

BTX TREATMENT FROM PETROCHEMICAL WASTEWATER USING  
*PSEUDOMONAS PUTIDA*

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for the award of the degree of  
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## ABSTRACT

The widely consumption of petrochemical as domestic and industrial product contributed to large production of petrochemical wastewater. The production of petrochemical wastewater generally consists of polycyclic and aromatic hydrocarbons, cyanide, oil, phenols, metal derivatives, sulphides, and other chemicals. Benzene, Toluene and Xylene are part of the aromatic hydrocarbon that contain in the petrochemical wastewater. The present of BTX in wastewater cause the severity and toxicity to the human, animal and environment. Thus this proposal will propose a proper treatment that should be constructed in order to obtain the standards that have been regulated by the Environment Protection Agency (EPA) before the industrial discharge the petrochemical wastewater. The chosen of *Pseudomonas Putida* as microorganism in biological treatment based on the ability to degrade the aromatic compound and produce a clean and safe treatment without compose other hazardous chemical after the treatment. The experiment that carryout in laboratory will study the effect of shaking speed, temperature shaker and ratio of bacteria to solvent BTX in order to reduce the concentration of BTX in the waste solution. The bacteria growth and percentage removal of BTX will analyze using UV-VIS Spectrophotometer and Gas Chromatography (GC). From the data obtain it show that the optimum condition for *Pseudomonas Putida* to achieve the highest percentage removal are 37°C, 180rpm and 1:9 ratio of bacteria to solvent used. Unfortunately, from the calculation obtain only xylene has satisfied the EPA standard.

## ABSTRAK

Penggunaan petrochemical samada dalam kegunaan domestic mahupun industrial menyumbang kepada masalah sisa air buangan petrochemical yang berleluasa. Pengeluaran air sisa buangan petrochemical kebiasaannya mengandungi polycyclic dan aromatic hidrokarbon, cyanide, minyak, phenols, besi, sulfur juga beberapa jenis bahan kimia yang lain. Benzene, Toluene dan Xylene adalah sebahagian daripada aromatic hidrokarbon yang terkandung didalam sisa air buangan. Kewujudan BTX didalam sisa air buangan mengundang masalah dan toksik kepada manusia, haiwan dan alam sekitar. Disebabkan itu, kajian ini bertujuan untuk mencari penyelesaian bagi merawat sisa air buangan secara effective agar sisa air buangan tersebut dapat memenuhi syarat-syarat yang telah ditetapkan oleh Environment Protection Agency (EPA) sebelum ia dilepaskan kealam sekitar. Pemilihan *Pseudomonas Putida* mikroorganisma dalam rawatan secara biologi adalah disebabkan keupayaannya yang mampu menghuraikan aromatic hidrokarbon dan menyediakan rawatan yang bersih dan selamat tanpa menghasilkan bahan kimia yang berbahaya semasa juga setelah proses rawatan selesai. Kajian yang dijalankan merangkumi kesan kelajuan goncangan, suhu dan nisbah bacteria kepada larutan BTX bagi mengurangkan kepekatan BTX didalam sisa air buangan. Pertumbuhan bacteria dan peratusan pengurangan BTX dikaji menggunakan uv-vis spectrophotometer dan Gas Chromatography (GC). Daripada data yang diperolehi menunjukkan keadaan optimum bagi *Pseudomonas Putida* untuk mempunyai peratusan pengurangan yang tinggi adalah pada 37°C, kelajuan 180rpm dan 1:9 nisbah bacteria kepada larutan BTX. Walaubagaimanapun, berdasarkan kiraan yang telah dibuat hanya Xylene yang Berjaya memenuhi syarat yang ditetapkan EPA.

