MERCURY REMOVAL USING *PSEUDOMONAS PUTIDA* (ATTC: 49128)

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A thesis submitted in fulfillment of the requirement for the award of the Degree of Bachelor of Chemical Engineering

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DECLARATION

"I declared that this thesis entitled "Mercury Removal using *Pseudomonas putida* (ATTC: 49128)" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Name of Candidate	: Khorsiah Binti Sabar
Date	: 18 April 2008

DEDICATION

Special dedicated to my.....

Beloved Parent:

Haji Sabar Bin Sharib
Hajah Noriah Binti Haji Jumli

All my Family members

All my Friends and Lecturers

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ABSTRACT

Microbes have been used to solve environmental problems for many years. The use microorganism to sequester, precipitate or alter the oxidation state of various heavy metals has been extensively studied. Processes by which microorganism interacts with toxic metal are very diverse. The objective of this research is to remove the mercury using *Pseudomonas putida*.In this research, Pseudomonas *putida* strain, a mercury resistant bacterium, which is able to reduce ionic mercury to metallic mercury, was studied.This study has been carried out using mercury nitrate in the concentration 1-5ppm. In this study the *P.putida* must observed first to know the best condition that can remove mercury with effectively. Maximum removal capacity for bacterium was found to be 80%.Thus; bacterial removal of mercury is a potential biological treatment for mercury environmental pollutants.

ABSTRAK

Mikroorganisma telah lama digunakan untuk menyelesaikan pelbagai masalah pencemaran alam sekitar. Banyak kajian telah di buat yang membuktikan mikroorganisma mampu mengubah tahap oksidasi pelbagai jenis logam berat. Proses yang melibatkan logam berat dan mikroorganisma sangat luas. Salah satu proses tersebut ada terkandung dalam kajian ini.Objektif kajian ini adalah untuk menukarkan logam merkuri yang toksik kepada ion merkuri yang tidak toksik,dengan menggunakan *Pseudomonas putida.Pseudomonas putida* merupakan salah satu mikroorganisma yang tahan rintang terhadap merkuri. Dalam kajian ini merkuri yang digunakan adalah merkuri nitrate yg berkepekatan satu hingga lima ppm.Bagi memastikan kejayaan kajian ini *Pseudomonas putida* perlu dikaji dengan lebih mendalam terlebih dahulu.Telah terbukti melalui kajian ini bahawa maksimum kapasiti yang mampu ditukarkan adalah sebanyak 80 peratus.oleh itu mikroorganisma mampu menyelesaikan masalah pencemaran yang berpunca daripada merkuri.

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CHAPTER 1

INTRODUCTION

1.1 Overview Of Research

Mercury pollution of the environment by mining activities and industrial has resulted in worldwide contamination of large areas of soils and sediments (Miller, Rowland, Lechler, Desilets and Hsu, 1996) ;(Degetto, Schintu, Contu and Sbrignadello, 1997) ;(Suchanek, 1998) ;(Horvat, 1999) and let to elevated atmospheric mercury levels (Ebinghaus and Slemr, 2000). Because of lack of suitable cleanup technologies, efforts to deal with polluted sites are directed toward the mechanical removal of contaminated material and its deposition elsewhere (Hosokawa, 1993) ;(Miserocchi, Langone and Guerzoni, 1993). Such processes are costly and often result in remobilization of toxic mercury compounds during the dredging process (Skej, 1992).

Mercury is one of the most toxic elements. It binds to the sulfhydryl groups of enzymes and proteins, thereby inactivating vital cell functions (Quig, 1998). After discharge into the environment, mercury enters the sediments where it persists for many decades. It is taken up by aquatic organisms in the form of highly toxic methylmercury and is subsequently biomagnified through the food chain. The health of top predators, e.g. birds, fish, seals, and man, is thereby threatened (Braune, 1999); (Muir, 1999). At high concentrations, mercury vapor inhalation produces acute necrotizing bronchitis and pneumonitis, which is lead to death from respiratory failure. Long term exposure to mercury vapor primarily affects the central nervous system. Mercury also accumulates in kidney tissues, directly causing renal toxicity, including proteinuria or nephritic syndrome (Chang, Chao and Law, 1998). High concentration of Hg^{2+} cause impairment of pulmonary function and kidney, chest pain and dyspnousea (Manohar,D.M,Anoop and Anirudhan,2002). Therefore, the discharge of mercury into the environment needs to be prevented by efficient and cost-effective end-of-pipe treatment technologies for mercury emitting industries.

