UNIVERSITI PUTRA MALAYSIA

DEGRADATION OF BENZENE, TOLUENE, ETHYLBENZENE, AND XYLENE BY ISOLATED MICROORGANISMS FROM PETRONAS GAS PLANT AT KERTIH, TERENGGANU

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By

HAMID REZA SALSALI

Thesis submitted in Fulfillment of the Requirements for the Degree of Master of Science in the Faculty of Engineering
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LIST OF ABBREVIATIONS

BTEX	Benzene, toluene, ethylbenzene, xylene
CO_2	Carbon dioxide
cfu/mL	Colony forming unit/mL
g	gram
GC	Gas chromatograph
GC-MS	Gas chromatograph mass spectra
HPLC	High Performance Liquid Chromatography
mg/L	Milligram/litre
v/v	volume/volume
w/v	weight/volume
TLV	Threshold limit value
IDLH	Immediately dangerous to life and health
M	Mixture of benzene, toluene, ethylbenzene, and xylene

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

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November 1999

Chairman : Assoc Prof Dr Azni Hj Idris

Faculty : Engineering

A total of 38 isolates originating from five different sources of sediments, and contaminated soils from Petronas Gas Plant at Kertih, Terengganu were obtained through enrichment culture method using 0.1 %(v/v) of benzene, toluene, ethylbenzene, and xylene or mixture of them as their sole energy and carbon sources. Most of the isolates were from S_5 (Residential area) and the least from S_1(Equalization basin). Out of this, 11 good isolates were able to grow in varying hydrocarbon concentration up to 50% (v/v) BTEX. Only isolate No.4 showed extremely good growth in concentration up to 80 % (v/v) of BTEX.

Using benzene at 60% (v/v), the growth rate was observed to be highest during the first 2 hours, while degradation rate and percentage of removal by using isolate No.4 showed 1.5E-04 mM/s and 75 % respectively. For toluene at 60% (v/v), the growth rate

was highest during the first 3 hours, while degradation rate and percentage of removal were 5E-05 mM/s and 70 % respectively. In the case ethylbenzene at 60% (v/v), the growth rate was highest during the first 7hours, while degradation rate and percentage of removal by using same isolate showed 1E-04 mM/s and 98% respectively. For xylene at 60%(v/v), the growth rate was highest during the first 5hours, while degradation rate and percentage of removal achieved were 5.35E-04 mM/s and 8.9 % respectively. In general, all the isolates had tolerated the organic solvent (BTEX), which may be due to the adaptability of the cell membrane through a metabolic or enzymatic properties.

Both High- Performance Liquid Chromatography and Gas Chromatography Mass-Spectra profile had clearly showed a BTEX-peaks area ratio reduction, where the isolates were shown to biodegrade the hydrocarbon either by mineralization or biotransformation. However, based on the almost complete peak reduction as well as appearance of unidentified peaks, it may be suggested that BTEX can be degraded by naturally present microorganisms which was identified at Petronas Gas Berhad site.

Abstrak tesis yang dikemukakan kepada Senat Universiti Puta Malaysia sebagai memenuhi keperluan untuk Ijajah Master Sains

PEREPUTAN BENZENE, TOLUENE, ETILBENZENE, DAN EXYLENE DENGAN MENGGUNAKAN PERGASINGAN MIKROORGANISMS MENGGUNAKAN PETRONAS GAS KILANG, KERTIH TERENGGANU

OLEH
HAMID REZA SALSALI

November 1999

Pengerusi : **Prof.Madya.Dr. Azni Hj Idris**

Fakulti : **Kejuruteraan**

Terdapat lima jenis "inocular" ia termasuk pemendapan, enapan pasir, hasil keluaran proses yang digunakan untuk mengefektifkan kaedah kultur bakteria bagi benzene, toluene, etilbenzene, dan exylene. Sejumlah 38 bahan tersingkir yang diperolehi menggunakan 0.1 %(v/v) samada benzene, toluene, etilbenzene, dan exylene atau campuran bahan-bahan tersebut sebagai sumber utama tenaga dan karbon kebanyakkan singkiran adalah daripada S_5(Luas Penempatan) dan yang lain daripada S_1(Tangki Penyamarataan)dari kaedah kultur.