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## LIST OF SYMBOLS

$^{\circ}\text{C}$	degree Celcius
%	Percentage
g	gram
g/L	gram per liter
mg	Miligram
mL	mililiter
$R^2$	Correlation Coefficient

## LIST OF ABBREVIATIONS

GC	Gas Chromatography
UV-VIS	UV –VIS Spectrophotometer
ACGIH	American Conference of Governmental Industrial Hygienists
IUPAC	International Union of Pure and Applied Chemistry
ppm	Part per million
rpm	revolution per minute

# CHAPTER 1

## INTRODUCTION

### 1.1 Background of study

Petrochemical is the derivative product from Crude Oil (Petroleum) and Natural Gas. Processing of petrochemical from the petroleum or by product of petrochemical involving distillation, catalytic cracking, platforming, hydrocarbon production and others. The petrochemical wastewater production strongly depends on this process configuration. According to A.U. ISRAEL(2007) and André due to the ineffectiveness of purification systems wastewaters released by petrochemical industries are characterized by the presence of large quantities of polycyclic and aromatic hydrocarbons, cyanide, oil, phenols, metal derivatives, sulphides, and other chemicals.

Benzene, toluene and xylene are part of the hydrocarbon aromatic that contain in the petrochemical wastewater (Manahen and Noah 1988). According to the Environmental Protection Agent (EPA) and Occupational, Safety and Health Administration (OSHA) these toxic compounds are cause environmental and health hazard whether to human or animal in term of exposure, breathing, and drinking water.

Benzene has been widely used as a multipurpose organic solvent but now the use is discouraged due to its high toxicity and had be proved as the carcinogenic agent. The over exposure of benzene it may affect cancer since it has been proved as carcinogenic agent as well as cause Central Nervous System (CNS) depression and Irregular heart rhythm (EPA).

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Toluene is a major aromatic constituent of gasoline. It is used in household aerosols, nail polish, paints and paint thinners, and solvent based cleaning agents (OEHHA). Toluene may affect the nervous system tiredness, confusion, memory loss, nausea and loss of appetite. These symptoms usually disappear when exposure is stopped. Inhaling High levels of toluene in a short time can make you feel light-headed, dizzy, or sleepy. But, high levels of toluene may affect your kidneys (ATSDR, 2001).

Xylene may be found in solvents for gums and resins, manufacture of plastics and synthetic fibers, insecticides and other pesticides(DHS,1989).According to Department of Health Service(DHS,1989) Xylene can enters human body rapidly while breathing in its vapors. It can also be absorbed through skin, particularly prolonged exposure. Overexposure to xylene most commonly affects nervous system and respiratory system. The effect of xylene becomes more noticeable and serious as the level or length of time of exposure increases.

Untreated wastewater from petrochemical generally contains high levels of organic material and toxic compounds such benzene, toluene and xylene that cause environmental and health hazards. Consequently, it must immediately be conveyed away from its generation sources and treated appropriately before final disposal (United Nations). Wastewater treatment is the process of removing contaminants from the effluent streams and runoff. It includes physical, chemical and biological processes to remove physical, chemical and biological contaminants. Its objective is to produce a treated effluent and a solid waste or sludge suitable for reuse or discharge back into the environment (Arcadio, 2003)

Physical treatment just involving change in the appearance like size but not in the substance, the example of process is filtration and membrane process. While chemical treatment use chemical compound to remove the contamination by chemical precipitation or coagulation. Sometimes these two processes are combining to complete the process of wastewater treatment (Pradeep.P, 2008).

Biological treatment is the modern alternative in wastewater treatment, it basically the same that would occur naturally in the receiving water but need to place under controlled condition. Most biological treatment use bacteria as primary microorganisms and degradation of organic compound. It occurs as it use waste as food by microorganism during growth process to produce protoplasm for new cell (Pradeep, 2008).

Microbes in the soils and water such *Pseudomonas*, *Rhizobium*, and *Agrobacterium*, have a natural ability to breakdown many of the hydrocarbon compounds and any hydrocarbon which is exposed to the air will also have an affinity to volatilize. (Harwood, 1989 & Reeves, 2000). As well, reactions including photochemistry and the various transformations of the hydrocarbon through these reactions can enhance the hydrocarbon decomposition. The selected of *Pseudomonas putida* used in the biological treatment of BTX in petrochemical wastewater because the capability of *Pseudomonas putida* to breakdown the aromatic hydrocarbon to carbon dioxide (CO<sub>2</sub>) and water (H<sub>2</sub>O) since *Pseudomonas putida* is aerobic metabolisms. Furthermore, it is a non pathogenic which is not bring any dieses while reaction occur (Harwood, 1989 & Cornelis, 2008)