Purification of areas polluted by heavy metals such as mercury is difficult, because the metals cannot be transformed into harmless elements. Over a few decades, community is devoting concentrated efforts for the treatment and removal of heavy metals in order to face this problem (Chandra, S.K., C.T. Kamals and Chary, 2003). Various types of technology is available for removing of mercury in water and wastewater including chemical precipitation, conventional coagulation, reverse osmosis, ultrafiltration, magnetic filtration, ion exchange and activated carbon adsorption and chemical reduction(Derek, R.L. and Coates, 1997).

Biological systems have been thought to be adapted for removal of toxic heavy metals (Zeroul, Moutaouakkil and Blaghen, 2001). Bioremoval is biological systems for removal of metals ion from polluted water has the potential to achieve greater performance at lower cost than nonbiological wastewater treatment (Kondoh, Fukuda and Azuma, 1998). Developments in the field of environment biotechnology indicate the bacteria, fungi, yeasts and algae can remove heavy metals from aqueous solution by adsorption (Saglam, Aviles and Cervantes, 2000).

In bacteria resistance to mercury is related to enzymatic reduction of Hg^{2+} to volatile Hg^0 (Devars, Aviles and Cervantes, 2000). Mercury detoxification process originated from *mer* operon located on either plasmids or transposable elements in the mercury resistant microorganisms. Specific transport of bulk mercury across the cell membrane is achieved by two *mer* operon genes *mer*P and *mer*T, which express cystein-rich protein to deliver ambient mercuric toward intracellular mercuric reductase for subsequent reduction of mercuric ions to volatile Hg^0 (Weon, Rajesh and Ashok, 2001).In the present investigation after isolation bacteria from petrochemical wastewater the ability of isolated bacterium, *Pseudomonas putida,* has been assessed for removal,biosorption and up take, mercury ion.

1.2 Problem Statement

Mercury is one of the heavy metal of concern and has been found in the wastewaters coming from oil refinery, chloralkali manufacturing industry, paint, pharmaceutical, paper and battery manufacturing industries. Mercury and mercurial compounds are highly toxic contaminants in aquatic system and soils. They are dangerous pollutants because they are able to disperse widely into environment due to their high mobility and potentially concentrated through the food chain.

Mercury contamination of hydrocarbon production and processing systems can be more than a mere nuisance. Early detection and accurate quantification of mercury is necessary to assure equipment integrity, to comply with regulations and to insure worker safety. DOE is responsible for thousands of cubic meters of mercury waste that will require treatment to meet EPA regulations before it can be accepted into landfills. High concentrations of mercury are found in several regions of the world and operators have developed measures to cope with the major ramifications but all such measures benefit from early recognition of potential problems. Routine maintenance and inspection activities become non-routine when mercury is present in fluids above a few ppb and become problematic when mercury concentrations reach approximately 100 ppb. Mercury in crude oil or gas affects quality and price of salable products and raises equipment integrity concerns in proportion to concentration that may be present. In rare cases (SE Asia), mercury can be present in sufficient quantities to interfere with the normal function of heat exchangers, separators and conditioning systems (amine, glycol).

Mercury in crude oil above certain limits can be problematic to refining operations. Mercury poisons catalysts and reduces the quality of refined products. For waste with mercury concentrations below 260 ppm, EPA specifies stabilization before disposal. Environmental impacts are also important because running mercury-laden crude's can produce wastewater and solid waste streams having mercury concentrations that exceed regulatory limits. Mercury originating in crude feeds can deposit in equipment and thus can become an important health and safety issue during inspection and maintenance operations. Refiners have the need to know exactly how much mercury is in the refinery crude diet to allow blending to acceptable limits or to develop contingencies. Attaching a certain range to the concentration of mercury in a purchased crude oil is not an easy task and significant errors can be encountered that can produce negative impacts on refinery operations and profitability.

