UNIVERSITI PUTRA MALAYSIA

ISOLATION, SCREENING AND GROWTH OF MONOAROMATIC HYDROCARBON UTILISING BACTERIA

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ISOLATION, SCREENING AND GROWTH OF MONOAROMATIC HYDROCARBON UTILISING BACTERIA

By

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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.

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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. Nowdays, 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.....

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LIST OF ABBREVIATIONS

BTEX	Benzene, toluene, ethylbenzene, *p*-xylene
CO_2	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 fulfillment of the requirements for the degree of Master of Science.

ISOLATION, SCREENING AND GROWTH OF MONOAROMATIC HYDROCARBON UTILISING BACTERIA

By

PALSAN A/L SANNASI @ SANNACH

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 CO_2 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

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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 berbeze nilai log *P* -nya. Bakteria 145yw memberikan kadar pertumbuhan yang tertinggi iaitu 0.22 j^{-1} di dalam 0.1 % (i/i) benzena. Keboleh-upayaan 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 municipial 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

Chemical class	Number of compounds
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 m^3 of hazardous wastes are discharged annually into the environment. Of this oil and grease account 19,896 m^3, sludge, paint, dye, pigment in the form of solvent 950 m^3 and spent solvents (non - halogenated) 2,471 m^3 (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 arised 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 ubiquitious 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 alkanesor 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).