## 1.2 Problem statement

BTX are among frequently of the hazardous chemical that presence in Petrochemical wastewater. Based on their toxicity and carcinogenic potential that will harm human, animal and environment a proper treatment is needed to reduce the hazard of BTX before discharge to the environment . The standards of discharge petrochemical wastewater will guided by the regulation that have been constructed by EPA and OSHA to protect the safety and health.

## 1.3 Research objectives and scope.

The objective of this proposal is to treat BTX from Petrochemical Wastewater using *Pseudomonas putida*. In order to achieve the objectives stated, the following scopes of study have been listed.

- i. To study *Pseudomonas putida* growth curve.
- ii. To determine the cell dry weight of *Pseudomonas putida*.
- iii. To investigate the effect of temperature shaker on BTX removal.
- iv. To study the effect of shaking speed on BTX removal.
- v. To investigate the effect of *Pseudomonas putida* ratio to solvent on BTX removal.

#### **1.4 Significance of study**

According to the research scopes mentioned above, the following significance that have been outlined are:-

- i. It shall reduce the hazard of BTX petrochemical wastewater.
- ii. Alternative way to treat BTX in petrochemical wastewater in order to.
- iii. It shall reduce factory's waste disposal costs.
- iv. It shall reduce pollution and environmental problem.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.0 HYDROCARBON

A hydrocarbon is one of the most simple and primitive of organic compounds. They are a common and natural happen in the environment and have varying concentrations in storm water and effluent water. Hydrocarbons in water can be found as free floating, emulsified, dissolved, or adsorbed to suspended solids. Basically hydrocarbons compound contain only carbon and hydrogen atoms. Hydrocarbons are broken down into three main classes; aliphatic, alicyclics, and aromatics (Reeves, 2000).

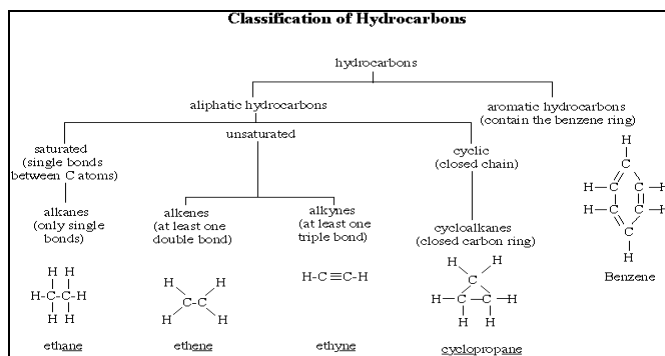


Figure2.1: Three Main Classes of Hydrocarbon

Aliphatics is an open chain compounds, bonded in a linear chain. This hydrocarbon can be whether in saturated or unsaturated phase. Saturated aliphatic consist of single bond aliphatics. While, unsaturated aliphatic consist of double and triple bond of aliphatic.



Typically aliphatics include ethane, actelyene, and 1,2 butadiene, and the most popular; methane (Alan & Wilkinson, 1997).

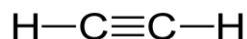


Figure 2.2: Unsaturated Triple Bond of Aliphatic Compound (acetylene)

Alicyclics or cyclic as indicated by their name, contain rings of carbon atoms in their structure. The ring size and number of hydrocarbon can vary which increases the number and classes of this compound. Examples of alicyclics include cyclopropane and cyclopentane (Alan & Wilkinson, 1997)

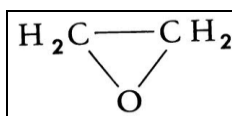


Figure2.3:Alicyclic Compound(Ethylene)

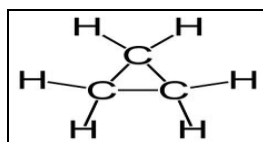


Figure2.4: Alicyclic Compound(Cyclopropane)

