE-LIVING AMOEBAE FROM
GROUNDWATER CONTAMINATED WITH CRUDE PETROLEUM

CHE KU DAHLAN BIN CHE KU DAUD

FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2006

1100046009

ISOLATION AND IDENTIFICATION OF FREE-LIVING AMOEBAE FROM
GROUNDWATER CONTAMINATED WITH CRUDE PETROLEUM

By

Che Ku Dahlan Bin Che Ku Daud

Research Report submitted in partial fulfillment of
the requirement for the degree of
Bachelor of Science (Biological Sciences)

Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2006

This project should be cited as:

Che Ku Dahlan, C.K.D. 2006. Isolation and identification of free-living amoebae from groundwater contaminated with crude petroleum. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 68p

No part of this project report may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor of the project.



**JABATAN SAINS BIOLOGI
FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: ISOLATION AND IDENTIFICATION OF FREE-LIVING AMOEBAE FROM GROUNDWATER CONTAMINATED WITH CRUDE PETROLEUM, oleh Che Ku Dahlan Bin Che Ku Daud, no. matrik: UK 8436 telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: **PROF. MADYA DR. NAKISAH BT. MAT AMIN**
Pensyarah

Cop Rasmi: **Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu**

Tarikh: 21/5/06

Ketua Jabatan Sains Biologi

Nama: **PROF. MADYA DR. NAKISAH BT. MAT AMIN**
Ketua

Cop Rasmi: **Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.**

Tarikh: 21/5/06

ACKNOWLEDGEMENTS

BISMILLAH RAHMANI RAHIM,

In the name of Allah, Most Gracious, Most Merciful.

I would like to especially thank to my respective supervisor, Assoc. Prof Dr. Nakisah Mat Amin for her help, guidance, encouragement and patient, without whom, I would not be able to though my final year project successfully. Above all, thanks you so much for being so generous with ideas. To Mr. Munawir, who give a lot of help during sampling activities in sampling areas, thank you very much.

Special thanks to all staffs of Faculty Science and Technology, KUSTEM especially to Cik Azlina, Kak Che Ku Naiza, Kak Fatimah from Biochemistry Laboratory; Kak Ina and Kak Tie from Microbiology Laboratory; Puan Kartini from Biodiversity Laboratory, to all the staffs of Institute Oceanography KUSTEM (INOS), especially to Kak Suhaila, Kak Ati, Encik Nasir and Kak Ita from Electron Microscopy Preparation Laboratory and Assoc. Prof. Dr. Norhayati Mohd Tahir from Department of Chemical Science, I am truly grateful for the kindness and guidance from which I was able to complete my project work successfully.

Not forgetting, I gratefully acknowledge the following people for their times, advices and generous contribution for making this piece of work possible, students master in Biotechnology 3 Laboratory who shared working with me at INOS especially Kak Siti Faezah, Kak Fatimah, Kak Ida and Kak Eiffa; Kak Nori, Abang Faiz, Jee and Lina from Department of Chemical Sciences, KUSTEM. Last but not least to my fellow

group of final year project, Norfazliza Ishak (Gja). All above, thanks a zillion for sharing the work load of keeping the lab tidy with me.

To all my course mates or house mates Mior, Izani, Yoe, Firdaus, Elmi, JayB, Joe, Shafie, Mullem, Rasul and Zairul who always giving me support and encouragement. Your advices are always in my mind.

Special grateful also to my beloved mom, Puan Lijah Binti Ab. Rahman, my brother, Abang Man and his wife, Kak Tini; my aunties, my uncles and all my siblings who always giving me support and advices without felling, all the loved and support given each time have strengthened up my soul gave me confident to accomplish this study.

Lastly to all my course mates and persons, who did not mention here, thanks for helping and being supportive to me. May Allah bless all of you, thanks you.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF ABBREVIATIONS	ix
LIST OF APPENDICES	xi
ABSTRACT	xii
ABSTRAK	xiii
CHAPTER 1 INTRODUCTION	1
1.1 Introduction	1
1.2 Important of Study	6
1.3 Objectives of Study	6
CHAPTER 2 LITERATURE REVIEW	7
2.1 Classification of Amoebae	7
2.1.1 The ultra structure of amoeba cells.	12
2.1.2 Reproduction and life cycle	14
2.2 Crude Petroleum (oil)	15
2.3 Bioremediation (Microbial Degradation)	17
2.3.1 Microbial degradation of petroleum hydrocarbons in groundwater systems	18
2.3.2 Case studies of bioremediation	19
2.4 The Roles of Amoebae in Environment	23