1.3 Objective of The Project

The main objective in this project is to remove mercury by using mercury resistance bacterial strain, *Pseudomonas putida*

1.4 Scope of Research Work

i) To study on kinetics bacteria growth in shake flask.
ii) To determine the screening of environmental factors like temperature, pH, agitation and incubation time.
iii) To study on performance of *P.putida* in removing mercury.

CHAPTER 2

LITERATURE REVIEW

2.1 Mercury Overview

There are three important types of mercury which is the pure element, inorganic compounds such as mercuric nitrate and organic mercury compounds such as phenyl mercuric propionate. Elemental mercury is a liquid and gives off mercury vapor at room temperature. This vapor can be inhaled into the lungs and passed into the blood stream. Elemental mercury can also pass through the skin and into the blood stream. If swallowed, however, this form of mercury is not absorbed out of the stomach, and usually passes out of the body without harm. Inorganic mercury compounds can also be inhaled and absorbed through the lungs, and may pass through the skin. But the compounds can also be absorbed through the stomach if swallowed. Many inorganic mercury compounds are irritating or corrosive to the skin, eyes and mucus membranes as well. Organic mercury compounds can enter the body readily through all three routes-lungs, skin and stomach.

2.1.1 History of Mercury

Mercury was known to the ancient Chinese and Hindus, and was found in Egyptian tombs that date from 1500 BC. In China, India and Tibet, mercury use was thought to prolong life, heal fractures, and maintain generally good health. China's first emperor, Qin Shi Huang Di said to have been buried in a tomb that contained rivers of flowing mercury, representative of the rivers of China was driven insane and killed by mercury pills intended to give him eternal life. The ancient Greeks used mercury in ointments and the Romans used it in cosmetics. By 500 BC mercury was used to make amalgams with other metals. The Indian word for alchemy is *Rasavatam* which means ‘the way of mercury’. Alchemists often thought of mercury as the First Matter from which all metals were formed. Different metals could be produced by varying the quality and quantity of sulfur contained within the mercury. An ability to transform mercury into any metal resulted from the essentially mercurial quality of all metals. The purest of these was gold, and mercury was required for the transmutation of base (or impure) metals into gold as was the goal of many alchemists.

Hg is the modern chemical symbol for mercury. It comes from *hydrargyrum*, a Latinized form of the Greek word *`Υδραργυρος* (*hydrargyros*), which is a compound word meaning 'water' and 'silver' — since it is liquid, like water, and yet has a silvery metallic sheen. The element was named after the Roman god Mercury, known for speed and mobility. It is associated with the planet Mercury. The astrological symbol for the planet is also one of the alchemical symbols for the metal (above left). Mercury is the only metal for which the alchemical planetary name became the common name.

Sometime prior to the autumn of 1803, the Englishman John Dalton was able to explain the results of some of his studies by assuming that matter is composed of atoms and that all samples of any given compound consist of the same combination of these atoms. Dalton also noted that in series of compounds, the ratios of the masses of the second element that combine with a given weight of the first element can be reduced to small whole numbers (the law of multiple proportions). This was further evidence for atoms. Dalton's theory of atoms was published by Thomas Thomson in the 3rd edition of his *System of Chemistry* in 1807 and in a paper about strontium oxalates published in the *Philosophical Transactions*. Dalton published these ideas himself in the following year in the *New System of Chemical Philosophy*. The symbol used by Dalton for mercury is shown below. (History *of Chemistry*, Sir Edward Thorpe, volume 1, Watts & Co, London, 1914)

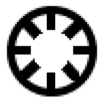

Figure 2.1 Symbol of mercury by Dalton

2.1.2 Mercury Properties and Characteristic

Mercury is the only common metal liquid at ordinary temperatures. Mercury is sometimes called quicksilver. It rarely occurs free in nature and is found mainly in cinnabar ore (HgS) in Spain and Italy. It is a heavy, silvery-white liquid metal. It is a rather poor conductor of heat as compared with other metals but is a fair conductor of electricity. It alloys easily with many metals, such as gold, silver, and tin. These alloys are called amalgams. Its ease in amalgamating with gold is made use of in the recovery of gold from its ores.

Mercury is a chemical element in the periodic table that has the symbol Hg (Latinized Greek: *hydrargyrum*, meaning *watery* or *liquid silver*) and atomic number 80. A heavy, silvery transition metal, mercury is one of five elements that are liquid at or near room temperature and pressure (Fred Senese, 2001).