Aromatic hydrocarbons typically contain at least one 6-membered benzene ring which is called Polycyclic aromatic hydrocarbons (PAHs). This aromatic compound can be bonded with other aliphatic, alicyclic or with aromatic itself. As the name is aromatic, these compounds typically related to odor and fragrance. Benzene, toluene and xylene (BTX) is part of common aromatic compound (Alan & Wilkinson, 1997)

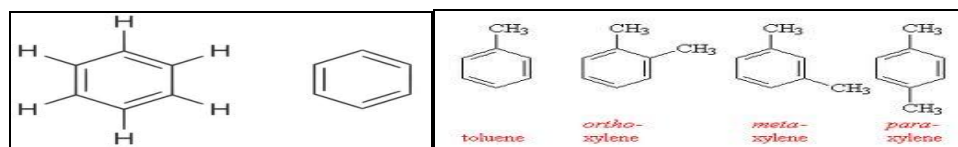


Figure 2.5: Aromatic Compound (BTX)

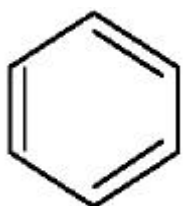
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## 2.1 AROMATIC HYDROCARBON

“Aromatic” term started use as chemical when it introduced by August Wilhelm von Hofmann who is a German chemist in 1855. Aromatic term always apply to compounds that contain the phenyl radical. In organic chemistry, the structures of some rings of atoms are unexpectedly stable. Aromaticity is a chemical property in which a conjugated ring of unsaturated bonds, lone pairs, or empty orbital exhibit a stabilization stronger than would be expected by the stabilization of conjugation alone. It can also be considered a manifestation of cyclic delocalization and of resonance (Schleyer, 2001).

## 2.2. BENZENE

### Benzene



(Benzol; Benzole; Cyclohexatriene)

**CAS Registry Number: 71-43-2**

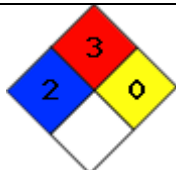
Figure 2.6: Benzene Structure

### 2.2.1 PHYSICAL AND CHEMICAL PROPERTIES

Table 2.1 Physical and Chemical Properties of Benzene (HSDB, 1994; 1999)

PROPERTIES	VALUE
Appearance	Colorless liquid
Molecular formula	C <sub>6</sub> H <sub>6</sub>
Molecular weight	78.1 g/mol
Density at 25° C	0.879 g/cm <sup>3</sup>

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Melting point	5.5 °C
Boiling point	80.1°C
Solubility	Soluble in ethanol, chloroform, ether, carbon disulfide, acetone, oils, and glacial acetic acid; slightly soluble in water
Vapor Pressure at 25°C	0.13 atm.
Flash point (closed cup)	-11.1°C
Conversions at 25°C	1 ppm = 3.25 mg/m <sup>3</sup> 1 mg/liter = 313 ppm
Viscosity at 20 °C	0.652 cP
NFPA 704	

### 2.2.2 APPLICATION

Benzene has commonly used as a multipurpose organic solvent. Styrene monomer is the largest use of benzene, followed by cumene/phenol, cyclohexane, and nitrobenzene. Besides, benzenes also use in the manufacture of various plastics, resins, and detergents. Furthermore Syntheses of pesticides and pharmaceuticals also involve benzene as a chemical intermediate (HSDB, 1994).

