UMAIMAH BACHELOR OF CHEMICAL ENGINEERING 2012 UMP

BTX TREATMENT FROM PETROCHEMICAL WASTEWATER USING *PSEUDOMONAS PUTIDA*

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UMAIMAH BINTI MOHAMED KHIR

Thesis submitted in fulfillment of the requirements
for the award of the degree of
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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this project report and in our opinion this project is satisfactory in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

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STUDENT'S DECLARATION

I hereby declare that the work in this report is my own except for quotations and summaries which have been duly acknowledged. The report has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Date : 20th JANUARY 2012

DEDICATION

Specially dedicated to my beloved parents and best friends for their full support and love throughout my journey of education. You are forever in my heart .God bless them.

ACKNOWLEDGEMENT

Assalamualaikum w.b.t.

Firstly, I am thankful to ALLAH S.W.T for blessing me in finishing this final year project (FYP) with successful complete and in achieving the objectives of this project. Hopefully, this project will be benefit to all.

In this opportunity, I would like to give my sincere gratitude to my Family, Mohamed Khir Bin Abdullah my father, Zakiah Binti Mokhtar, my mother and all of family members for morale support, motivation and encouragement in completing this final year project and in finishing my study in UMP with successful.

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My special thanks to the friend that gives support and always company me in making and complete this project.

Thank you.

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^{O}C,180rpm and1:9 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 hirdokarbon, 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 Agecy (EPA) sebelum ia dilepaskan kealam sekitar . Pemilihan *Pseudomonas Putida* mikroorganisma dalam rawatan secara biologi adalah disebabkan keupayaannya yang mampu menghuraikan aromatic hidrikarbon dan menyediakan rawatan yang bersih dan selamat tanpa menghasilkan bahan kimia yang merbahaya semasa juga setelah proses rawatan selesai.Kajian yang dijalankan merangkumi kesan kelajuan goncanagn,suhu dan nisbah bacteria kepada larutan BTX bagi mengurangkan kepekatan BTX didalam sisa air buangan. Pertumbesaran bacteria dan peratusan pengurangan BTX dikaji mengunakan 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^{O}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

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

LIST OF ABBREVIATIONS

GC	Gas Cromatography
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).

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 ($CO_{2)}$ and water (H_20) 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).

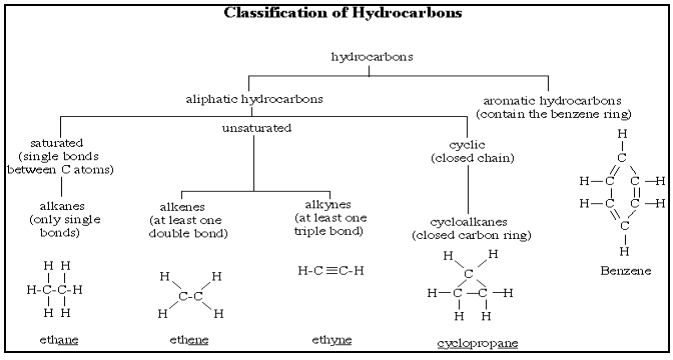


Figure2.1: Three Main Classes of Hydrocarbon

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).

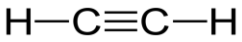


Figure 2.2: Unsaturated Triple Bond of Aliphatic Compound (acetylene)

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

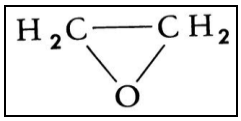


Figure2.3:Alicyclic Compound(Ethylene)

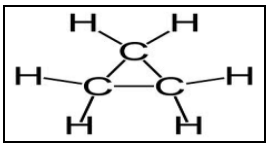


Figure2.4: Alicyclic Compound(Cyclopropane)

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)

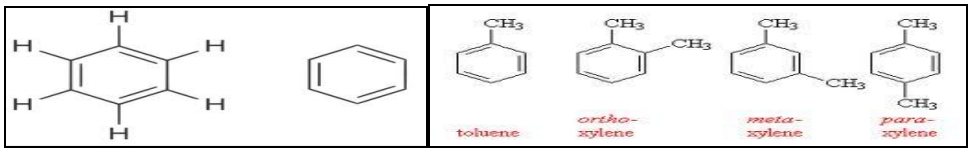


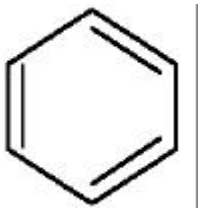
Figure 2.5: Aromatic Compound (BTX)

2.1 AROMATIC HYDROCARBON

"Aromatic" term started use as chemical when it introduced by August Wilhelm von Hofmann who is a German chemist in1855. 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*)

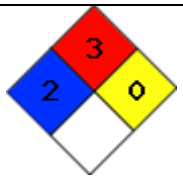
CAS Registry Number: 71-43-2

Figure 2.6: Benzene Structure

2.2.1 PHYSICAL AND CHEMICAL PROPERTIES

Table2.1 Physical and Chemical Properties of Benzene (HSDB, 1994; 1999)

PROPERTIES	VALUE
Appearance	Colorless liquid
Molecular formula	C_6H_6
Molecular weight	78.1 g/mol
Density at 25° C	0.879 g/cm3

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/m3 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).

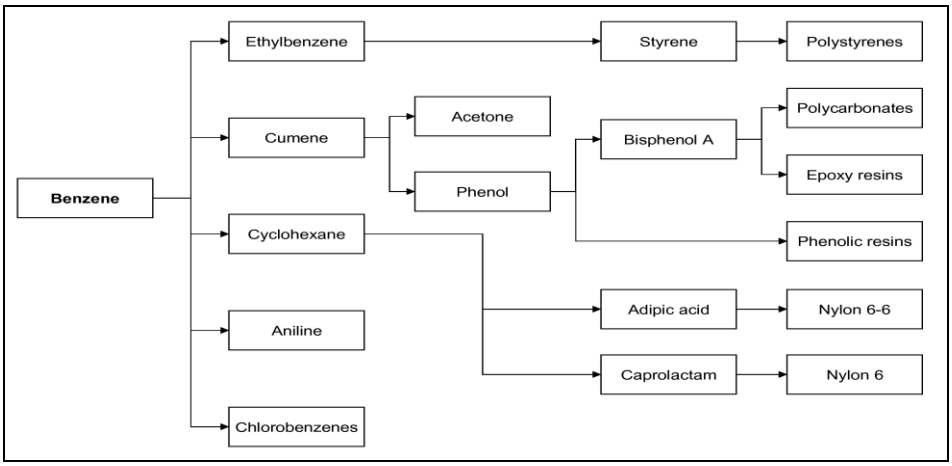


Figure2.7: Major Commodity Chemicals and Polymers Derived from Benzene

2.2.3 EXPOSURE

The media exposure of toxic benzene can be occur whether from indoor air, ambient air or in food and drink. The indoor air exposure happen mainly from cigarette smoke, smoke from burning wood and some from benzene household product like cigarette lighter fluid, lacquer thinner, ink markers and some glue. The ambient air exposure commonly occur when someone expose 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 happen but it still needs to prevent. Some of diet supplements and vitamin C are detected contain a lower concentration of benzene which is approved but the elimination of benzene is better. Vegetables that watering with water that contaminating with benzene also is one part of media to benzene expose (EPA, 2009).

2.2.4 EFFECT OF EXPOSURE

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

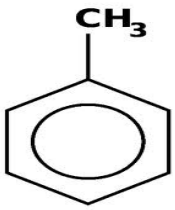
2.2.5 PROCEDURE EXPOSURE

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



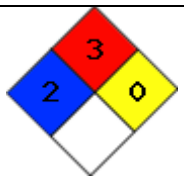
(*Methyl benzene; methyl benzol; phenyl methane; toluol*)

CAS Registry Number: 108-88-3

Figure 2.8: Toluene Structure

2.3.1 PHYSICAL AND CHEMICAL PROPERTIES

Table2.2: 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/m3
Viscosity at 20°C	0.590 cP
NFPA 704	

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).

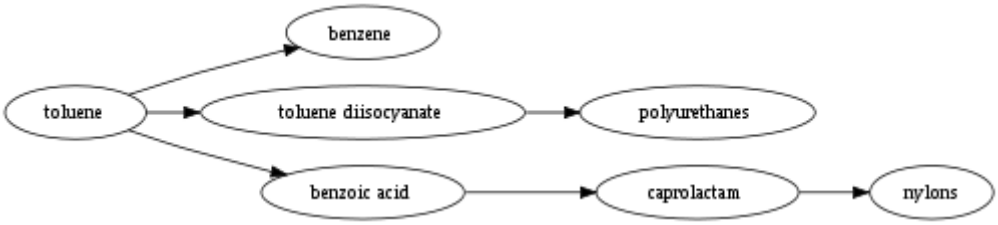


Figure 2.9: Chemicals and Polymers Derived from Toluene.

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

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 & OEHHA)

2.3.5 PROCEDURE EXPOSURE

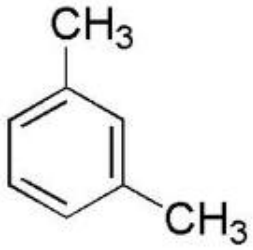
The Clean Air Act Amendments of 1990 list toluene as a hazardous air pollutant. Thus, Occupational Safety and Health Administration have set a limit of 200 ppm of toluene for air in the workplace that averaged for an 8 hour exposure per day over a 40 hour work week. But American Conference of Governmental 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

1,000 pounds of this chemical to the environment must be reported to the National Response Center (ATSDR,2000).

When toluene enters human body it will directly transfer to blood from lung. It takes 12 hours to discharge toluene from human body whether from exhalation or urine. Sometimes human body turns toluene into less harmful chemicals such as hippuric acid (ATSDR, 2000)

2.4 XYLENE

XYLENES



Xylol or m-,o- and p-xylenes

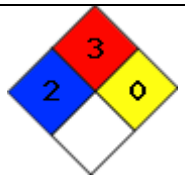
CAS Registry Numbers: 1330-20-7 (technical mixture of o-, p-, and m-xylene); 95-47-6 (o-xylene); 108-38-3 (m-xylene); 106-42-3 (p-xylene)

Figure 2.10: xyleneStructure

2.4.1 PHYSICAL AND CHEMICAL PROPERTIES

Table2.3: Physical and Chemical Properties of Xylene (OEHHA & EPA)

PROPERTIES	VALUE
Appearance	clear, colorless liquid
Molecular formula	C_8H_{10}, $C_6H_4(CH_3)_2$ or $C_6H_4C_2H_6$
Molecular weight	106.16 g/mol
Density at 20 °C	0.864 g/cm3 (technical mixture); 0.881 (o-); 0.860 (m-); 0.861 (p-)
Melting point	-25.2 °C (o-); -47.8°C (m-); +13.2 °C (p-)

Boiling point at 1atm	137-140°C (technical mixture); 144.5 °C (o-); 139.1°C (m-); 138.3 °C (p-)
Solubility	• practically insoluble in water • miscible with absolute alcohol, ether and Soluble in non-polar solvents such as aromatic hydrocarbons
Vapor Pressure at 25°C	6.6 torr (o-); 8.39 torr (m-); 8.87 torr (p-)
(Flash point (closed cup)	17 °C (o-); 25°C (m-); 25 °C (p-)
Conversions at 25°C	1 ppb = 4.34 mg/m3
Viscosity at 20°C	0.812 cP (o-); 0.62 cP (m-); 0.34 cP (p-)
NFPA 704	

2.4.2 APPLICATION

Xylene often use in the industry as chemical solvent in production of ink,thinner, pharmaceuticals, perfumes, fabricated items and pesticide formulations, paints, and varnishes. Xylene also functions as produce a monomer called terephthalic acid that usually use in the polymer production. It is a good cleaning agent for silicon wafers and steel. Xylene is used as a feedstock in the production of petrol and small proportions in gasoline and jet fuel. In histology, xylene use to clean tissue for the preparation of paraffin wax and to prepare very thin slice of tissues for microscopic examination by making them hydrophobic so a coverslip can be used. Xylene is also used in breathing devices as mask(OEHHA).

