



UNIVERSITI PUTRA MALAYSIA

**ISOLATION, SCREENING AND GROWTH OF MONOAROMATIC
HYDROCARBON UTILISING BACTERIA**

PALSAN AIL SANNASI @ SANNACH

FSAS 1998 23

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By

PALSAN A/L SANNASI @ SANNACH

**Thesis submitted in Fulfillment of the Requirements for
the Degree of Master of Science in the Faculty of Science
and Environmental Studies,
Universiti Putra Malaysia**

June 1998



ACKNOWLEDGEMENTS

Firstly, I would like to extend my deepest gratitude to God for making me who I am and for being able to be here, to write and complete this project.

It is that time of the year again, when you sit down and try to write about how it all started in the summer of '96 and how it was made possible, the rest is history.

I would like to thank my parents for all their undivided support and guidance throughout my life ; my brothers, Sanpall for the patchwork and Sanpagan for the marvellous shades.

To my dearest supervisor, Assoc. Prof. Dr. Che Nyonya Abd. Razak, I am truly grateful for the advice, guidance, patience, ideas, critics and encouragement throughout the study and life in general (still do). To the committee members, Prof. Dr. Abu Bakar Salleh, Assoc. Prof. Dr. Mahiran Basri and En. Mohamad Pauzi Zakaria, thank you for everything and not forgetting Dr. Misri Kusnan for the help during GC analysis. All the departmental staffs, K. Ros, K. Wok, K. Nya, K. Long, K. Ida, Pak Pin, En. Baim, Yeop, Nuar, En. Karim, En. Hidir and En. Azmi (Biology Dept.).

It surely is hard to express how grateful you are to so many in so little time and space. My housemates, Praba and Ugan, I owe you all a lot, my labmates, F.M. Ali, K. Yam, Sue, Moon and Leha.

My mega bubble friends and fun friends, Azman Ihsan, Azhan, Zahidi, Mr. Tembok, Joe, Gombak, Joe BS, Cikgu Jamal, Ayam, Alai, Kotai, En. Harndan (ICM) and just about everyone involved in the project directly and indirectly, I could go on..

Last but not least, the one person who made all the difference, whom believed before I did, words may not be enough to say how much I appreciate every single little thing you did. For your kindness, sincereness, companion, friendship and for just being there. You jump .. I jump, remember ? Thank You Shidah.

DEDICATIONS

To my parents..
brothers (Sanpall & Sanpagan)..
and just about everyone ..

When you live life in the fast lane, to live life to the fullest, everything's fast and nothin' seems to last, when you have had everything only to lose it, sometimes you wanna believe so badly but then always end up being deceived, in the darkest hour of the darkest night you ask, were these doubts ever seem to be reasonable ?

You live day to day, you don't really look too far into the future, you just take every day as it comes, based part in reality, part in fantasy mostly in escapism.

Some walk the straight and narrow, some walk the rocky road, in a losing fight when you're feeling down and can't go on, it's easy to lose sight of what you think is right.. and this wicked world sometimes makes you wanna crawl then you bleed just to know you're still alive. Nowadays, it's hard to keep an open heart, when even friends seem out to harm you .. in this society, you might get wasted, elegantly. Nobody wants to be themselves no more.

You can't let them clip your wings, coz' I believe that you can fly and my heart beats as loud as thunder, for the things that I've thought and believed, what you get in life you take it, you've gotta hold on, make it last and don't let go. Believe in constant change, everyday is another day, life must go on, you just have to keep reinventing yourself. No one's pinned their dreams on me. I'm the man I want to be .. someday .. It has always been about people, the everyday people like Tommy and Gina out there and they know how it feels to be living on the edge, living a lie, surviving, trying to make ends meet, keeping their dreams alive and they understand, they always have and that is all that matters .. not the critics, not the righteous society. Revenge has always been sweet .. ride on, the show must go, don't you think so.....



TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
DEDICATIONS	v
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF PLATES	xiii
LIST OF ABBREVIATIONS	xv
ABSTRACT	xvi
ABSTRAK	xviii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
Hydrocarbon of Interest	5
Benzene	6
Toluene	7
Ethylbenzene	7
<i>p</i> -Xylene	8
Exposure Routes, Metabolism and Health	8
Waste Treatment Methods	9
Chemical and Physical Treatments	9
Biological Treatment	10
Microbial Metabolism of Pollutants	11
Plasmids and Metabolism	11
Metabolic Pathways	13
Biodegradation	19
Aerobic Biodegradation	21
Anaerobic Biodegradation	21
Biotransformation of Organic Compounds	23
Hydrocarbon Uptake	24
Biotransformation Rates of Organic Compounds	25
Mineralisation	27
Co - metabolism	27

Factors Affecting Microbial Growth	32
Adaptation, Acclimatization and Microbiota Changes	35
Microbial Activity Measurements	37
Evaluating Biodegradability	38
Analytical Procedures	38
Growth Rates and Kinetics	41
Isolation and Screening of Microbes	41
Substrate Specificity	46
Mixed Cultures	48
Hydrocarbon Toxicity	50
3 MATERIALS & METHODS	52
Chemicals / Materials	52
Instruments	53
Reagent Preparation	53
Media Preparation	54
Hydrocarbon Preparation	55
Isolation and Screening	55
Primary Screening	56
Screening of Isolates	56
Secondary Screening	57
Inoculum Preparation	57
Active Inoculum Size Determination	58
Effect of Different Hydrocarbon Concentrations	58
Substrate Specificity	58
Growth Study	59
Metabolic Activity	59
The Effects of Temperature	59
The Effect of Sea Salinity	60
Solvent Susceptibility Test	60
Growth Rate Study	61
Mineralisation Study	62
Gas Chromatography Procedures	64
Cold Extraction for Samples	64
Degradation Study of BTEX	65
Oxygen Consumption Study	65
Laboratory Scale Biodegradation Study	66
Description of Study Site	66
Sample Collection and Handling	66
Sample Analysis	72
Biodegradation Study of Site Samples	73
Biodegradation of Individual Samples	73
Biodegradation of Mixed Samples	73
Mineralisation Study	74
Oxygen Consumption Study	74

4	RESULTS AND DISCUSSION	75
	Isolation and Screening	75
	Primary Screening	77
	Secondary Screening	80
	Effect of Different Concentrations	82
	Active Inoculum Size	85
	Substrate Specificity	86
	Metabolic Activity	95
	The Effects of Temperature	97
	The Effect of Sea Salinity	102
	Solvent Susceptibility Test	104
	Carbon Dioxide Evolution and Growth	108
	Mineralisation by Monoculture	108
	Mineralisation of BTEX by Mixed Culture	110
	Oxygen Consumption	113
	Growth Rate Study	114
	Biodegradation of BTEX	117
	Isolate Characteristics	126
	Laboratory Scale Biodegradation Study	129
	Physical Characteristics of Site Samples	129
	Biodegradation of Individual Site Samples	131
	Biodegradation of Mixed Site Samples	137
	Mineralisation Study	141
	Oxygen Consumption Study	142
5	CONCLUSION AND RECOMMENDATIONS	145
	Conclusion	145
	Recommendations	147
	BIBLIOGRAPHY	149
	APPENDICES	158
	A Health Effect	159
	B CO₂ Evolution and Growth Rate Studies Calculation	160
	C BTEX Peak Elution Reference	161
	D Site and Sample Description	162
	E Additional Tables	164
	F Abstract of Poster Presented at Conference	169
	BIOGRAPHICAL NOTE	170

LIST OF TABLES

Table	Page
1. Organic priority pollutants listed by EPA	3 ✓
2. Average make-up constituents of gasoline	6
3. Examples of plasmids involved in biodegradation	13
4. Solvents of interest and their respected log <i>P</i> values	61
5. Total bacterial population of the different soil samples	75
6. Total plate count of hydrocarbon utilizing bacteria in different soil samples	76
7. Growth of bacterial isolates in different concentration of hydrocarbon	83
8. Effect of inoculum size on the growth of bacterial isolates	85
9a. Growth of isolates in different substrate and concentration	88
9b. Growth of isolates in different substrate and concentration	89
10. Growth in naphthalene and mineral oil for the selected isolates	95
11. Use of INT as an indicator of viable cells	96
12. Effects of different temperature towards growth of isolates	98
13a. Effect of different hydrocarbon substrates (BTEX) on bacterial growth at 37 °C	101
13b. Effect of different hydrocarbon substrates (BTEX) on bacterial growth at room temperature	102
14. Growth performance of isolates in media supplemented with 0.4 M NaCl	103
15. Selection tier route	103
16. Calculated specific growth rates at different intervals	116