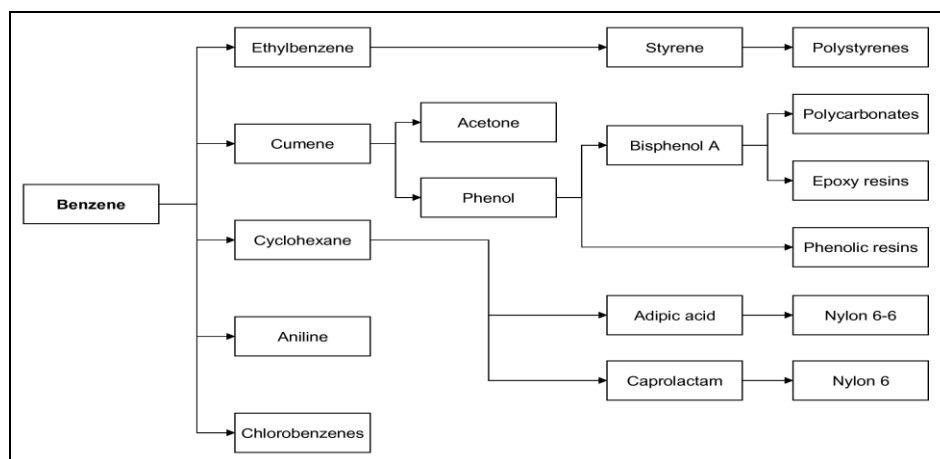


Figure2.7: Major Commodity Chemicals and Polymers Derived from Benzene

### 2.2.3 EXPOSURE

The media exposure of toxic benzene can occur whether from indoor air, ambient air or in food and drink. The indoor air exposure happens mainly from cigarette smoke, smoke from burning wood and some from benzene household products like cigarette lighter fluid, lacquer thinner, ink markers and some glue. The ambient air exposure commonly occurs when someone is exposed to vehicle exhaust or living in the industrial area. The exposures to drinking water happen when leaking of benzene storage to the soil and groundwater. Even though, this accident is least likely to happen but it still needs to be prevented. Some of the diet supplements and vitamin C are detected to contain a lower concentration of benzene, which is approved, but the elimination of benzene is better. Vegetables that are watered with water that is contaminated with benzene are also one part of the media to benzene exposure (EPA, 2009).

### 2.2.4 EFFECT OF EXPOSURE

The chronic effect of prolonged inhalation and exposure to benzene can damage marrow bone, which is the decreasing of red blood cells in the body that can lead to anemia or can cause leukemia, which is the increasing of white blood cells in the body.

(N G Abraham, 1996). Besides, US Department of Health and Human Services (DHHS) classifies benzene as a human carcinogen agent that can cause a fatal cancer of the blood-forming organs. Benzene also can affect in menstrual cycle and contaminate in breast milk that can harm to child (EPA, 2009).

The range acute effect of benzene exposure is dizziness to death. Inhale Lower concentration of benzene will effected human by dizziness, drowsiness, headache and rapid heart rate. The effect of taking food or drink that contains benzene will have the same effect but with high concentration dose of benzene the fatal lose is possible to happen because it direct damage the body and immunity system(SA Health,2008). The effect of carcinogenic agent does not affect human but as well as animal and the effect is slightly similar to human (EPA, 2009).

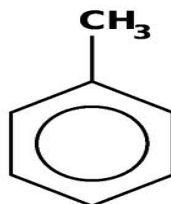
### **2.2.5 PROCEDURE EXPOSURE**

According to the US Environmental Protection Agency and Occupational Safety and Health Administration the maximum permissible level of benzene in drinking water is 0.005 milligrams per liter (0.005 mg/L) and a permissible exposure air limit of 1 part of benzene per million parts of air (1 ppm) in the workplace (OSHA). Besides that, the spills or accidental releases of more than 10 pounds (4.5 kg) of benzene into the environment required to inform to the EPA.

When benzene enters human body it will temporary store in bone marrow or fat before it breakdown or metabolized by liver and further will discharge in the urine. This process took 48 hours to complete but it still has a side effect to human body. Exposure of benzene in the air takes more than 8 days to breakdown to other compound (SA Health,2008).