2.4.3 EXPOSURE

Exposure of xylene can occur similar to benzene and toluene. It can be exposing by inhalation and skin contact. Since xylene is easily evaporate to the air

the can be happen whether at working place or in ambient environment. From the California Environmental Protection Agency study they found that 60% of xylene exposure comes from the route inhalation oh household product or air in working place and the rest of 40% is comes from drinking water. The amount of xylene in soil is found much higher than water because the leaking of storage or pipes that

2.4.4 EFFECT OF EXPOSURE

Xylene has a strong odor and many exposed persons react negatively at concentrations above a few parts per million in airs the odor threshold is about 1 ppm. Commonly xylene effect on central nervous system as the same way of alcohol drinks effect. Human reactions at about 1 to 100 ppm of xylene in air can effect include nausea, headache and eye, nose, throat and lung irritation. exposure to more than a few minutes to concentrations in air of 100 ppm or more can cause the previously listed symptoms, plus sedation, disorientation and muscular in coordination. With prolonged or repeated inhalation exposures to xylene in the concentration range of about 50 to 2,000 ppm in air for several hours per day can cause damage in the liver and kidney. Based on the study previously they have proved that xylene cannot cause a cancer (EPA).

2.4.5 PROCEDURE EXPOSURE

Occupational Safety and Health makes and enforces regulations for chemical exposure in the workplace. The Permissible Exposure Limit (PEL) for the amount of xylene in your breathing zone. The PEL for xylene is 100 parts of xylene per million parts of air or 100ppm. Your exposure may legally be above 100 ppm at times, but only if it is below 100 ppm at other times, so that your average exposure for any 8-hour work-shift is 100 ppm or less. OSHA has also adopted an "excursion"

limit of 200 ppm, which may be exceeded for no more than 30 minutes during any 8-hour shift, and a "ceiling" limit of 300 ppm, which must never be exceeded for any period of time. These limits are set at levels intended to protect against effects on nervous system, eyes, nose, and throat (DHS)

2.5 MICROORGANISM

Microorganism is the organism that can live as unicellular or in colony cellular which is called multicellular organism. This microorganism is very tiny and visible to the naked eye, therefore it required microscope to see the microorganism. The first microorganism was found by Anton van Leeuwenhoek in 1675 with his own microscope design. This discovery was contributed to the microbiology subject and also revealed the secret of decades why grapes can turn to wine and milk can change into cheese as well as why food can be poisoning (Eddleman, 1997).

Microorganism had variety of group consist of bacteria, fungi, archaea, and protists, microscopic plants like green algae and in animals such as plankton and the planarian. Besides, some of microbiologists believe that virus is included but part of them considers these as non-living thing. But sometimes one microorganism can be placed into many group since there are varies method to identifying microbe whether use temperature or categorized either in prokaryote or eukaryote group. Microorganism can be found in all part of biosphere since it can be living in soil, desert, salt or fresh water, in the air or even in the earth crust. As cited by Jim Deacon (2003) the total of microorganism in our body is more than the cell that built up our body. He also reported around 200 miles per hour of microorganisms will leave the lungs when a human sneezes.

Microorganism can be divided into two parts whether it can be pathogenic microbes or not. Pathogenic is the microbe that will bring dieses and harm to human, plant and

animal while non-pathogenic is vice verse. This type of microbe is depend to each other because the dieses is affect by the pathogenic thus the non-pathogenic microbe is needed to heal the dieses which is use as antibiotics it like the life cycle of microbe. The production of microorganism can be from sexual or asexual (single parent) reproduction. Sexual production require both male and female microbe but asexual just need one of them which is production happen by cell division, mitosis (Eddleman, 1997 & Deacon 2003).

2.5.1 BACTERIA

The oldest fossils known nearly 3.5 billion years old are fossils of bacteria. Bacteria are importance in life cycle because of their extreme flexibility, capacity for rapid growth and reproduction and great age. Bacteria can defined as single-cell organisms have neither a membrane-enclosed nucleus nor other intracellular organelles like mitochondria and chloroplasts. It simpler than eukaryotes cause consist of single circular DNA chromosome that is found within the cytoplasm of the cell as they do not have a nucleus. Bacteria have varies of length and shape in range from spheres to rods and spirals, besides some of them has flagella as medium of movement (Kimball, 2011).

As other creature bacteria get energy or eat such as sugars, proteins and vitamins to live. Part of the method to obtain food is by photosynthesis some of bacteria have chlorophyll like blue-green algae that can make their own food from light energy and carbon dioxide. Some of them have red chlorophylls that have same function to produce sugar. Besides they also can oxidize iron or sulfur and digest proteins down to amino acids or breakdown complex hydrocarbon to simple compound to obtain energy and mineral (Eddleman, 1997).

Kimball (2011) stated that differ Bacteria have greatly differ life style in order to growth in optimum condition. Some grow best in cool places such as soil or bodies of water, but some are able to grow in hot springs, hot water heaters, or undersea volcanoes. The bacteria grow best at body temperatures usually is pathogenic that can harm to mammals and birds. Some bacteria effect diseases in hydra, snakes, turtles, and other cold-

blooded animals, are not able to cause disease in birds or mammals because the high body temperatures kill these bacteria or limit their growth. Besides, the growth of bacteria depends on effect of pH, temperature, osmotic pressure, oxygen concentration, and other environmental factors (Eddleman, 1997 & Deacon 2003).

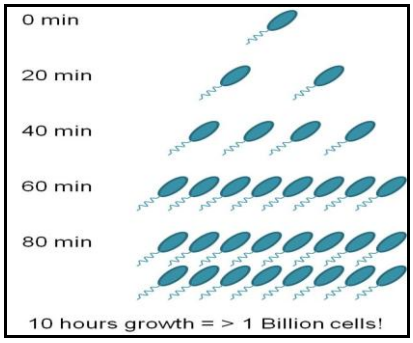


Figure 2.11: Bacterial Reproduction

Bacteria cell in biological treatment usually reproduction by binary diffusion the mother of cell will produce two daughter and continuously growth. The growth can classify as batch culture (close system) or continuous culture (open system) .Batch cultures occur in the present of limited substrate while continuous batch is supply with continuous substrate. The growth of bacteria is proportional to the mass of bacteria after the treatment. The optimum growth of bacteria can be assumed similar like the picture above:

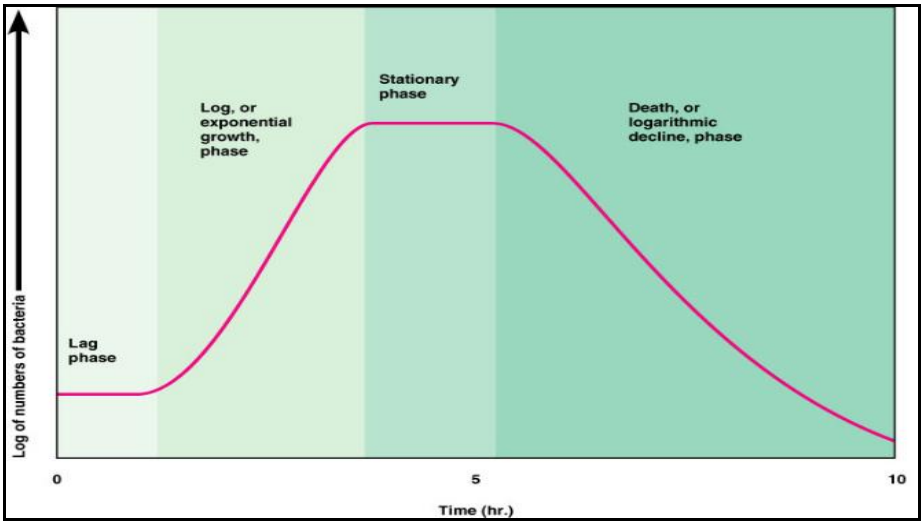


Figure 2.12: Bacterial Growth Curve

The events at each of phase are concluded as:

Table2.4: Phase and Event of Bacteria Growth

PHASE	EVENT
Lag	– Begin expose to substrate (compound to degrade) – Adjust the life style to the new habitat – Slightly no mass increase.
Log , Exponential	– After comfortable they start to degrade the substrate – Rapidly increase in mass
Stationary	– Growth enough – Lack of substrate – The number of bacteria remain constant
Death	– Accumulation of waste – Death rate is faster than growth rate – Mass decrease

2.5.2 *Pseudomonas Putida*

Pseudomonas putida can be describe as Gram-negative rod-shaped and have one or more polar flagella that help they o move faster. Besides *Pseudomonas putida* are fluorescent, aerobic, non spore forming, oxidize positive bacteria. They mostly found in moist environments, such as soil and water habitat in the present of oxygen since it aerobic microorganism. The optimum growth are usually at room temperature or in range of 25-30^{0}C. Certain strains in *Pseudomonas putida* have the ability to grow on and break down many dangerous pollutants and aromatic hydrocarbons such as toluene, benzene, and ethylbenzene. Thus, *Pseudomonas putida* can also be used in petroleum plants to purify fuel. It also use in formation of biodegradable plastic Polyhydroxyalkanoates (PHA) from

styrene oil. Consequently help in degrade the polystyrene foam which is non-biodegradable. Besides particularly found around the roots of plants that protect plants from disease by other microorganisms Due to the strong appetite of aromatic hydrocarbon *Pseudomonas putida* widely use in laboratory for research on bacteria-remediated soil processes (Cornelis, 2008)

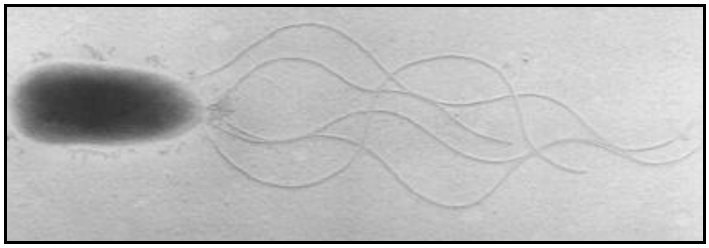


Figure 2.13: *Pseudomonas putida*

Table2.5: Scientific Classification *Pseudomonas putida*

Domain	Bacteria
Phylum	*Proteobacteria*
Class	*Gamma proteobacteria*
Order	*Pseudomonadales*
Family	*Pseudomonadaceae*
Genus	*Pseudomonas*
Species	*Pseudomonas putida*

Pseudomonas putida consists of genome of any known species involved in breaking down aromatic hydrocarbons. Aromatic hydrocarbons are hazardous chemicals generated by the burning of coal, gas, tobacco, meat and other organic matter. Research is being done on the difference in genome sequencing of *Pseudomonas putida* and its relative *Pseudomonas aeruginosa* in relation to cystic fibrosis. *P. aeruginosa* infects and kills those with the disease while *Pseudomonas putida* lacks the genes that cause such destruction, like the genes that code for enzymes that it cannot degrade cellular membranes or release toxins.