17.	Biochemical tests and morphology summary of the isolates 145yw, 113i & 205y	127
18.	Some physical characteristics of the collected effluent sample	130
19.	Isolates origin	164
20.	Summary of isolates origin and their respective hydrocarbons	165
21.	Isolates capable of growing in different concentration	166
22.	Physical characteristics of selected hydrocarbons	167
23.	Amount of hydrocarbon present in mineral media as a multi phase system	167



LIST OF FIGURES

Figure	Page
1. Summary of general pathway of hydrocarbon metabolism	14
Summary of general pathway of hydrocarbon metabolism (contd.)	15
2. Toluene degradation pathway map	17
3. Ethylbenzene degradation pathway map	18
4. Metabolism of <i>p</i> -xylene by <i>Pseudomonas</i> species	19
5. Relevant biotransformational steps involved in the biodegradation of aromatic hydrocarbon	20
6. Mineralisation study diagrammatic set-up	62
7. Site sample collection station	71
8. No. of morphologically different isolates isolated from different soil samples	78
9. No. of isolates and their growth ability in 1 % (v/v) hydrocarbon during secondary screening	81
10. Effect of organic solvents of varying log <i>P</i> on isolates growth	105
11. CO ₂ evolved and growth depicted by different isolates	111
12. Mineralisation pattern derived from the mmol of CO ₂ evolved with BTEX	112
13. Oxygen consumption by consortium utilizing BTEX	114
14. Biodegradation of BTEX by monoculture	117
15. Biodegradation of BTEX by mixed culture	118
16. Chromatogram of isolates 205y + 113i in benzene 10 % (v/v)	119
17. Chromatogram of isolate 145yw in benzene 10 % (v/v)	120
18. Chromatogram for mixed culture of 113i + 145yw + 205y in benzene 5 % (v/v)	121



19.	Chromatogram for mixed culture of 113i + 145yw + 205y in benzene 10 % (v/v)	122
20.	Chromatogram of isolates 205y + 145yw in benzene 5 % (v/v)	123
21.	Chromatogram of isolates 113i + 205y in benzene 10 % (v/v)	124
22.	Chromatogram of isolates 113i + 145yw in benzene 10 % (v/v)	125
23.	Chromatogram of isolate 113i in toluene 1 % (v/v)	125
24.	Chromatogram of isolates 113i + 145yw in benzene & toluene 25 % (v/v)	126
25.	Growth observed by OD in different site samples.....	133
26.	Growth observed by OD in different site samples	133
27.	Growth observed by OD for mono and mixed culture inoculated in site sample mixtures	138
28.	Mineralisation pattern derived from the mmol of CO₂ evolved with site samples by isolate consortia	141
29.	Oxygen consumption by isolate consortia utilizing site samples	142
30.	Biodegradation of site samples using mono culture	143
31.	Bioedgradation of site samples using mixed culture	144

LIST OF PLATES

Plates	Page
1. Location of site 1	67
2. Sample collection. Location of site 2	67
3. Location of the pond designated as site 3	68
4. Location of site 4	68
5. Location of site 5	69
6. Site samples ; site 6 and site 7	69
7. Location point of site 6	70
8. Growth of different isolates in 33 % (v/v) toluene at 37 °C after 15 days	84
9. Growth of isolate 145yw at 37 °C after 15 days	84
10. Growth of different isolates in 10 % (v/v) toluene at 37 °C after 15 days	90
11. Growth of different isolates in 10 % (v/v) benzene at 37 °C after 15 days	90
12. Growth of isolate 145yw in <i>p</i> -xylene	91
13. Growth of isolate 145yw in ethylbenzene	91
14. Growth of isolate 113 in toluene	92
15. Growth of isolate 205y in <i>p</i> -xylene	92
16. Growth of isolate 113i in <i>p</i> -xylene	93
17. Control BHA plate for the vapor method	93
18. Growth of isolate 113i on 1 % (v/v) benzene	94
19. The use of INT	97