2.4.1	Potential as parasites or pathogens	23
2.4.2	Food chain's structure	25
CHAPTER 3 METHOD AND MATERIALS		26
3.1	Sources of Samples	26
3.2	Isolation and Identification of Amoebae from Contaminated and Non-Contaminated Groundwater with Crude Petroleum	26
3.3	Media Preparations	27
3.3.1	Non-nutrients agar (NNA)	27
3.3.2	Page's amoebae saline (PAS)	27
3.3.3	Nutrient agar (NA)	28
3.4	Maintenance of The Amoebae Culture	28
3.4.1	Sub-cultivation of amoebae	28
3.3.2	Heat-killed <i>Escherichia coli</i> (<i>E. coli</i>)	29
3.5	Determination of Hydrocarbon Concentration in Groundwater	29
3.5.1	Cleaning of glassware	29
3.5.2	Cleanup step (Alumina, Silica, Glass wool and Na ₂ SO ₄)	30
3.5.3	Liquid-liquid extraction	31
3.5.4	Sample fractionation	31
3.5.5	Gas Chromatography-Flame Ionization Detector (GC-FID)	33
3.5.6	Identification and quantification	33
CHAPTER 4 RESULTS		34
4.1	Isolation and Identification of Amoebae from Non-Contaminated and Contaminated Groundwater with Crude Petroleum.	34
4.1.1	<i>Vahlkampfia</i> sp.	34
4.1.2	<i>Echinamoeba</i> sp.	39

4.2	Determination of Hydrocarbon Concentration in Groundwater	42
CHAPTER 5 DISCUSSION		45
5.1	Identification of Amoebae from Non-Contaminated and Contaminated Groundwater with Crude Petroleum	45
5.1.1	<i>Vahlkampfia</i> sp.	45
5.1.2	<i>Echinamoeba</i> sp.	47
5.2	Hydrocarbon Concentration in Contaminated and Non-Contaminated Groundwater with Crude Petroleum.	48
5.2.1	Aliphatic hydrocarbons	48
5.2.2	Polycyclic aromatic hydrocarbons (PAHs)	49
5.3	The Potential of <i>Vahlkampfia</i> sp. as Crude Petroleum Degradar.	51
CHAPTER 6 CONCLUSION AND RECOMMENDATION		52
REFERENCES		53
APPENDICES		58
CURRICULUM VITAE		67

LIST OF TABLES

Table		Page
2.1	Typical fractionation of a crude oil	16
2.2	Advantages and disadvantages of bioremediation	18
4.1	Concentration of Aliphatic hydrocarbons compound in each samples ($\times 10^4$ ppm)	43
4.2	Concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in each samples ($\times 10^2$ ppm)	43

LIST OF FIGURES

Figure		Page
2.1	Amoebae structure	13
2.2	The general life cycle of an amoeba	14
2.3	Life cycle of <i>Naegleria fowleri</i>	14
2.4	Petroleum processes and products	16
2.5	Schematic diagram of aerobic biodegradation in soil	17
4.1	Cysts of <i>Vahlkampfia</i> sp.	36
4.2	Trophozoites of <i>Vahlkampfia</i> sp.	38
4.3	Scanning EM images of <i>Vahlkampfia</i> sp.	39
4.4	Cysts of <i>Echinamoeba</i> sp.	40
4.5	Trophozoites of <i>Echinamoeba</i> sp.	41
4.6	Scanning EM image of <i>Echinamoeba</i> sp.	42
4.7	Concentration of polycyclic aromatic hydrocarbons (PAHs) in contaminated and non-contaminated groundwater	44

LIST OF ABBREVIATIONS

°C	-	Degree celcius
µm	-	Micron meter
Ace	-	Acenaphthene
Acy	-	Acenaphthylene
Anth	-	Anthracene
BaA	-	Benz(a)anthracena
Bap	-	Benzo(a)pyrene
Bbf	-	Benzo(b)fluoranthene
Bghip	-	Benzo(g,h,i)perylene
Bkf	-	Benzo(k)fluoranthene
Chry	-	Chrysene
DiahA	-	Dibenz(a, h)anthracene
Flt	-	Fluoranthene
Flu	-	Fluorene
GAE	-	Granulomatos Amebic Encephalitis
GC-FID	-	Gas Chromatography –Flame Ionization Detector
INPY	-	Indeno(1,2,3-cd)pyrene
mg/L	-	milligram per Liter
NA	-	Nutrient Agar
Naph	-	Napthalene
NNA	-	Non-Nutrient Agar
PAHs	-	Polycyclic Aromatic Hydrocarbons
PAM	-	Primary Amebic Meningoencephalitis