Generally, *Pseudomonas putida* has been designated as a safe bacterium for use in the cloning of genes for bioremediation work as degrade pollutants in water or soil. It has a very versatile metabolism, and can degrade an array of toxic chemicals, including gasoline components. Other strains can convert packing peanuts, made of polystyrene foam, into a biodegradable plastic. Furthermore it also important in maintaining plant health and improves plant health by produces molecules that reduce iron from the area around the plant.

2.6 WASTEWATER TREATMENT

Wastewater treatment is not the modern practice that created recently but this activity had been implementing as common in ancient Rome and widely use started on the early of 19^{Th} century after all the citizen able to understand the necessary of reducing the amount of pollutant that they discharged into the nature. Naturally, fresh water had the ability to cleanse themselves from the material that disturbed their purity but late of 1850 the civilian become threatening by the high concentration of water pollution that chaos their life since at that time they commonly use the water river directly from it source (Guelph City).

Until mid 19^{th} century the relationship between human waste, drinking water and disease was documented as the first step to approach the development of wastewater treatment as the alternative way to offer a protection and preservation of our natural water resources instead of protect the human health. From the documentation their release that the content of pathogenic in water had contributed to the water polluted. Thus the wastewater treatment is develop to encounter the cholera cases that comes from the consumption of water. Since the water can naturally treat themselves so that the function of waste treatment is needed to speed up the natural cleansing process by implementing biological, physical, chemical and mechanical techniques that available and suitable. The combination of this

technique result the public health and water quality become better and more protected compare than ever before (Guelph City).

Source of wastewater comes from the water discharge from homes, businesses, industries, commercial activities and an institution which is directly transfer to treatment plants through the systematically designed and engineered network of pipes. The largest amount of waste comes from domestic wastewater that refers to flows discharged that basically comes from residential sources generated by such daily activities as food preparation, laundry, cleaning and personal hygiene. The industrial or commercial wastewater is flow generated and discharged from manufacturing and commercial activities such as printing, food and beverage processing and production. Institutional wastewater characterizes wastewater generated by large institutions such as hospitals and educational facilities (Howard. et al, 1985).

The waste water treatment process means the series of action and activities that combine the incorporate numerous processes in order to achieve the desired water quality. The water treatment consists of separation, removal and disposal the pollutant and undesired material that present in the wastewater. The waste water treatment basically comes from the combination of basic techniques of physical, biological and chemical. Basically the physical treatment is use as the primary treatment in wastewater to remove the solid material from the fresh wastewater before it proceeds to the biological and chemical treatment. The process units that usually use in physical treatment are sedimentation, separation and filtration. The function of physical treatment just to separate the water from the solid material without concern about the other parameter (Predeep ,2008).

Biological process had found little use in the treatment of water supplies cause the low level of biodegradable organics in the raw water. However, the biological process is use extensively to convert the biodegradable organics and other nutrient into a more manageable and beneficial form. Biological is consider as the secondary treatment after the

physical treatment, which is function to dissolved and colloidal organic are converted into biomass that is subsequently separated from the liquid stream. This stage is design to optimize the contact between microorganisms and organic under the most favorable environmental condition (Howard. et al, 1985).

Chemical treatment usually consider also as secondary stage of wastewater treatment. This method enhances the efficiency of other process operations and provides specialized treatment as a result of their addition at various treatment stages. In this stage chemicals may added to alter the equilibrium condition and cause the precipitation of undesirable species. The chemical treatment adjust the parameter of wastewater such pH, hardness, colour, taste and odor to fulfill the requirement of water quality. Some chemical maybe use in the process for the special purpose like chlorination and coagulation to obtain the effectively treatment. Chemical processes are conversion processes that actually remove the contamination by physically separation of solid liquid and gaseous from the chemical reaction (Arcadio & Gregorio 2003).

2.6.1 Preliminary

Municipal wastewater comes from residential area, business, industrial and commercial activities are transfer to the waste treatment area through the sanitary sewer pipes line. The gravitational force is naturally applied on the water flow so that the waste will flow along the pipeline.

Figure 2.14: Sanitary Sewer Pipelines

In the water treatment plant, the arrival of wastewater will go through screening process unit as the preliminary treatment. The purpose of the screening process through the bar screen is to separate the solid material like trash, tree branch and metal from the water. There are two bar screen placed in the Raw Wastewater Pump in order to effectively separate the water and solid material. Besides that, the installation of grit removal is needed in order to remove the coarse or small stone flow along with the wastewater. Furthermore it also functions to protect the pump and other equipment from damage and clog by the trash. The solid waste that collected will disposal properly to avoid environmental pollution. The water that passed the screening process will pump to the primary stage to go through the next treatment (Predeep ,2008).

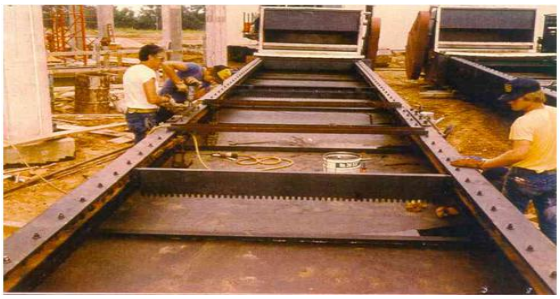


Figure 2.15: Screening process unit

2.6.2 Primary Treatment

Primary treatment is the second stage of the wastewater treatment plant. In the primary treatment it involves the removal of settle able organic and inorganic solids by sedimentation and the removal of float materials by skimming. During the process, the level of suspended solid and Biological Oxygen Demand (BOD) can be reduced. Basically this stage separates the small particle of organic or inorganic material from the wastewater (Arcadio & Gregorio 2003).

Primary sedimentation or clarifier commonly use in the sedimentation process unit. The settled solid or primary sludge are removed from the bottom of the tank by sludge rakes that gather the sludge to the center wall that then will be pump to the sludge process

unit. If the larger amount of water is treated the biological process by anaerobic digestion is applied (Arcadio & Gregorio 2003).

2.6.3 Secondary Treatment

The effluence from the primary treatment only removes 60 percent of the original suspended solid, organic and inorganic dissolve. The remaining contaminates material need to be reduced to meet the EPA requirement. During the secondary treatment there may consist the combination of physical-chemical or biological process only. The common process units of physical-chemical treatment are coagulation, chemical oxidation, carbon absorption and some unit need other process to reduce the suspended solid and BOD level. As the recently modification this operation unit demand high cost option with respect to both capital and operating expenses. Thus, the biological process is practically applied to encounter the problem of high cost of operation (Howard. et al, 1985).

During biological treatment, microorganism use the organic material in the wastewater as their food supply (substrate) and convert the organic material to biological cell or biomass. Besides, the microorganism able to produce waste ad by product during the process. In the wastewater there will be variety of organic material that also need the variety species of microorganism that is capable to treat the organic material through degradation process since a type of bacteria only able to treat specific organic material(Howard. et al, 1985).

2.6.4 Advance Treatment

The effluence from the secondary treatment will through the advance treatment as the effluence of wastewater not always adequate to meet the discharge requirement. This is often occurs when the larger quantities of effluence discharge into small stream or when delicate ecosystem are encounter. In this case the additional polish treatment or alternative method of wastewater disposal is needed (Predeep ,2008).

The treatment is refers to the removal of nitrogen and phosphorus compound, suspended solid, dissolve inorganic salt and refractory organic. The combination of process can be used to restore the wastewater to body water, although at considerable expense. Sometimes, the secondary or primary unit process will apply as advance treatment to increase the efficiency of wastewater treatment (Predeep ,2008).

2.7 Activated Sludge

Active sludge process is suspended culture system that has been in use since the early 1990s.The process derived the names from the fact that settled sludge containing living or active microorganism is returned to the reactor in purpose to increase the available of biomass and speed up the reaction. The active sludge process is a suspended culture process with sludge return and may either completely mixed or a plug flow process. The process is an aerobic with oxygen being supply by dissolution from entrained air (Howard. et al, 1985).

The organic material present in the primary effluent, which overflows from the primary settling tanks consists a certain characteristics which require additional forms of treatment. This organic material contain of dissolved and finely whether in form of suspended or colloidal solids which account for the turbid appearance of the primary effluent. Naturally, the dissolved organic material present in the influent will remain in solution during primary treatment. While the colloidal solids present are very small in size and mass and do not settle during primary treatment. Practical to increase the detention time of the wastewater in the primary tanks is an alternative way to remove these colloidal solids. Increased detention times would offer the development of septic conditions within the settling tanks and solids removal efficiencies would actually decrease (Howard. et al, 1985).

Secondary biological treatment process is used known as the activated sludge process is needed as the proceeding process to treat the effluence of primary treatment. This process effectively removes the dissolved organic material to a portion of the colloidal matter and converts the remaining colloidal material to a biological sludge which rapidly settles. Activated sludge consists of sludge particles produced by the growth of organisms in the presence of free dissolved oxygen. Activated sludge comes from the fact that the particles are alive and teeming with bacteria, fungi and protozoa. These microorganisms cleanse the wastewater by using the organic material present as a food source to grow and reproduce. The organisms stabilize soluble or colloidal solids by partial oxidation forming carbon dioxide, water, and other chemical compound (Predeep ,2008).