## 2.3 TOLUENE

### TOLUENE



(Methyl benzene; methyl benzol; phenyl methane; toluol)

**CAS Registry Number: 108-88-3**

Figure 2.8: Toluene Structure

#### 2.3.1 PHYSICAL AND CHEMICAL PROPERTIES

Table 2.2: Physical and Chemical Properties of Toluene (HSDB, 1999 & EPA)

PROPERTIES	VALUE
Appearance	Colorless liquid
Molecular formula	$C_7H_8$ or $C_6H_5CH_3$
Molecular weight	92.14 g/mol
Density at 20 °C	0.8669 g/mL
Melting point	-93 °C
Boiling point	110.6 °C
Solubility	miscible in most organic solvents
Vapor Pressure at 25°C	0.13 atm.
(Flash point (closed cup))	4 °C
Conversions at 25°C	1 ppm = 3.76 mg/m <sup>3</sup>
Viscosity at 20°C	0.590 cP
NFPA 704	

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### 2.3.2 APPLICATION

Benzene is the main product from toluene in industrial. The household product that comes from toluene are aerosols, nail polish, paints and paint thinners, lacquers, rust inhibitor, gums and as cleaning agents. Sometimes toluene is used as in printing operations, leather tanning and chemical processes like solvent extraction. The least of toluene product as well as spot removers, paint strippers, cosmetics, perfumes, and antifreeze (OEHHA).

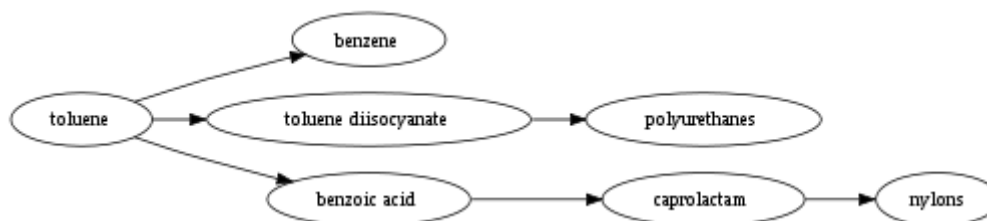


Figure 2.9: Chemicals and Polymers Derived from Toluene.

### 2.3.3 EXPOSURE

Toluene exposure not directly expose when it release from industrial or from their container to the environment. But the toluene exposure can happen while breathing, drinking, eating and skin contact with toluene compound from consumer product. Similar to the benzene exposure toluene can be exposing by inhalation. In ambient air toluene exposure occur from car exhaust fumes, evaporation of gasoline, printer ink and comes from odor of glue, paints, and lacquers. Indoor exposure of toluene comes from the area of living in the industrial area or from household product like paintbrush cleaners, stain removers, fabric dyes and some cosmetics. The amount of toluene in cigarette smoke is less than benzene. Besides toluene can be expose when use water for cleaning, shower or cooking. Toluene in food can be reducing by cooking but we maybe inhale it in air when toluene evaporates. Sometimes egg also can expose to toluene when wrapped it using

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polystyrene containers containing toluene. Toluene exposure also possible to expose from taking medicine like combine of toluene and aspirin can damage hearing (ATSDR, 2000)

#### **2.3.4 EFFECT OF EXPOSURE**

Exposure to toluene major cause on the human central nervous system (CNS) like unconscious, heartbeat, sleepiness, headache, tiredness, and loss of appetite, hearing and vision for a temporary and after a certain time it will back to normal. But this effect depend on the amount take, how long expose and genetic susceptibility like age, gender, health condition also in consideration. Long term of exposure is possible to have permanent effect in brain and body that can cause death.

Expose to the high concentration of toluene can damage kidney, lung and liver. Inhalations of toluene not affect the reproductive system but for pregnancy women it can lead to fatal problem like slow mental ability and growth. Animal have similar effect exposure of toluene. However Environment Protection Agent confirmed that toluene is not carcinogenic agent (ATSDR, 2000 & OEHA)

#### **2.3.5 PROCEDURE EXPOSURE**

The Clean Air Act Amendments of 1990 list toluene as a hazardous air pollutant. Thus, Occupational Safety and Health Administration have set a limit of 200 ppm of toluene for air in the workplace that averaged for an 8 hour exposure per day over a 40 hour work week. But American Conference of Governmental Industrial Hygienists (ACGIH) recommends that toluene in workplace air not exceed 50 ppm, and NIOSH recommends that toluene in workplace air not exceed 100 ppm in the same average of hour of work. While in the drinking water maximum contaminant level (MCL) for toluene that guided by Environment Protection Agent is 1 milligram per liter of water (1 mg/L). Any release of more than