Table 2.1: General properties of mercury

General properties of mercury	
Name	Mercury
Symbol	Hg
Number	80
Chemical series	Transition Metals
Group	12
Period	6
Block	d
Appearance	silvery
Standard atomic weight	200.59(2) $g \cdot mol^{-1}$
Electron configuration	[Xe] $4f^{14}$ $5d^{10}$ $6s^2$
Electron per shell	2, 8, 18, 32, 18, 2

Table 2.2: Physical properties of mercury

Physical properties of mercury	
Phase	liquid
Density (near r.t.)	(liquid) 13.534 $g \cdot cm^{-3}$
Melting point	234.32K -38.83°C -37.89°F
Boiling point	629.88 K 356.73°C 674.11°F
Critical point	1750 K, 172.00 MPa
Heat of fusion	2.29 $kJ \cdot mol^{-1}$
Heat of vaporization	59.11 $kJ \cdot mol^{-1}$
Heat capacity	(25°C) 27.983 $J \cdot mol^{-1} \cdot K^{-1}$

Vapor pressure						
P(Pa)	1	10	100	1 k	10 k	100 k
at *T*(K)	315	350	393	449	523	629

Table 2.3: Atomic properties of mercury

Atomic properties of mercury	
Crystal structure	rhombohedral
Oxidation states	**2**, 1 (mildly basic oxide)
Electronegativity	2.00 (Pauling scale)
Ionization energies	1st: 1007.1 kJ/mol
	2nd: 1810 kJ/mol
	3rd: 3300 kJ/mol
Atomic radius	150 pm
Atomic radius (calc.)	171 pm
Covalent radius	149 pm
Van der Waals radius	155 pm

Table 2.4: Miscellaneous of mercury

Miscellaneous of mercury	
Magnetic ordering	diamagnetic
Electrical resistivity	(25 °C) 961 nΩ·m
Thermal conductivity	(300K) 8.30 $W \cdot m^{-1} \cdot K^{-1}$
Thermal expansion	(25°C) 60.4 $\mu m \cdot m^{-1} \cdot K^{-1}$
Speed of sound	(liquid, 20 °C) 1451.4 m/s

Table 2.5: Selected isotopes of mercury

Isotopes of mercury					
iso	**NA**	**half-life**	**DM**	**DE (MeV)**	**DP**
^{194}Hg	syn	444 y	ε	0.040	^{194}Au
^{195}Hg	syn	9.9 h	ε	1.510	^{195}Au
^{196}Hg	0.15%	Hg is stable with 116 neutrons			
^{197}Hg	syn	64.14 h	ε	0.600	^{197}Au
^{198}Hg	9.97%	Hg is stable with 118 neutrons			
^{199}Hg	16.87%	Hg is stable with 119 neutrons			
^{200}Hg	23.1%	Hg is stable with 120 neutrons			
^{201}Hg	13.18%	Hg is stable with 121 neutrons			
^{202}Hg	29.86%	Hg is stable with 122 neutrons			
^{203}Hg	syn	46.612 d	β^-	0.492	^{203}Tl
^{204}Hg	6.87%	Hg is stable with 124 neutrons			

2.1.3 Mercuric nitrate

Mercury (II) nitrate is a toxic colorless or white soluble crystalline compound of mercury. It is sometimes, though not often, used to determine chlorides in the blood. It was also used to treat fur to make felt until the practice was banned in December 1941 by The United States Public Health Service.

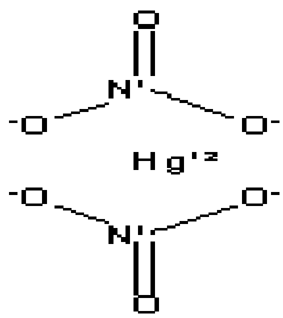

Figure 2.2 Mercuric nitrate [$Hg(N0_3)_2$]

Table 2.6: General properties of mercury dinitrate

General properties of mercury dinitrate	
Systematic name	Mercury dinitrate Mercury(II) nitrate
Other names	Mercuric nitrate
Molecular formula	$Hg(NO_3)_2$
Molar mass	324.7 g/mol
Appearance	colorless crystals or white powder
CAS number	[10045-94-0]