There are many factors which must consider in the design of the active sludge system. Combination of the process variation and reactor type are compatible with the wastewater characteristic and environmental constrain must be selected. Wastewater to be treated is thoroughly mixed with the activated sludge to form what is termed mixed liquor. The mixed liquor flows through large aeration basins which allow for detention times between 4 to 6 hours. High concentrations of biomass in the mixed liquor will reduce the detention time needed to produce good treatment efficiencies with respect to the soluble BOD. In the aeration basins, oxygen is dissolved into the mixed liquor by blowing air through the flow or by mechanical surface mixers which splash the mixed liquor into the air allowing oxygen from the atmosphere to be dissolved. Following this aeration period the aerobic organisms present in the mixed liquor are directed to a secondary clarifier where they flocculate and settle to form sludge. A portion of this settled sludge is sent back to the beginning of the process as return activated sludge to maintain and continue the process. Sludge produced in excess of process requirements is wasted or discharged from the treatment system back to the primary settling tanks or a separate sludge thickening operation (Howard. et al, 1985).

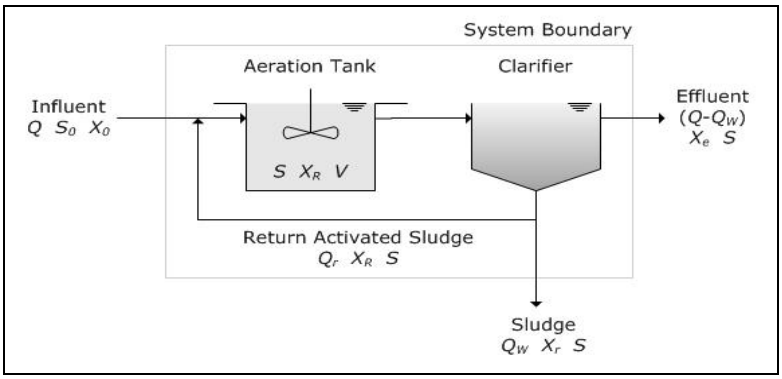


Figure 2.16 : Activted Sludge Unit Process

CHAPTER 3

METHODOLOGY

This chapter is elaborates the procedures that used in the experiment of BTX treatment from Petrochemical wastewater using *Pseudomonas putida*. The experiment is divided into three major scopes of studies which are study on effect of temperature, shaking speed and effect of different ratio of bacteria used. The methodology of this experiment consists about the preparation culture of *Pseudomonas putida*, *Pseudomonas putida* growth curve and cell dry weight, preparation of BTX stock solution, extraction and dilution procedure of sample and also analysis process by UV-Vis Spectrophotometer and Gas Chromatography (GC)

3.1 General flow of the process

Flow chart below indicates the general process of BTX treatment from Petrochemical wastewater using *Pseudomonas putida* started from the beginning until the end process. Before started any procedure each of glassware use in this experiment should be through autoclave and sterilize properly to avoid contamination. The present of contamination will affect the efficiency and result that obtain during the experimental. Besides as the precaution safety, the procedure that deals with microorganism should be carried out in the laminar flow hood.

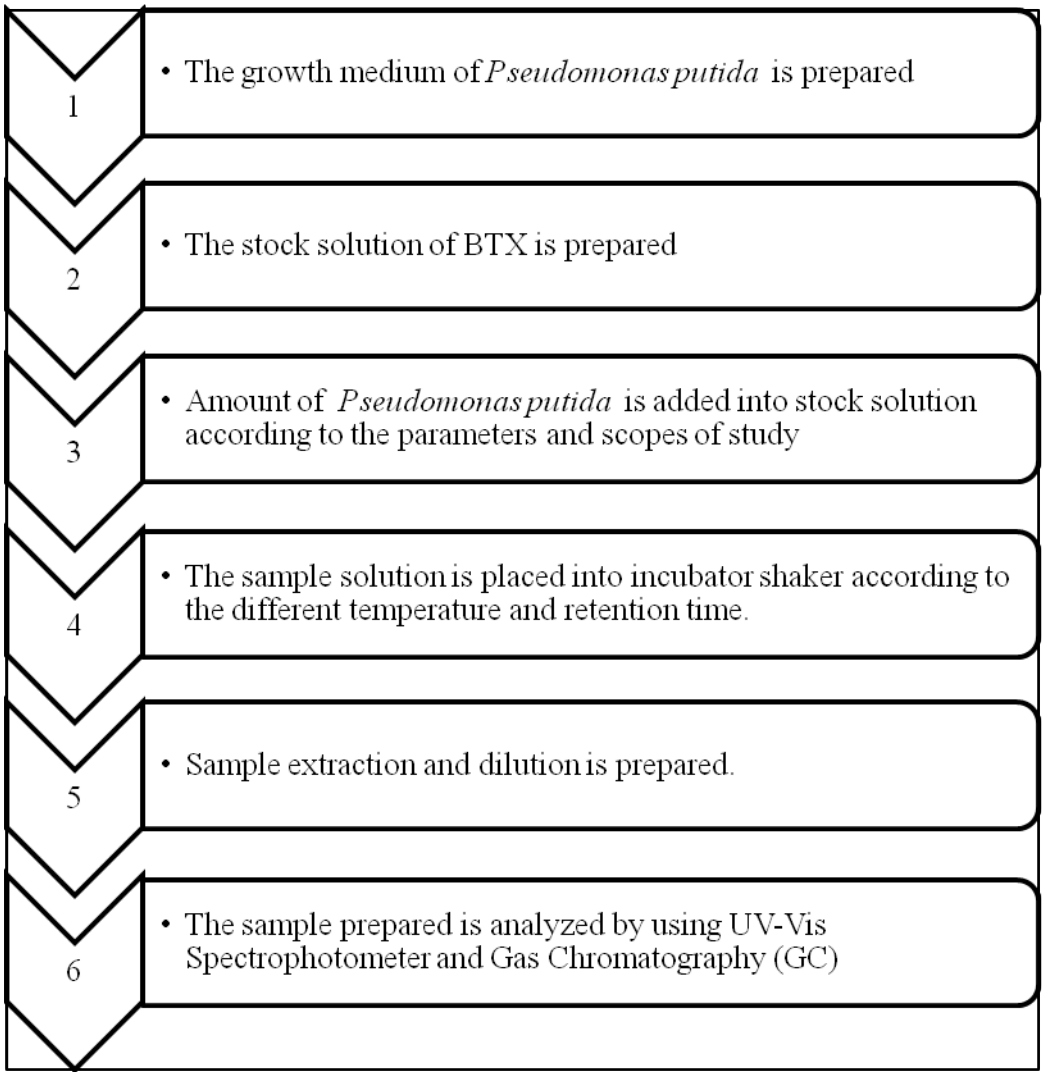


Figure 3.1: Procedure of BTX treatment from Petrochemical wastewater using *Pseudomonas Putida*

(Adapted from Haibo Yu,Byung J.K. & Bruce E.R., 2001)

3.2 PREPARATION OF CULTURE MEDIUM

The preparation of nutrient broth (BD 23400) is started by Weighed 8g of nutrient broth powder and added with 1L of distilled water in a 1L Schott bottle. After the powder dissolve completely in the distilled water the solution is sterile at 121°C for 20 minutes in an autoclave. The same procedure ids carried out in the preparation of nutrient agar (BD 213020) but it needed 23g of nutrient agar powder.

The agar plates is prepared by poured 15-20ml of warm sterile nutrient agar per petri plate and allow the nutrient agar to harden before use or keep in chiller to avoid contamination. While agar slants is prepared by added 5mL of a warm sterile nutrient agar into universal bottle and then Place the universal bottle in a bend position and allow the nutrient agar to harden in this position before use or keep in chiller to avoid contamination.

3.3 REVIVING FREEZE DRY CELL

The *Pseudomonas putida* that use in this experimental is obtain in freeze dry cell. The freeze dry cell condition is use by the supplier to deliver the bacteria cell in safety and guarantee the viable of living organism. In other word the reviving of freeze dry cell is the step to wake up of bacteria from their sleep mode. The reviving of freeze dry cell is needed to proceed the experiment to the next stage. The arriving of *Pseudomonas putida* should be placed in the chiller with 5^{o}C or lower if not it will immediately rehydrated.

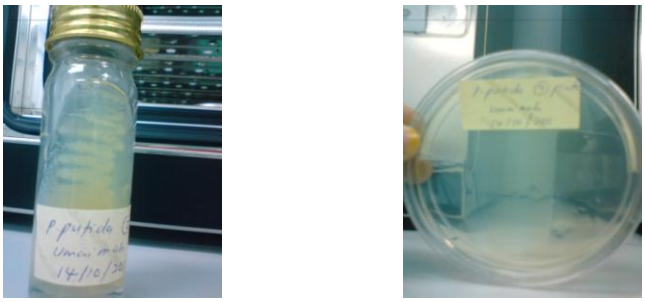
Figure 3.2: Freeze Dry Cell *Pseudomonas Putida*.

The process of reviving *Pseudomonas putida* is carried out in the laminar air flow for the safety purpose. A few drop of nutrient broth is added into the bottle of *Pseudomonas putida* and make sure all contain is saturate with nutrient broth. The solution of *Pseudomonas putida* is transferred to the 10ml of universal bottle that contain 5ml of nutrient broth and keeps in the oven with temperature 37^{o}C for 2days.

(a) (b)

FIGURE 3.2: (a) Fresh Reviving *Pseudomonas Putida*, (b) *Pseudomonas Putida* after 2 Days

After 2 days the present of *Pseudomonas putida* is detected by cloudy appearance of nutrient broth. Then the amount of *Pseudomonas putida* will transfer to agar slant for storage purpose and agar plate that will use in the next step of experiment. Both of them will place in 37^{o}C for 2 days.

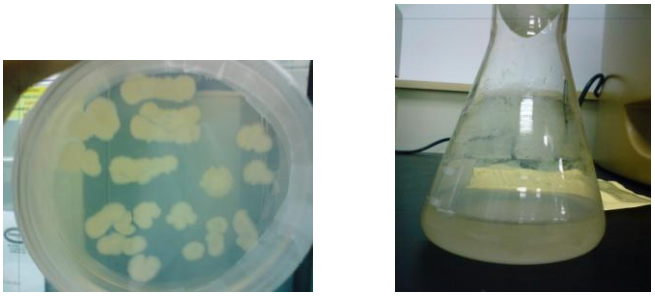


(a) (b)

Figure 3.3: (a) Agar Slant, (b) Agar Plate

The agar slant prepared will be store in 4^{o}C of chiller, while the agar plate will place in laminar flow for the next procedure. In laminar flow the colonial present will transfer to

the several agar plate to get the pure colonial of *Pseudomonas putida* and the plate was keep on the 37^{o}C oven . After 2 day the resent of single colonial *Pseudomonas putida* will observed and take out using inoculating needle then transfer to the100ml of nutrient broth that will placed in the 37^{O}c and 180rpm of incubator shaker for 24 hours.