20.	Growth of isolate 113i on 1 % (v/v) toluene	99
21.	Growth of isolate 113i on 1 % (v/v) ethylbenzene	100
22.	Growth of isolate 113 on 1 % (v/v) benzene	100
23.	Growth of isolate 113 on 1 % (v/v) <i>p</i> -xylene	101
24.	Viable cells of isolate 145yw	112
25.	Isolate 145yw cellular morphology	128
26.	Isolate 113i cellular morphology	128
27.	Isolate 205y cellular morphology	129
28.	Growth of isolates in different site samples	134
29.	Growth of isolates in mixed site samples	135
30.	Biodegradation of mixed site samples	136
31.	Biodegradation study of mixed site samples with monoculture	140



LIST OF ABBREVIATIONS

BTEX	Benzene, toluene, ethylbenzene, <i>p</i>-xylene
CO₂	Carbon dioxide
cfu / mL	Colony forming unit / mL
DO	Dissolved oxygen
g	gram
GC	Gas chromatograph
HC	Hydrocarbon
INT	Iodonitrotetrazolium chloride
mg / L	Milligram / Litre
PAH	Polycyclic aromatic hydrocarbon
TPC	Total plate count
v/v	volume / volume
w/v	weight / volume

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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June, 1998

Chairman : Assoc. Prof. Dr. Che Nyonya Abd. Razak

Faculty : Science and Environmental Studies

A total of 131 isolates originating from different soils were obtained through enrichment and direct plating methods using 0.1 % (v/v) of either benzene, toluene or mixture of benzene and toluene as their sole energy and carbon sources. Most of the isolates were from S5 (garden soil) and the least from S1 (ESSO refinery soil) obtained by the enrichment culture method. Out of this, 107 morphologically different isolates were rescreened in 1 % (v/v) of their respective hydrocarbon of either benzene or toluene. Out of the 34 good isolates grown in varying hydrocarbon concentrations up to 50 % (v/v), 23 gave good and moderate growth. These isolates were further grown in different concentrations of BTEX. Six isolates (145yw, 113i, 205y, 205w,



113 & 146) exhibited good growth withstanding up to 75 % (v/v) concentration of BTEX. The isolates were also able to grow in 0.4 M NaCl (35 p.s.u.) which is equivalent to sea salinity level. Studies done on the 3 isolates (145yw, 113i & 205y) showed that they were metabolically active throughout their growth in the hydrocarbon spiked media deduced from the INT stain test, increased oxygen consumption and increased plate counts. High colony forming units / mL percentage (50 % or more) were observed in different organic solvents of varying log *P* values. Isolate 145yw gave the highest growth rate of 0.22 h⁻¹ in 0.1 % (v/v) benzene. Biodegradability of the isolates were further confirmed by positive CO₂ production and the reduction in the hydrocarbon peaks observed by GC. Extensive degradative profiles were obtained with isolate 113i and mixed culture. Laboratory biodegradation studies showed that all the 3 isolates were able to grow in both single and mixture of site samples.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk mendapatkan Ijazah Sarjana Sains.