Daripada jumlah ini, hanya 38 bahan pemendap diskrinkan dalam 1 % (v/v) BTEX. Di antaranya, 11 pemendapan yang baik dibiakkan dalam berbagai kepekatan sehingga mencapai kepekatan 50 %(v/v) BTEX, hanaya pemendapan No.4 menunjukkan perkembangan ekstrem yang boleh menampung kepekatan sehingga 80 % (v/v) BTEX.

Kedua-dua "High Performance kromotografi cecair" dan gas profil spektra jisim telah jelas menunjukkan BTEX-pengurangan nisbah luas puncak, di mana pemendapan secara amnya membolehkan proses biodegradasi hidrokarbon samada melalui mineralisasi atau biotransformasi. Walaubagaimanapun, berdasarkan kepada puncak

pengurangan, dan puncak penonjolan yang tidak dapat dipastikan, adalah dicadangkan bahawa BTEX boleh didegradasikan oleh organisma asli di Petronas Gas Berhad.

CHAPTER I

INTRODUCTION

Aromatic hydrocarbons are components in petroleum products, which have been used extensively as degreasing agents. These compounds are widespread use common contaminants in ground water and soils. Both aerobic and anaerobic biodegradations of aromatic compounds have been studied. The microorganisms responsible for degradation have been identified and many of the enzymatic and genetic regulatory mechanisms have been determined (Smith, 1994).

Hazardous waste generated from three major sources, which are industrial, agricultural and domestic sectors. Potential entry points of pollutants into the environment are effluent from domestic, industrial and municipal waste treatment plants, emissions and solid wastes produced by the manufacturing sector. However chemical products and chemical wastes undergo different routes into the environment, either may enter the ecosystem directly or after passing through some sort of waste treatment system. The wide use of chemical products by consumers makes their release difficult to control, while the stream of concentrated waste chemicals released by manufacturing plants justify the development of sophisticated treatment techniques. A great variety of pollutant are released into environment by illegal dumping.

However organic compounds can be a major pollution problem in ground water. Their presence in water can create a hazard to public health and the environment. The BTEX group of contaminants consists of benzene, toluene, ethyl benzene, and three isomers of xylene. Previous reports have been shown that BTEX was accumulating in the

ground water for over 20 years. There is now a two - foot thick layer floating on ground water at approximately twenty feet below the surface (Gossett, 1987; Almedia, 1995). BTEX makes up one of the main groups of soluble organic compounds that find their way into our soil and ground water. The release of petroleum products such as gasoline (Evans, *et al.*, 1991), diesel fuel, lubricating and heating fuel from leaking oil tanks (Hutchins, 1991), and cause of their polarity and very soluble characteristics, these organic chemicals (BTEX) of petroleum products will be able to enter the soil and groundwater systems, and cause serious pollution problems.

In peninsular Malaysia alone in 1987, about 26% of the rivers monitored have been classified as polluted due to organic pollution and approximately 380,000m^3 of hazrdous waste are discharged annually into the environment.The oil and grease accounts 19,896m^3, sludge, paint, dye, pigment, in the form of solvent about 950m^3 and spent solvent (non-halogenated) about 2,471m^3 (Hamid and sidhu, 1993). However BTEX group of contaminants make up a significant percentage of petroleum products. The reason why the BTEX, entering our soil and ground water system, are considered such a serious problem that have acute and long term toxic effects, especially with benzene which is well known to be a carcinogen.

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 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.

Objective of the Study

This study will be focused on the degradation of BTEX group of contaminants with the following objectives:

(i) To isolate and screen of suitable microorganism sampled at Petronas Gas Berhad at Kertih Terengganu.