Table 2.7: Physical properties of mercury dinitrate

Physical properties of mercury dinitrate	
Density and phase	4.4 g/cm³, solid
Solubility in water	Soluble
Melting point	79 °C
Boiling point	N/A

Table 2.8: Safety data of mercury dinitrate

Safety data of mercury dinitrate	
EU classification	Very toxic (**T+**) Dangerous for the environment (**N**)
R-phrases	R26/27/28, R33, R50/53
S-phrases	(S1/2), S13, S28, S45, S60, S61

2.1.4 Application of Mercury

2.1.4.1 Electrical and Electronic

Mercury was a propellant for early ion engines in electric propulsion systems. Advantages were mercury's high molecular weight, low ionization energy, low dual-ionization energy, high liquid density and liquid storability at room temperature. Disadvantages were concerns regarding environmental impact associated with ground testing and concerns about eventual cooling and condensation of some of the propellant on the spacecraft in long-duration operations. The first spaceflight to use electric propulsion was a mercury-fueled ion thruster SERT-1 launched by NASA at NASA's Wallops Flight Facility in 1964. SERT stands for Space Electric Rocket Test. The SERT-1 flight was followed up by the SERT-2 flight in 1970. Mercury and Cesium were preferred propellants for ion engines until Hughes Research Laboratory performed studies finding Xenon gas to be suitable replacement. Xenon is now the preferred propellant for ion engines as it has a high molecular weight, little or no reactivity due its noble gas nature, and has a high liquid density under mild cryogenic storage.

2.1.4.2 Manufacture of industrial chemicals

It is used in some thermometers, especially ones which are used to measure high temperatures (In the United States, non-prescription sale of mercury fever thermometers is banned by a number of different states and localities).Mercury also used inside wobbler (fishing) lures. Its heavy, liquid form made it useful since the lures made an attractive irregular movement when the mercury moved inside the plug. Such use was stopped due to environmental concerns, but illegal preparation of modern fishing plugs has occurred. Liquid mercury was sometimes used as a coolant for nuclear reactors; however, sodium is proposed for reactors cooled with liquid metal, because the high density of mercury requires much more energy to circulate as coolant. Liquid mercury has been proposed as a working fluid for a heat pipe type of cooling device for spacecraft heat rejection systems or radiation panels.

2.1.4.3 Medicine and Health

Mercury and its compounds have been used in medicine for centuries, although they are much less common today than they once were, now that the toxic effects of mercury and its compounds are more widely understood.

Mercury (I) chloride (also known as calomel or mercurous chloride) has traditionally been used as a diuretic, topical disinfectant, and laxative. Mercury(II) chloride (also known as mercuric chloride or corrosive sublimate) was once used to treat syphilis (along with other mercury compounds), although it is so toxic that sometimes the symptoms of its toxicity were confused with those of the syphilis it was believed to treat (Pimple 2004); it was also used as a disinfectant. Blue mass, a pill or syrup in which mercury is the main ingredient, was prescribed throughout the 1800s for numerous conditions including constipation, depression, child-bearing and toothaches. In the early 20th century, mercury was administered to children yearly as a laxative and dewormer, and it was used in teething powders for infants. The mercury containing organohalide Mercurochrome is still widely used but has been banned in some countries such as the U.S.

Some vaccines have contained the preservative Thimerosal (partly ethyl mercury) since the 1930s FDA report. It has been widely speculated that this mercury-based preservative can trigger autism in children who are already genetically predisposed to it; however, recent studies have shown no evidence supporting any such link.

Mercury in the form of one of its common ores, cinnabar, remains an important component of Chinese, Tibetan, and Ayurvedic medicine. As problems may arise when these medicines are exported to countries that prohibit the use of mercury in medicines, in recent times, less toxic substitutes have been devised.