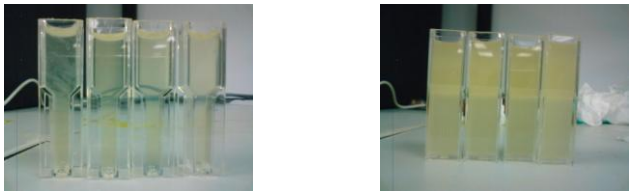


(a) (b)

Figure 3.4: (a) Observed Single Colonial of *Pseudomonas Putida,* (b) 100ml of Nutrient Broth

3.4 PREPARATION OF BACTERIA GROWTH AND CELL DRY WEIGHT

The procedure of bacteria growth and cell dry weight is started by added 20ml of *Pseudomonas putida* solution that obtain from the previous procedure to the conical flask that contain 180ml of nutrient broth. The ratio of bacteria to nutrient broth is 1:9. Then the sample solution will placed in 37^{O}c and 180rpm of incubator shaker. An amount of sample will take at certain hour and placed in the cuvette that will immediately analyze using UV-VIS with wave length use is 600µm.



(a) (b)

Figure 3.5: (a) sample for an hours, (b) sample for 24 hours

The procedure of cell dry weight is carried out simultaneously with bacteria growth.1.5ml of sample is transfer in the microbiological centrifuge tube that has been dry in 100^{o}C oven for 2 hours. The 1.5ml sample in tube will be centrifuge at 12000rpm for 20 minutes before placed in the oven for 2hours. The weight of tube with sample is recorded before and after the sample dry in 100^{o}C oven.

(a) (b)

Figure 3.6: (a) Microbiological Centrifuge, (b) Microbiological Centrifuge Tube

3.5 PREPARATION OF STOCK SOLUTION

The stock solution of BTX is prepared according to the data obtain from Industrial Pretreatment Department of El Paso. The simulation sample is prepared by dilute the pure solvent of BTX in the nutrient broth solution that prepared earlier. The stock solution that prepared is keeps in the freezer to avoid the contamination of bacteria and other microorganism.

Figure 3.7: Stock Solution of BTX

3.6 PREPARATION OF SAMPLE

There are three parameter that use in the experiment which are temperature, shaking speed and bacteria ratio to solvent. The procedure for each parameter is describes in the next subtopic.

2.6.1 Effect on Temperature

300ml of synthetic wastewater is added with bacteria solution with ratio 1:9. After through sterilize process the sample will placed in 180rpm of incubator shaker with variable temperature of 27^{o}C, 37^{o}C and 47^{o}C. At 24 hours and 48 hours 50ml of sample will retain in 100ml of vial and keeps in the freezer to avoid contamination.

2.6.2 Effect on Shaking Speed

300ml of synthetic wastewater is added with bacteria solution with ratio 1:9. After through sterilize process the sample will placed in 37^{o}C of incubator shaker with variable shaking speed of 120rpm, 180rpm and 220rpm. At 24 hours and 48 hours 50ml of sample will retain in 100ml of vial and keeps in the freezer to avoid contamination.

2.6.3 Effect on Ratio Bacteria to Solvent Solution

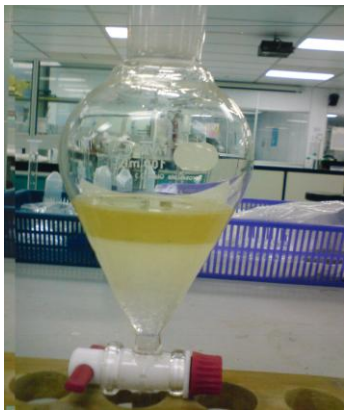
300ml of synthetic wastewater is added with bacteria solution with variable bacteria ratio of 1:9, 2:8 and 3:7 to solvent solution. After through sterilize process the sample will placed in 37^{o}C and 180 rpm of incubator shaker. At 24 hours and 48 hours 50ml of sample will retain in 100ml of vial and keeps in the freezer to avoid contamination. If 300ml of 1:9 sample is prepared, then the amount of 10% bacteria which is 30ml will added in 90% synthetic wastewater which amount 270ml to obtain 300ml of sample.

Figure 3.8: Sample solution that prepared

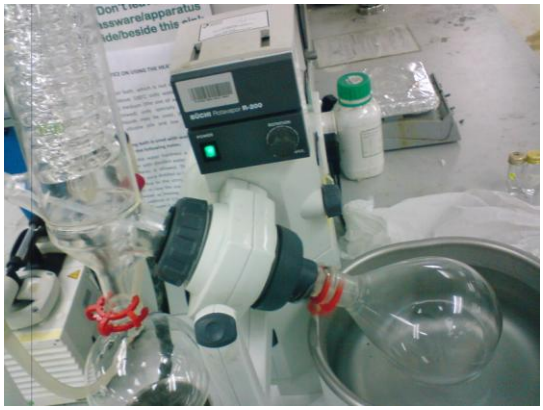
3.7 SAMPLE EXTRACTION AND DILUTION

Before go through the extraction procedure, the sample obtain will centrifuge at 1000rpm for 20minutes and filter using the 4.5 µmm of nylon filter to remove the biomass retain in the sample solution.

Each sample prepared will extract with 50ml of dichloromethane (DCM) using separator funnel to separate water from the BTX solvent. Then the solvent obtain from the separation process will transfer to the evaporator rotary and the solvent evaporated until around 2ml of sample remain in the evaporator. After that the 2ml of sample obtain will dilute with DCM in 10ml of volumetric flask.1.5ml sample prepared in the volumetric flask will transfer to GC vial to through the analysis procedure.



(a)



(b)

Figure 3.9: (a) Separator Funnel, (b) Rotary Evaporator

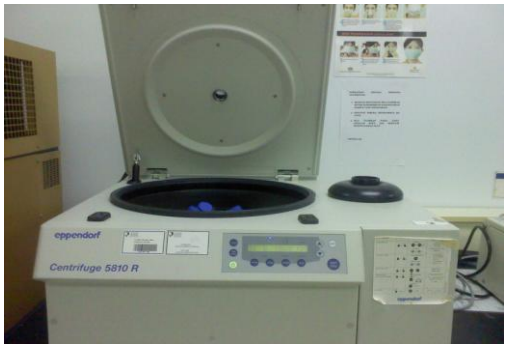


Figure3.10: Centrifuge

3.8 GAS CHRAMATHOGRAPHY (GC)

To analyze the sample prepared using GC the specific condition should be apply to obtain the result. The condition that should apply to analyze the BTX solvent is:

Table 3.1: Specification of GC

Types of Column	BD-Wax
Detector	FID
Carrier gas	Helium
Sample inlet	split
Temperature Detector	300^{o}C
Temperature column	250^{o}C

CHAPTER 4

RESULT AND DISCUSSION

In this chapter there will be talk about the result and discussion that obtain from the experimental. The subtopics that will be discussed are the *Pseudomonas putida* growth curve and cell dry weight. Besides there will be about the effect of temperature, shaking speed and ratio of *Pseudomonas putida* in the benzene, toluene and xylene treatment.

4.1 *PSEUDOMONAS PUTIDA* GROWTH CURVE

The data for *Pseudomonas putida* curve that obtain from the experimental is:

Table 4.1: ABS Data

Time, hour	ABS
0	0
1	0.135
2	0.3755
3	0.682
4	0.976
5	1.5245
6	1.6955
8	1.8410
10	1.9270
12	1.9890
14	2.1030
16	2.1270
18	2.1927
20	2.2270
22	2.2390
24	2.2445
26	2.2495
28	2.2475
30	2.2600
32	2.2618
34	2.2626
36	2.2776
38	2.2927
40	2.3078
42	2.3229
44	2.3380
46	2.3355
48	2.3335
50	2.3273
52	2.2973
54	2.2865
56	2.2865
64	1.9836

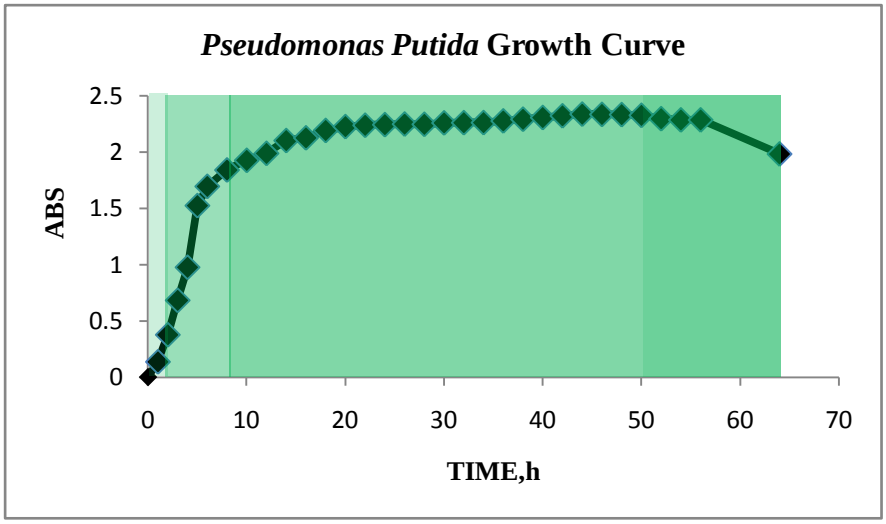


Figure 4.1 *Pseudomonas Putida* Growth Curve

The growth curve of bacteria commonly determine by the simply condition of batch culture. The *Pseudomonas putida* originally suitable with condition of incubator shaker with 37^{o}C and shaking speed of 180rpm.The bacteria growth curve basically consists of four phase which are lag, exponential growth, stationary and death phase. From Figure 4.1, it shows that the graph of ABS against time. The ABS (absorbance) is referring to the viable or living bacteria at certain time. The growth of *Pseudomonas putida* obtain is quite similar to the standard growth of bacteria that claim from Widdel (2007).

Lag phage occur in the period of time 0 to 1 hour, this phase happen in very short of period. Compared to the lag phase in Widdel (2007) written it show that the lag phase is occur in several hour before it proceed to exponential phase. It means that *Pseudomonas putida* take a shorter time to encounter with the new environment that supply to them.

The second phase is exponential phase, in this stage the living bacteria is rapidly increased within time. From the graph obtain it show that the exponential phase occur in the period of 1 to 7 hours. During this phase the bacteria is comfortable with the environment supply as it called as the optimum condition for the *Pseudomonas putida* to growth up and create as much as colonial possible by degrade the substrate.

The stationary phase occur in the period of 7 to 48 hours. During this phase the growth rate is equal to death rate so that the growth rate becomes slower. Since the process occurs in batch culture, the substrate supply will decrease cause degradation that occur through the exponential phase. Besides that, *Pseudomonas putida* is the living thing that will produce waste and secondary metabolic products in this phase.

The death phase occur in the period of 48 to 64 hours. During this phase the number of living bacteria will decrease within time as the death rate is faster than growth rate. The substrate supply is consider finish since the *Pseudomonas putida* is dead. Furthermore the toxic that may produce as waste and by product will harm the weak bacteria that force them die.