(ii) To study of growth performance of native isolate at different BTEX concentration.

(iii) To investigate the biodegradability of BTEX compound using HPLC and GC-MS respectively.

CHAPTER II

LITERATURE REVIEW

Hydrocarbon of Interest

Organic chemical are widely used in all facets of industries including machine and electric manufacturing, metal finishing, automotive and engine repair, dry cleaning, asphalt operations, dye manufacturing, agricultural activities, and food processing. The usage of organic chemicals is of increasing concern to regulators and the industry in general because of the contamination of soil and ground water resulting from the mishandling and disposal of these chemicals.

Typically, these organic chemicals are stored in drums and tanks. Many industries, especially gasoline fueling stations and trucking companies, store their chemical in under ground storage tanks. The presence of large quantities of chemicals, gasoline, and diesel fuel on-site is often an indicator of the potential for soil and ground water contamination. The specific organic compounds are the petroleum hydrocarbons and organic functional groups. The majority of sites an engineer has to deal with will probably involve one of these contaminants, with gasoline being the most common at the smaller site.

The carbon-containing compounds are classified as organic compounds. All organic compound contain carbon; virtually all contain hydrogen and possess at least one C-H bond in their chemical structure. Hydrocarbons is the most basic organic compound group contain only the elements hydrogen and carbon.

Hydrocarbons are divided into two classes, aliphatic hydrocarbon, which is a straight chain hydrocarbon without a benzene ring, and aromatic hydrocarbon, which contains benzene ring. A benzene ring contains six carbons joined in a ring structure with alternative single and double bonds.

Aromatic hydrocarbons, also called alkyl hydrocarbons are characterized by carbon-carbon ring structures, and as suggested by their name, a distinctive odor. Benzene, C_6H_6, is the simplest compound of this group of hydrocarbons. Joining functional groups to the basic benzene ring can form other molecules. If there is only one functional group is not specified because all six carbon atoms are equivalent.

Table2.1 lists the physical properties of some aromatic hydrocarbons that are commonly encountered in soil and ground water contamination.

Table2.1: Physical properties of selected aromatic hydrocarbons (BTEX)

	Benzene	**Toluene**	**m-Xylene**	**o-Xylene**	**p-Xylene**	**Ethyl benzene**
Chemical formula	C_6H_6	C_7H_8	C_8H_{10}	C_8H_{10}	C_8H_{10}	C_8H_{10}
Molecular weight [g/mole]	78	92	106	106	106	106
Water solubility [mg/l]	1700	515	-	175	198	152
Vapor pressure (at 20 C) [mm Hg]	95.2	28.4	-	6.6	-	9.5
Specific density (at 20 C)	0.8787	0.8669	0.8642	0.8802	0.8610	0.8670
Octanol-water partition coeff. (at 20 C) [log Kow]	2.13	2.69	3.20	2.77	3.15	3.15
Henry's law constant (at 25 C) [kPa*m^3/mole]	0.55	0.67	0.70	0.50	0.71	0.80
Polarity	Non-polar	Non-polar	Non-polar	Non-polar	Non-polar	Non-polar
Biodegradability	Aerobic	Anaerobic /Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Maximum Contaminant Level(MCL)(mg/l)	0.005	1	10	10	10	0.7

Adapted from (Jimmy et al.,1997).

Petroleum is composed chiefly of saturated hydrocarbons, but may also contain unsaturated hydrocarbons and their derivatives, nitrogen compounds, and sulfur compounds.Regular gasoline is mixture of more than 100 organic compounds Table 2.2 gives an analysis of fresh and weathered gasoline (Jimmy *et al.*, 1997).