LIST OF APPENDICES

Appendix		Page
1	Flow chart of methodology	59
2	General classification of hydrocarbons	60
3	Polycyclic aromatic hydrocarbons (PAHs) standards (80 ppm)	61
4	Molecular structure for 16 compounds of PAHs	62
5	Aliphatic Standard (150 ppm)	63
6	Maximum contaminant level (MCL) mg/L and water health based limits (HBLs) for aromatics hydrocarbons (PAHs)	64
7	Samples non-contaminated and contaminated water with crude petroleum	66
8	Gas Chromatography-FID, evaporator rotatory and oven for hydrocarbon glassware	67

ABSTRACT

In this study, several species of amoebae were isolated and identified from contaminated and non-contaminated groundwater with crude petroleum. The identification of amoebae was done based on morphology of cyst and trophozoites and also its locomotion based on Key Page's (1988). Results from this study indicated that at least one amoeba species isolated and identified from contaminated water, that is *Vahlkampfia* sp. and in non-contaminated groundwater, at least two species of amoebae were counted, they are *Vahlkampfia* sp. and *Echinamoeba* sp. This study is an initial step to identify the amoebae species that can be used to clean oil spills either in open or close sites. The concentrations of hydrocarbons (Polycyclic Aromatic Hydrocarbons (PAHs) and aliphatic hydrocarbons) in contaminated and non-contaminated water were determined using Gas Chromatography-Fluid Ionization Detection (GC-FID) method. In contaminated groundwater with crude petroleum, six PAHs compounds were detected. The compounds are Naphthalene (0.12×10^2 ppm), Acenaphthylene (0.075×10^2 ppm), Anthracene (0.067×10^2 ppm), Benz(a)anthracene (0.033×10^2 ppm), Benzo(b)fluoranthene (0.114×10^2 ppm) and Benzo(a)pyrene (0.193×10^2 ppm). Only one aliphatic compound in contaminated groundwater sample, it is C₂₂ (6.535×10^2 ppm). Meanwhile, in non-contaminated groundwater with crude petroleum, only one PAH compound was detected. It is Anthracene (0.063×10^4 ppm), but no aliphatic compound was found in this sample.

PENGASINGAN DAN PENGECEMAN AMOEBAE DARIPADA AIR BAWAH TANAH YANG TERCEMAR DENGAN PETROLEUM MENTAH

ABSTRAK

Dalam kajian ini beberapa spesies amoeba telah diasingkan dan dikenalpasti dari air bawah tanah yang tercemar dan yang tidak tercemar dengan petroleum mentah. Pengecaman amoebae adalah berdasarkan morfologi sista dan tropozoit serta pergerakannya berdasarkan kekunci Page (1988). Hasil kajian mendapati terdapat sekurang-kurangnya satu spesies amoeba, diasingkan dan dikenalpasti dari air tercemar iaitu *Vahlkampfia* sp. dan sekurang-kurangnya dua spesies amoeba dalam air tidak tercemar iaitu *Vahlkampfia* sp. dan *Echinamoeba* sp. Kajian ini adalah satu langkah permulaan untuk digunakan sebagai cara membersihkan tumpahan minyak samada di kawasan terbuka atau tertutup. Kepekatan kandungan hidrokarbon (hidrokarbon polisiklik aromatik (PAH) dan alifatik) dalam air tercemar dan air tidak tercemar telah ditentukan menggunakan kaedah gas kromatografi-pengesanan cecair ionik (GC-FID). Dalam sampel air tercemar, enam sebatian aromatik dikesan iaitu Naftalena (0.12×10^2 ppm), Acenaphtilena (0.075×10^2 ppm), Anthrasena (0.067×10^2 ppm), Benz(a)anthrasena (0.033×10^2 ppm), Benzo(b)fluoranthena (0.114×10^2 ppm) and Benzo(a)pyrena (0.193×10^2 ppm). Hanya satu sebatian alifatik dalam air tercemar iaitu C₂₂ (6.535×10^2 ppm). Sementara itu, dalam air tidak tercemar dengan petroleum mentah, hanya satu sebatian PAH telah dikesan iaitu Anthracena (0.063×10^4 ppm), tetapi tiada sebatian alifatik dikesan dalam sampel ini.