4.2 CELL DRY WEIGHT

The cell dry weight of *Pseudomonas putida* that obtain from the experimental is:

Table 4.2: Cell Dry Wright Data

No	Time	*Pseudomonas Putida*		
		Weight	Weight	net
0	0			0
1	1	1.0030	1.0036	0.0006
2	2	1.0114	1.012	0.0006
3	3	1.0184	1.0213	0.0029
4	4	1.0131	1.0147	0.0016
5	5	1.0216	1.0235	0.0019
6	6	1.0158	1.0179	0.0021
7	9	1.0109	1.0169	0.0060
8	12	1.0025	1.0047	0.0022
9	15	1.0097	1.0139	0.0042
10	18	1.0142	1.0162	0.0020
11	21	1.0123	1.0154	0.0031
12	24	1.003	1.0046	0.0016
13	27	1.0131	1.0159	0.0028
14	30	1.0218	1.0249	0.0031
15	33	1.0187	1.0219	0.0032
16	36	1.0136	1.0176	0.0040
17	39	1.0143	1.0163	0.0020
18	42	1.0128	1.015	0.0022
19	45	1.0214	1.0235	0.0021
20	48	1.0149	1.0174	0.0025

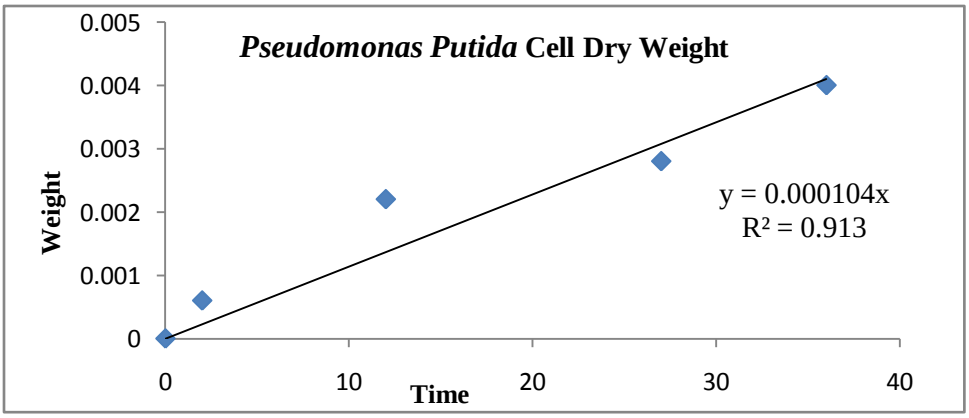


Figure 4.2 *Pseudomonas Putida* Cell Dry Weight

From figure 4.2 it shows that the mass of cell dry weight is proportional to the time of experimental. The increasing of cell dry weight is effected also by the growth that occurs during exponential and stationary phase. During the exponential and stationary phase the weight cell keeps increase due to the generation on new living bacteria through reproduction instead of the amount of waste and by product that produce. Meanwhile during the death phase the growth rate is decrease but the cell weight still keeps increase due to the culture of bacteria that occur in the batch process .Thus the amount of mass will keep remaining in the batch process until the experimental end.

4.3 Standard Curve of Benzene, Toluene and Xylene

The standard solution value for each solvent that obtain from the analysis of Gas Chromatography will use to construct the standard curve for each solvent so that the graph will use to determine the exact value of solvent concentration after the treatment done. The standard curve of benzene that obtain is:

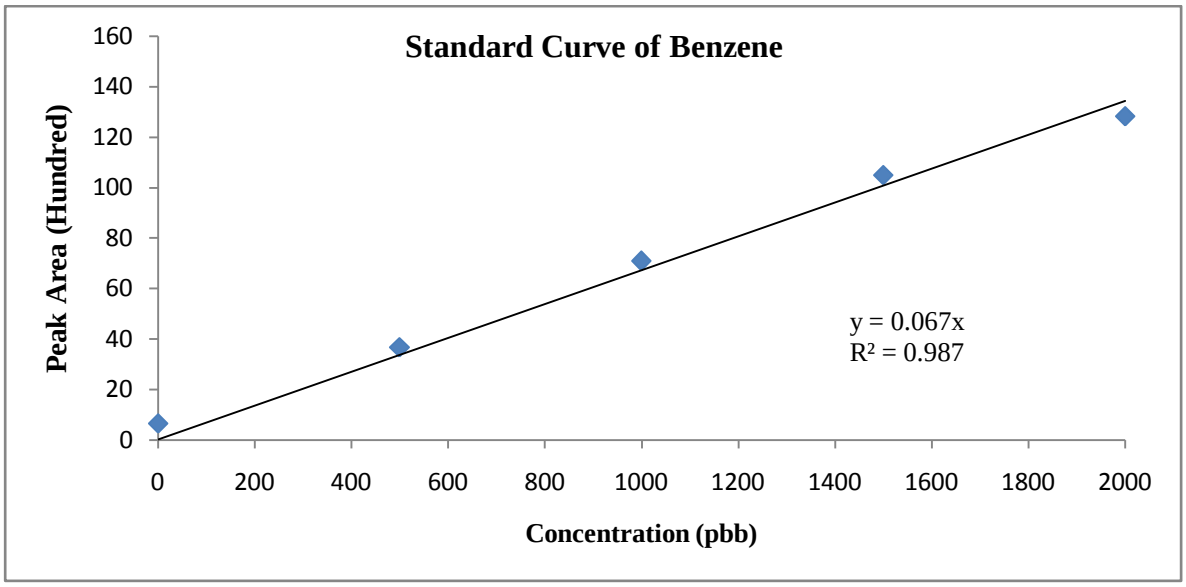


Figure 4.3 Standard Curve of Benzene

The standard curve of toluene that obtain is:

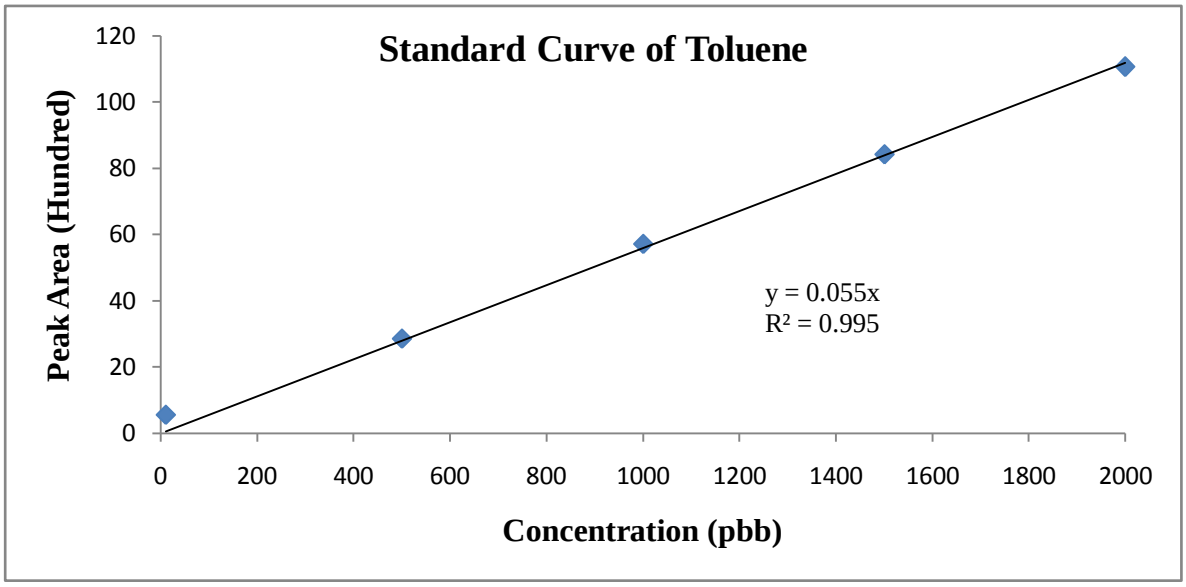


Figure 4.4 Standard Curve of Toluene

The standard curve of xylene that obtain is:

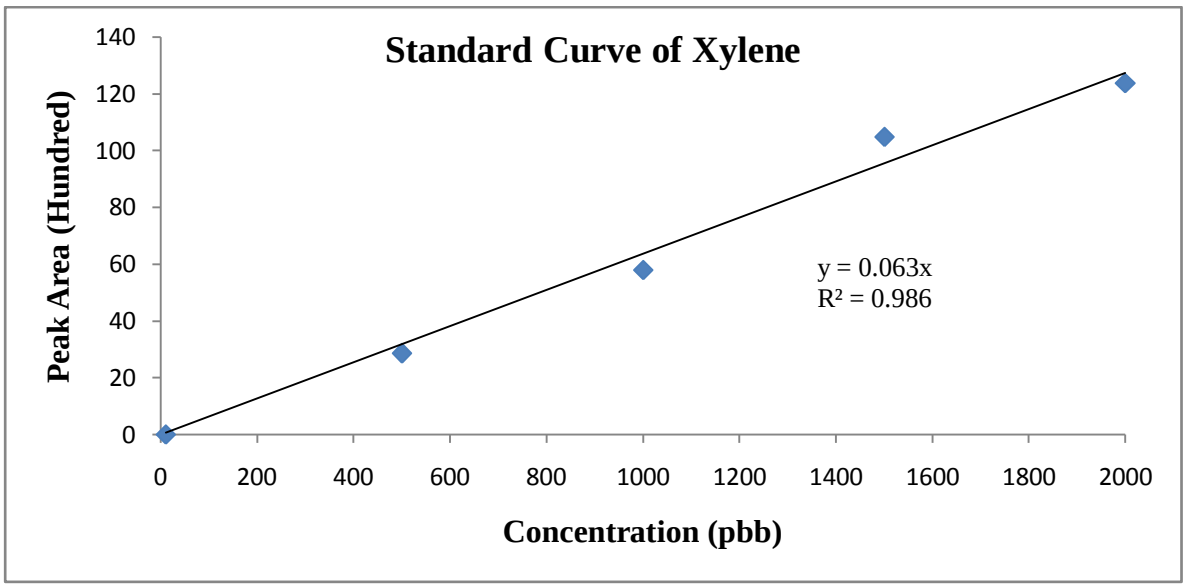


Figure 4.5 Standard Curve of Xylene

4.4 Effect on Temperature

For the variable temperature effect the shaking speed used is 180rpm while the bacterium to the solvent ratio is 1:9.The variable temperature that use is 27^{o}C, 37^{o}C and 47^{o}C.The data obtain from the experimental are:

Table4.3: Effect on Temperature Data

Sample	Temperature ^{o}C	Hour	Peak Area	Concentration (ppb)	Concentration X Dilution Factor	% Removal
Benzene	27	24	2829.323	189.565	947.823	89.378
Benzene	27	48	2061.925	138.149	690.745	92.259
Benzene	37	48	1405.228	94.150	470.751	94.724
Benzene	37	24	1048.846	70.273	351.363	96.062
Benzene	47	24	1915.198	128.318	641.591	92.810
Benzene	47	48	1365.911	91.516	457.580	94.872
Toluene	27	24	1222.970	77.047	385.235	61.446
Toluene	27	48	887.418	55.907	279.537	72.024
Toluene	37	24	600.100	37.806	189.031	81.082
Toluene	37	48	450.518	28.383	141.913	85.797
Toluene	47	24	821.501	51.755	258.773	74.102
Toluene	47	48	584.956	36.852	184.261	81.559
Xylene	27	24	6735.402	370.447	1852.236	28.827
Xylene	27	48	4905.063	269.778	1348.892	48.168
Xylene	37	24	3337.716	183.574	917.872	64.730
Xylene	37	48	2486.959	136.783	683.914	73.720
Xylene	47	24	4543.209	249.877	1249.383	51.992
Xylene	47	48	3253.002	178.915	894.576	65.626

From the calculation, the summary result for the variable effect of temperature on the BTX treatment is:

Table4.4: Summary Result of Effect on Temperature Data

Temperature	Benzene		Toluene		Xylene	
	24h	48h	24h	48h	24h	48h
27^{o}C	89.378	92.259	61.446	72.025	28.827	48.168
37^{o}C	94.724	96.062	81.082	85.798	64.73	73.72
47^{o}C	92.81	94.872	74.103	81.56	51.992	65.626

From the summary result variable effect of temperature on the BTX treatment, the graph of percent removal against temperature obtain is:

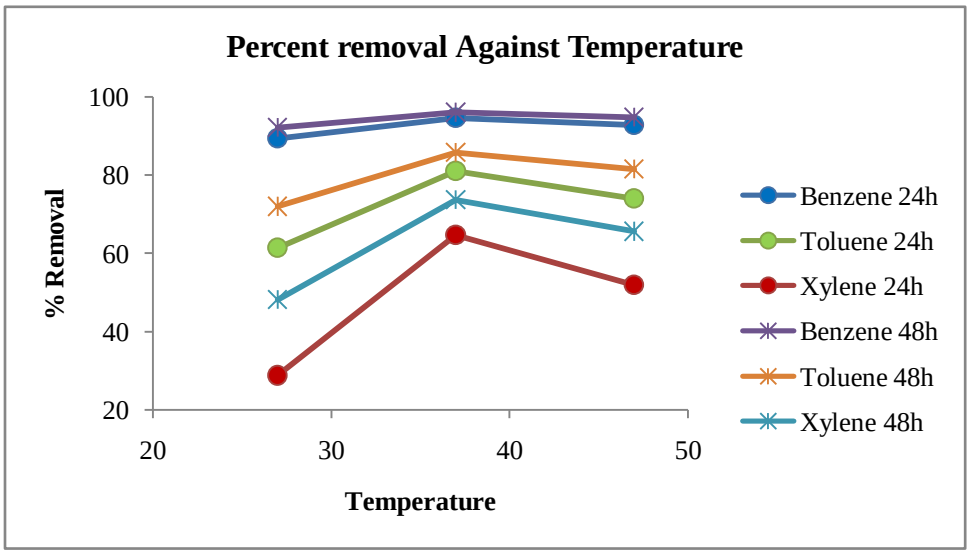


Figure 4.6 Graph of Percentage Removal against Temperature

From the graph obtain in figure 4.6, it shows that the temperature that had high percentage removal of BTX using *Pseudomonas putida* is 37^{o}C. As claimed by Teresa (1999), low and modest temperature will slow the growth rate but high temperature might kill the microbes. The temperature 27^{o}C had the lowest percentage removal of BTX. It seem at this temperature is unable to active the *Pseudomonas putida* immediately to achieve the optimum condition in shorter time. Since the *Pseudomonas putida* cannot tolerate the temperature the growth rate will becomes slow that cause less of colonial will produce and result lower percentage of removal. The temperature 47^{o}C in the modest position since the value of percent removal is always in between variable temperature that used in this experimental. Since 47^{o}C is quite hot, some of *Pseudomonas putida* may not growth well and some of them may death faster that cause the treatment not done properly. Therefore temperature 37^{o}C is selected as the optimum temperature for *Pseudomonas putida* to growth and obtain high percent removal of BTX.

4.5 Effect on Shaking Speed

For the variable shaking speed effect the temperature used is 37^{o}C while the bacterium to the solvent ratio is 1:9.The variable shaking speed that use is 120rpm, 180rpm and 220rpm.The data obtain, the result can be summaries as :

Table4.5: Effect on Shaking Speed Data

Sample	Shaking Speed (rpm)	Hour	Peak Area	Concentration (ppb)	Concentration X Dilution Factor	% Removal
Benzene	120	24	1343.730	90.030	450.150	94.955
Benzene	120	48	779.715	52.241	261.205	97.073
Benzene	180	24	1405.228	94.150	470.751	94.724
Benzene	180	48	1048.846	70.273	351.363	96.062
Benzene	220	24	1598.454	107.096	535.482	93.999
Benzene	220	48	1311.341	87.860	439.299	95.077
Toluene	120	24	1199.457	75.566	181.358	81.850
Toluene	120	48	697.460	43.940	105.456	89.446
Toluene	180	24	600.100	37.806	189.031	81.082
Toluene	180	48	450.518	28.383	141.913	85.797
Toluene	220	24	1434.418	90.368	216.884	78.294
Toluene	220	48	1168.465	73.613	176.672	82.319
Xylene	120	24	3198.145	175.898	879.490	66.205
Xylene	120	48	1860.613	102.334	511.668	74.339
Xylene	180	24	3337.716	183.574	917.872	64.730
Xylene	180	48	2486.959	136.783	683.914	73.720
Xylene	220	24	3800.205	209.011	1045.056	59.843
Xylene	220	48	3118.780	171.533	857.664	67.044

From the summary result variable effect of retention time on the BTX treatment, the graph of percent removal against shaking speed that obtain is:

Table4.4: Summary Result of Effect on Shaking Speed Data

Shaking Speed	Benzene		Toluene		Xylene	
	24h	48h	24h	48h	24h	48h
120rpm	94.955	97.073	81.850	89.446	66.205	74.339
180rpm	94.724	96.062	81.082	85.797	64.730	73.720
220rpm	93.999	95.077	78.294	82.319	59.843	67.044

From the summary result variable effect of shaking speed on the BTX treatment, the graph of percent removal against temperature obtain is:

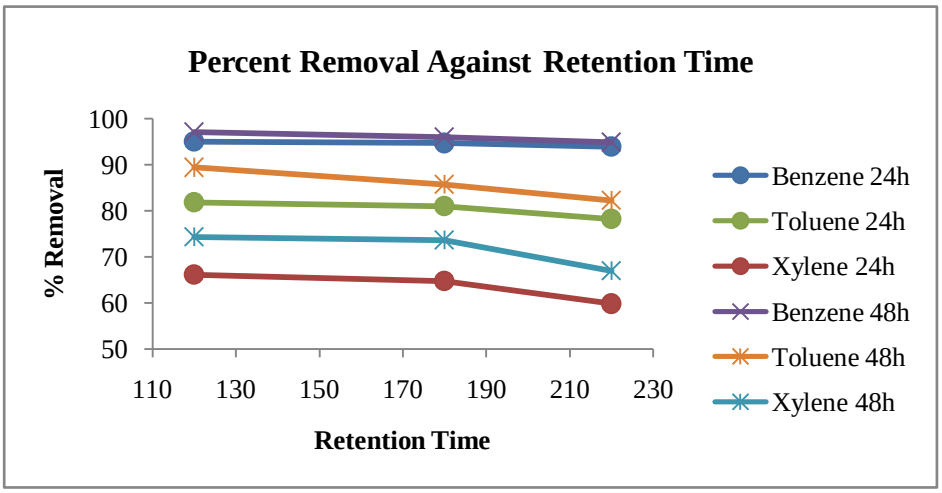


Figure 4.6 Graph of Percentage Removal against Shaking Speed

From the graph obtain in the figure 4.6, it show that the percent removal for 120rpm is the highest among the three variable shaking speed that had be tested. Thus, it can be used as the optimum shaking speed in BTX treatment. The ranking is followed by shaking speed of 180rpm and 220rpm. As mention by Elizabeth A.J. and Margaret A. J. (2004), high speed of shaking affect the lag phase duration shorter, increasing the growth rate and earlier reach stationary phase. Thus at speed of 220rpm the stationary phase will obtain in the shorter time that will cause the treatment not done properly consequently the percent of removal becomes the lowest. During the speed of 120 rpm, even the lag phase become longer and slightly decrease the growth but lower speed will delay the point of plateau that may the treatment done properly as the result the percent removal be the highest. Even though there are slightly differences of percent removal between180rpm and 120 but still the 120 rpm is the optimum shaking speed for *Pseudomonas putida* to growth and obtain high percent removal of BTX.

4.6 Effect on Bacteria to Solvent Ratio

For the variable bacteria to solvent ratio effect the temperature used is 37^{o}C while the retention time is 180rpm.The variable bacteria to solvent ratio that use is 3:7, 2:8 and 9:1.The data obtain from the experimental are:

Table4.7: Effect of Bacteria Ratio to Solvent Data

Sample	Ratio	Hour	Peak Area	Concentration (ppb)	Concentration X Dilution Factor	% Removal
Benzene	3:7	24	1520.571	101.878	509.391	94.291
Benzene	3:7	48	1510.043	101.173	505.864	94.331
Benzene	2:8	24	1441.369	96.572	482.859	94.589
Benzene	2:8	48	1341.260	89.864	449.322	94.964
Benzene	1:9	24	1048.846	70.273	351.363	96.062
Benzene	1:9	48	1405.228	94.150	470.751	94.724
Toluene	3:7	24	650.728	40.996	204.979	79.486
Toluene	3:7	48	645.840	40.688	203.440	79.640
Toluene	2:8	24	615.811	38.796	193.981	80.586
Toluene	2:8	48	572.274	36.053	180.266	81.959
Toluene	1:9	48	600.100	37.806	189.031	81.082
Toluene	1:9	24	450.518	28.383	141.913	85.797
Xylene	3:7	24	3615.909	198.875	994.375	61.791
Xylene	3:7	48	3585.922	197.226	986.129	62.108
Xylene	2:8	24	3423.477	188.291	941.456	63.824
Xylene	2:8	48	3186.344	175.249	876.245	66.330
Xylene	1:9	24	3337.716	183.574	917.872	64.730
Xylene	1:9	48	2486.959	136.783	683.914	66.720

From the calculation, the summary result for the variable effect of bacteria ratio to solvent on the BTX treatment is:

Table4.8: Summary Result of Effect on Bacteria Ratio to Solvent

Ratio	Benzene		Toluene		Xylene	
	24h	48h	24h	48h	24h	48h
3:7	94.291	94.331	79.486	79.64	61.791	62.108
2:8	94.589	94.964	80.587	81.959	63.824	66.33