Table 2.2: Composition (mass fraction) of BTEX in gasoline

Aromatic Hydrocarbons	Molecular Weight(g)	Fresh gasoline %	Weathered gasoline%
B	78.1	0.0076	0.0021
T	92.1	0.055	0.0359
E	106.2	0.0000	0.0156
X	106.2	0.0957	0.0151

Benzene

Benzene (or Benzin) is a member of the closed ring group of aromatic solvent. Commercially produced from petroleum feed stock reformation and hydrodealkylation of substituted aromatic. Petroleum naphtha from the craking fractionating of crude oil is partially converted to benzene and other aromatic by exposing into a platinum catalyst at elevated temperatures. Some possible reactions are:

$C_6H_{14} \rightarrow C_6H_6 + 4H_2$ (2.1)

Hexane

$(CH_3)2C_6H_{10} \rightarrow C_6H_6 + 2CH_4 + H_2$ (2.2)

Dimetyl cyclohexane

$CH_3C_9H_9 \rightarrow C_6H_6 + 3H_2$ (2.3)

Metyl cyclopentane

Overall benzene content maybe 5 to 10 percent. This recovered and purified using processes such as fractional distillation and solvent extraction. Benzene is used in the production of ethylbenzene/styrene, cumene, cyclohexane, maleic anhydride, vinyl copolymers and some acrylics and rubber based products (Sedivec , 1976).

Benzene Exposure Routes and Health

Benzene exposure routes and health are summarized in Table2.3.

Table2.3: Summary of benzene exposure routes and health

Sources	Gasoline, some food, drugs, pesticides, paints, plastic industries, ethylbenzene, styrene, cumene, cyclohexane and maleic anhydride.
Human Health	TLV:10 ppm in the blood, IDLH:2000 ppm,and Carcinogen
Immediate Action	Spill Control: Restrict access to spill site. Issue warning: "FLAMMABLE". Call Fire department and notify manufacture. Eliminate sources of ignition including traffic and equipment. Stop the flow and contain spill, if safe to do so. Avoid contact with liquid and vapour; stay upwind of release. Keep contaminated water from entering sewers or watercourses. Fire control: Use foam, dry chemical or carbon dioxide to extinguish. Don't extinguish fire unless release is stopped. Cool fire- exposed containers with water. Stay clear of ends to tanks.
Irritation and Inhalation Data	Irritation and inhalation data in appendix A

Adapted from Environmental and technology information: Benzene, 1984.

Toulene

Toluene (or metylbenzene) is used in the production of benzene, benzoic acid, phenol, trinitro toulene, lacquers, resin solutions, lacquer thinners, as a gasoline component and as a solvent in formulations of rubber cements paints, inks and pesticides. Toluene is primarily produced by catalytic reformation of naphthene.-Rich petroleum

feedstock fractions, generally in conjunction with the manufacture of benzene and xylenes.

Dehydrogenation of naphtha feed stock is the most common process used to produce toluene (Sedivec, 1976). The feedstock is preheated vaporized, mixed with hydrogen-rich recycled gas, and passed through the reactor for a contact time of about 15 seconds. Large quantities of it are consumed in the production of styrene, cumene (for manufacturing phenol and acetone) and cyclohexane (for manufacturing nylon).

Toluene Exposure Routes and Health

Toluene exposure routes and health are summarized in Table 2.4.

Table2.4: Summary of toluene exposure routes and health

Sources	Gasoline additive, manufacturing and solvent factories, benzoic acid/phenol.
Human Health	TLV:100 ppm ,and IDLH:2000 ppm
Immediate Action	Spill Control: Restrict access to spill site. Issue warning :"FLAMMABLE". Call fire department and notify manufacture. Eliminate sources of ignition including traffic and equipment. Stop the flow and contain spill, if safe to do so.Avoid contact with liquid and vapour; stay upwind of release. Fire control: Use foam,dry chemical or carbon dioxide to extinguish. Cool fire- exposed containers with water. Stay clear of ends to tanks.
Irritation and Inhalation Data	Irritation and inhalation data in appendix B.

Adapted from Environmental and technology information: Toluene, 1984.