PEMENCILAN, PEMILIHAN DAN PERTUMBUHAN BAKTERIA YANG BERUPAYA MENGGUNAKAN HIDROKARBON MONOAROMATIK

Oleh

PALSAN A/L SANNASI @ SANNACH

Jun, 1998

Pengerusi : Prof. Madya Dr. Che Nyonya Abd. Razak

Fakulti : Sains dan Pengajian Alam Sekitar

Sejumlah 131 pencilan bakteria dari tanah telah diperolehi melalui teknik pengkayaan dan pencilan terus dengan menggunakan 0.1 % (i/i) samada benzena, toluena atau campuran kedua-duanya. Jumlah bakteria tertinggi diperolehi dari S5 (tanah kebun) dan paling rendah dari S1(tanah kilang penapis ESSO) melalui teknik pengkayaan kultur. Daripada jumlah ini, 107 pencilan yang berbeza morfologinya telah diuji semula dengan 1 % (i/i) hidrokarbon yang sama. Daripada 34 pencilan yang diuji dalam kepekatan hidrokarbon yang berbeza sehingga 50 % (i/i), 23 pencilan memberikan pertumbuhan yang baik dan sederhana. Bakteria ini kemudiannya ditumbuhkan pula di dalam pelbagai kepekatan benzena, toluena, etilbenzena dan *p*-xilena. Enam pencilan (145yw, 113i, 205y, 205w, 113 dan 146) berjaya memberikan

pertumbuhan yang baik sehingga mampu hidup dalam 75 % (i/i) BTEX. Ia juga masih mampu bertahan di dalam 0.4 M NaCl (35 p.s.u.) yang menyamai kepekatan garam lautan. Kajian dengan 3 pencilan (145yw, 113i dan 205y) menunjukkan sel aktif sepanjang berada di dalam media yang mengandungi hidrokarbon. Ini terbukti melalui ujian INT, peningkatan pengambilan oksigen dan peningkatan bilangan bakteria. Peratus bilangan koloni / mL yang tinggi (50 % atau lebih) juga diperolehi daripada berbagai larutan organik yang berbeza nilai log P -nya. Bakteria 145yw memberikan kadar pertumbuhan yang tertinggi iaitu 0.22 j^{-1} di dalam 0.1 % (i/i) benzena. Kebolehpayaan biodegradasi bakteria tersebut telah dibuktikan dari penghasilan positif CO_2 dan penurunan puncak hidrokarbon yang dicerap melalui kromatografi gas. Profil degradasi maksimum diperolehi dengan bakteria 113i dan kultur campuran. Kajian biodegradasi makmal menunjukkan ketiga-tiga bakteria ini mampu hidup di dalam sampel sisa tunggal dan campuran.

CHAPTER 1

INTRODUCTION

Majority of compounds that cause pollution problems are synthesized in chemical production plants which convert raw materials into products and chemical waste. About 65,000 chemicals are believed to be in daily use thus making the monitoring of the environment for increased levels of all or even a large proportion of the commercially produced chemicals not feasible. To control pollution effectively, potentially hazardous chemicals have to be identified and their concentration-limit standards in the environment have to be specified (Leisinger, 1987). This involves an interdisciplinary approach by toxicologists, epidemiologists, analytical chemists, microbiologists and ecologists.

The Resource Conservation and Recovery Act (RCRA) 1976 and 1980 with Hazardous and Solid Wastes Amendments in 1986 of the United States defines hazardous waste as any solid waste or combination of solid wastes, spent materials, sludges, by-products and commercial chemical products that because of its quantity, concentration, or physical, chemical, or infectious characteristics, may cause or significantly contribute to an increase in mortality or an increase in serious irreversible or incapacitating reversible illness, or pose a substantial present or potential hazard to human health or the environment when improperly managed (Edelman, 1987).



Hazardous wastes are generated from three major sources which are industrial, agricultural and domestic sectors. Potential entry points of pollutants into the environment are effluents from domestic and industrial municipal waste treatment plants, emissions and solid wastes produced by the manufacturing sector. Chemical products and chemical wastes undergo different routes into the environment. Both may enter the ecosystem directly or after passing through some sort of waste treatment if there is any. The wide use of chemical products by consumers makes their release difficult to control (nonpoint sources), while the streams of concentrated waste chemicals released by manufacturing plants (point sources) justify the development of sophisticated treatment techniques. A great variety of pollutants are deliberately released into the environment by illegal dumping.