1:9	94.724	96.062	81.082	85.798	64.73	66.72

From the summary result variable effect of bacteria ratio to solvent on the BTX treatment, the graph of percent removal against bacteria ratio to solvent that obtain is:

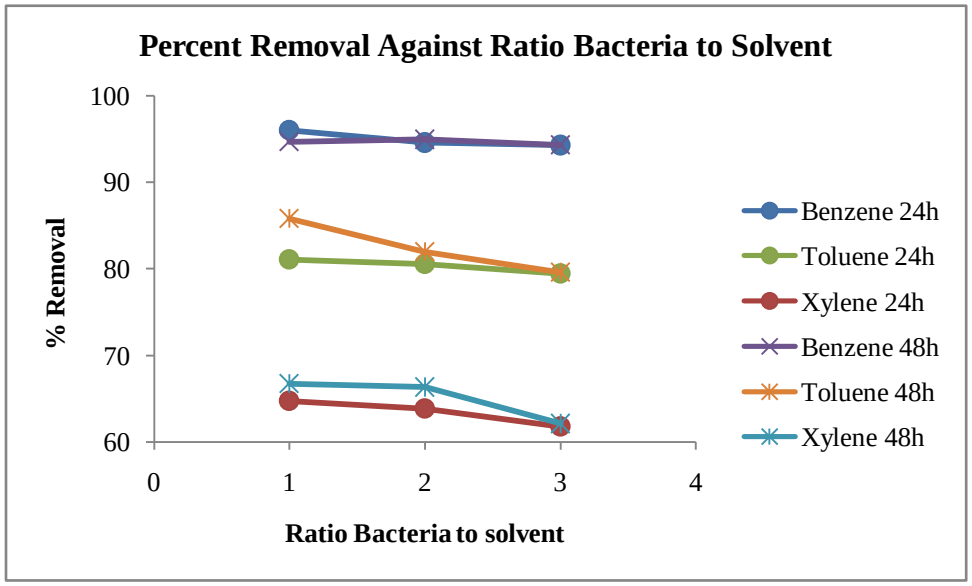


Figure 4.6 Graph of Percentage Removal against Ratio Bacteria to Solvent

Teresa T. (1999) stated that the growth rate optimum when the optimal amount of nutrient provided. From the graph obtain in figure 4.6, it show that the value for each condition of variable is not had very large differences in number but still the ratio of 1:9 is selected as the optimum ratio as it able to achieve the highest value of percent removal for BTX treatment. The result is followed by 2:8 and 3:7 ratio of bacteria to solvent. Even thought, the ratio 3:7 provide a large amount of bacteria but still the percent removal is the lowest cause the amount of bacteria is not compatible with the amount of substrate that provided in the solvent solution thus it make the exponential phase of the *Pseudomonas Putida* not growing at the optimum growth rate that lead the treatment process not perform properly. As well as 2:8 the same condition happen but the living bacteria can encounter the crisis well cause the amount of *Pseudomonas putida* supply can still tolerate to had high growth in exponential phase.

4.7 Percent Removal

From the graph constructed in figure 4.4, 4.5 and 4.6, it shows that there are slightly difference value for each sample at 24hours and 48 hours. Since the experimental is carried out in the batch process, the living bacteria of *Pseudomonas putida* was perform efficiently in the first 24hours the go through of lag and exponential phase. During the first 24 hours *Pseudomonas putida* is workout to encounter the new environment through the lag phase and then proceed to the exponential phase to increase the growth rate to provide amount of colonial that will treat the BTX solvent contain in the sample solution.

For the 48h later, the removal percent increase gradually because the living bacteria had reached the stationary phase. It means, during the phase some of living bacteria will death to complete the live cycle thus reduce the amount of colonial that able to perform. Furthermore, since the experimental occur in the batch process might be at this level the amount of substrate supply is too little to provide enough nutrients to *Pseudomonas putida* to continuous the growth rate.

From all the graphs obtain, it shows that benzene always has the highest value of percent removal compared to toluene and xylene. Howard et al (1985), point out that microorganism must be adjusting with their surrounding and the nutrient provided. The adjusting period refer to lag phase, which is differ in length depending on the history of the seed organism. If the organism familiar with the nutrient the lag phase become shorter and continuous growth rapidly. In this experimental, *Pseudomonas putida* might be prefer to consume benzene rather than toluene and xylene.

The process of removal BTX is done by degradation of *Pseudomonas putida*. Under aerobic condition BTX were transformed by dioxygenase reaction that produce catechol intermediate that will further break down into carbon dioxide (CO_2) and water that are not toxic compound to human and environment. Actually the present of catechol not support the *Pseudomonas putida* growth rate. Thus, catechol from benzene and 3-methylcatechol from toluene should through mineralization process to obtain the intermediate product that

support the *Pseudomonas putida.* Unfortunately, xylene produces 3, 5-methylcatechol as dead-end product that cause the biodegradation rate. The mechanism of dioxygenase reaction is:

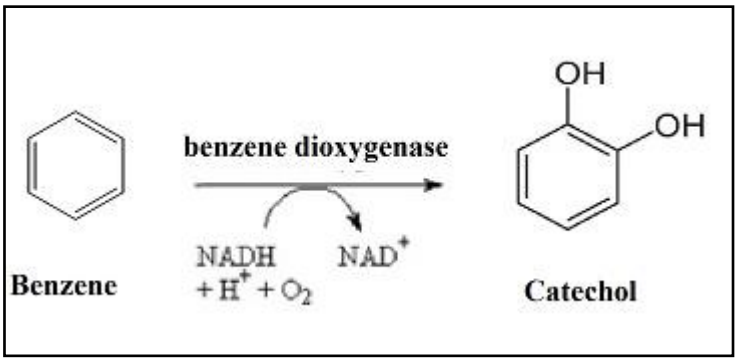


Figure 4.7: Dioxygenase of Benzene

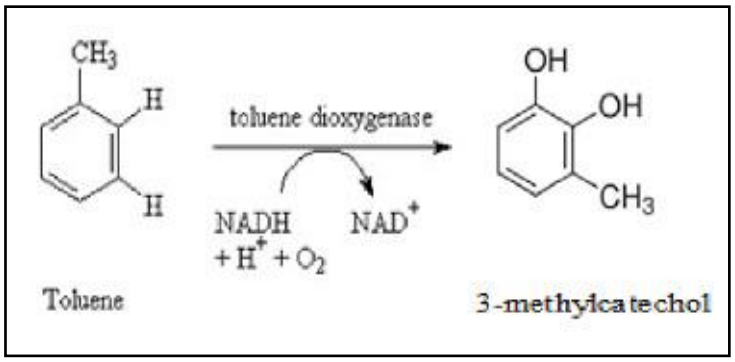


Figure 4.8: Dioxygenase of Toluene

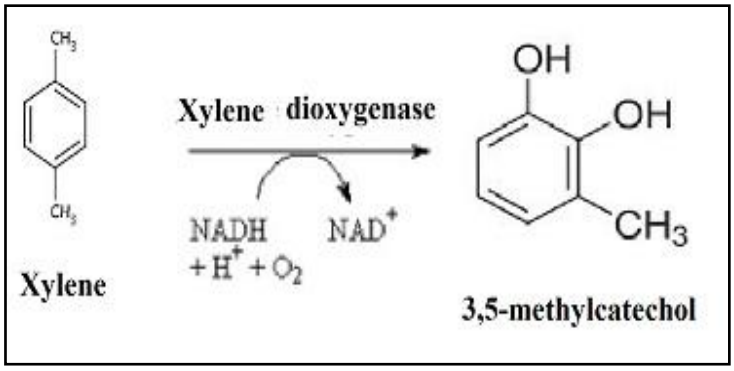


Figure 4.7: Dioxygenase of Xylene

From the BTX treatment that has been conducted the highest percent removal of BTX is determine at the temperature of 73°C, 180 rpm and 1:9 ratio of bacteria. The result of final concentration as follow:

Table 4.9: Comparison of BTX Concentration

Compound	Initial Concentration	After Treatment Concentration	Percent of Removal	Limit That Approve (EPA)
Benzene	20 ppm	0.56ppm	97.073	0.005ppm
Toluene	17 ppm	3.08ppm	89.446	1ppm
Xylene	17 ppm	5.74ppm	74.339	10ppm

The initial concentrations of BTX in obtain from the Industrial Pretreatment Department of El Paso that had analyzed the common concentration of BTX in the wastewater that discharge from the industrial around of El Paso. While the approved limit value of BTX is obtain from the EPA standard that has been established. From the graph constructed above it show that the percentage removal of BTX in the highest at the condition of 37°C, 120 rpm and 1:9 ratio of bacteria. Even though, Benzene had the highest percent removal compared to toluene and xylene but interestingly only xylene meet the requirement that regulated by EPA. It easier for xylene to satisfy the EPA standard since the limit concentration approval of xylene is quite high which 10ppm compared to benzene and toluene.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

From this research it can be conclude that *Pseudomonas putida* successfully obtain four phase of microorganism growth and able to achieve high percent removal of BTX from petrochemical wastewater.

The growth curve of *Pseudomonas putida* has complete the bacteria life cycle in period 64 hours. The lag phase occur in 0 to 1 hours and continuous with exponential phase at 1 until 7 hours. Then proceed to stationary phase at 7 hours until 48 hours and last phase which is death phase that occur at 48 hours until 64 hours. The cell dry weight obtain is proportional to the time of experimental.

The optimum temperature used is 37^{o}C, since low the temperature is not enough to activate the *Pseudomonas putida*.While high temperature able to kill the microbes.

120 rpm is selected as the optimum shaking speed in the BTX treatment. The selection is considered based on the high speed of shaking affect the lag phase duration shorter, increasing the growth rate and earlier reach stationary phase.

The optimum ration used is 1:9 ratio of bacteria to the solvent used. Since, the growth rate optimum when the optimal amount of nutrient provided. Other parameter of ratio is not balance in the bacteria supply and the nutrient provided.

Even though the optimum temperature, shaking speed and ratio of bacteria is successfully obtain but unfortunately from the calculation it show that only Xylene had satisfied the EPA standard although the percent removal of benzene and toluene is higher compare to xylene. The result turn out like that cause the limit concentration of xylene is quite high compared to the benzene and toluene. Thus the adjustment of parameter used is needed to determine the optimum condition to treat BTX effectively in order to meet the EPA requirement.

5.2 RECOMMENDATION

Some recommendations can be made for future improvement in this research:-

I. Use directly petrochemical wastewater as the stock solution.
II. Adjust the parameter so that the percent removal can be improved.
III. Study on the other parameter of waste treatment such COD, BOD, suspended solid and pH

This recommendation will be a guideline in improving the study of BTX treatment from petrochemical wastewater using *Pseudomonas putida.*

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