Accidents, spills during transport, leaks from waste disposal sites, surface and storm water run-off also contribute to the direct entry of untreated chemical waste from production sites into the environment (Hunter 1975 ; Haigler *et al.*, 1992 ; Fries *et al.*, 1994). The accumulation of point sources releasing chemicals leads to critical environmental situations in highly industrialized areas. Contamination of soil by toxic compounds may lead to potential threat to underground aquifers (Fries *et al.*, 1994; Chen and Taylor, 1995). In the United States, for example, volatile organic compounds have been detected in 45 % of the large public water supplies which distribute ground water.

Great concern have been given to such matters as proper waste disposal practices especially in the developing countries. Dawn of the environmental era urges safe, healthful, productive and esthetically and culturally pleasing surroundings to be promoted. Microbiology and biotechnology will play an important role in the development of alternate technologies to produce less waste and treating pollutants.



The Environmental Protection Agency (EPA) in the United States had identified significant and potential dangerous pollutants and came up with the “Toxic Pollutant list” which contained 65 compounds and classes of compounds (Table 1).

Table 1 : Organic priority pollutants listed by EPA

<u>Chemical class</u>	<u>Number of compounds</u>
Aliphatics	3
Halogenated aliphatics	31
Nitrosamines	3
Aromatics	14
Chloroaromatics (including TCDD)	16
Nitroaromatics	7
Polynuclear aromatic hydrocarbons	16
Pesticides and metabolites (including DDT)	17
Polychlorinated biphenyls (PCBs)	7

Source : Adapted from Leisinger, (1987).

In Peninsular Malaysia alone in 1987, about 26% of the rivers monitored have been classified as polluted due to organic pollution and approximately 380,000 m³ of hazardous wastes are discharged annually into the environment. Of this oil and grease account 19,896 m³, sludge, paint, dye, pigment in the form of solvent 950 m³ and spent solvents (non - halogenated) 2,471 m³ (Hamid and Sidhu, 1993).

The importance of the waste as potential pollutants of both soil and inland waters is well described under Act 127 and in the 5th and 6th Schedule of the Environmental Quality Act 1974, Environmental Quality, (Sewage and Industrial Effluents), Regulation 1978.

Great interest has arisen in the field of environmental biotechnology to find microbes capable of biological breakdown or conversion of organic compounds into simpler compounds of minimal environmental significance. Microbes are known to carry out biodegradation, mineralisation or biotransformation on vast array of organic compounds.

Studies to be called as our own should be done owing to the fact that our climate and condition are different from temperate countries. Research carried out in local conditions would then prove to be more fruitful, practical and promising for our hazardous waste remediation in the near future.

The objectives of this study are to isolate, screen and determine the growth performance and biodegradation or mineralisation capabilities of the potential BTEX (benzene, toluene, ethylbenzene and *p*- xylene) utilising bacteria.

In this study, focus will be given to hydrocarbon contaminated waste specifically on mono ring aromatic hydrocarbon. Potential BTEX utilising microbe isolates were screened and assessed microbiologically, followed by the degradation and transformation studies. Primary laboratory scale biodegradation study of site samples will also be carried out to evaluate the potential of the isolates for field applications.

CHAPTER 2

LITERATURE REVIEW

Hydrocarbon of Interest

Hydrocarbons being an ubiquitous natural compound could be classed into various classes according to their structures. In this context they would be classed into only two. First, the non - aromatic aliphatic group which include the normal, branched and cyclic alkanes or paraffins, alkenes or olefins and alkynes. The second group are the aromatics with unsaturated hydrocarbon which have distinctive, not unpleasant odors and mostly toxic. This group could be further classified to mono ring aromatics comprising of the basic benzene and alkylbenzene and the polycyclic aromatic hydrocarbons (PAH) which pose the major problem (Cole, 1994). Aromatic hydrocarbon generated is a cause of great concern due to their potential hazard to both plants and animals.

Petroleum products in which they are mainly encountered have a vast array of uses, such as fuels for vehicles and industry, heating oils, lubricants, raw materials in manufacturing petrochemicals and pharmaceuticals and solvents. Benzene, toluene, ethylbenzene and xylenes (collectively known as BTEX compounds) are among the 50 largest - volume industrial chemicals produced, with production figures of the order of millions of tonnes per year. They are also natural constituents of crude oil, gasoline, polymer adhesives and degreasers (Table 2).