Today, the use of mercury in medicine has greatly declined in all respects, especially in developed countries. Thermometers and sphygmomanometers containing mercury were invented in the early 18th and late 19th centuries, respectively. In the early 21st century, their use is declining and has been banned in some countries, states and medical institutions. In 2002, the U.S. Senate passed legislation to phase out the sale of non-prescription mercury thermometers. In 2003, Washington and Maine became the first states to ban mercury blood pressure devices.[15] Mercury compounds are found in some over-the-counter drugs, including topical antiseptics, stimulant laxatives, diaper-rash ointment, eye drops, and nasal sprays. The FDA (FDA) has “inadequate data to establish general recognition of the safety and effectiveness,” of the mercury ingredients in these products. Mercury is still used in some diuretics, although substitutes now exist for most therapeutic uses.

2.1.5 Exposure to Mercury

2.1.5.1 Metallic Mercury Exposure

Inhalation of mercury vapor poses the greatest risk to health and safety because mercury is absorbed more rapidly through the lungs than through the digestive tract or skin. Metallic mercury is highly lipophilic (has a high affinity for body fat) and is absorbed almost completely by the lungs upon inhalation. A few drops of mercury can raise the vapor concentration in surrounding indoor air to a dangerous level. Air saturated with mercury vapor at 20˚ C contains a concentration that greatly exceeds toxic limits for humans. Inhaled mercury enters the bloodstream, where it can accumulate and stay in the kidney and brain for weeks to months. One study of Japanese workers exposed to metallic mercury indicated high mercury levels in the brain ten years after their last exposure to metallic mercury (Ryan, R. P., Terry, C. E., Eds., Taylor and Francis, 1997).

Dermal absorption is much slower than inhalation, but mercury exposure may produce skin irritations and allergic reactions. Absorption by ingestion is much slower than for inhalation. Ingested mercury does not enter the blood stream easily, and is mostly expelled in the feces. Mercury is also expelled from the body via exhalation, saliva, bile, and sweat. The half-life of metallic mercury in the human body is approximately one to two months in the body as a whole. The half-life in blood ranges from two days to approximately one month.

2.1.5.2 Organic mercury exposure

Renal toxicity from exposure to organic forms of mercury has only been reported in cases of severe poisoning accompanied by symptoms of neurologic toxicity. Cinca et al., 1979, reports that two children who died of complications from severe poisoning after consuming pork contaminated by ethyl-Hg were found to have high urinary protein, urinary sediment, and blood urea at the onset of their symptoms, and severe nephritis on autopsy (NAS, 2000). In addition 62 out of 86 cases of ethyl mercury poisoning from Iraqi grain seed exhibited clinical symptoms of kidney damage such as, oliguria, polydipsia, polyuria, and albuminuria (Jalili and Abasi, 1961).

2.1.5.3 Inorganic mercury exposure

Inorganic mercury can damage the stomach and intestines, producing symptoms of nausea, diarrhea, or severe ulcers if swallowed in large amounts. Effects on the heart have also been observed in children after they accidentally swallowed mercuric chloride. Symptoms included rapid heart rate and increased blood pressure. There is little information on the effects in humans from long-term, low-level exposure to inorganic mercury.

2.1.6 Physical Damage from Mercury

Chronic and acute mercury poisoning can produce irreversible physical damage to the kidneys, lungs, spinal cord, and central nervous system. Developing fetuses may also be damaged if the mother is exposed to mercury. Mercury poisoning has not been shown to cause cancer in animals, but it produces a variety of other types of damage, often irreversible. Victims may experience digestive disturbances, skin irritation, eye damage, leg cramps, loss of sensation around the lips, ataxia (inability to control voluntary muscle movements), or tunnel vision.

2.1.7 Symptoms of Mercury Exposure

Acute mercury poisoning produces any of a wide variety of symptoms. These include irritation and burning of the skin and eyes and skin allergies, including a condition known as acrodynia (Boca Raton, FL, 1997).The symptoms of acrodynia include flushing, itching, swelling, pink palms and soles of the feet, excess perspiration, and rashes.Exposure to mercury may also produce symptoms of respiratory distress, including lung irritation with coughing, chest pain or chest tightness, shortness of breath, and pulmonary edema (lung swelling due to excess fluid buildup) (Boca Raton, FL, 1997); (Ryan, R. P., Terry, C. E., Eds., Taylor and Francis, 1997) .Mercury poisoning may lead to chemical pneumonia, which can be fatal. Acute mercury poisoning can also produce symptoms of neurological damage, including tremors, irritability, weakness, chills, headaches, and disturbances in vision(Boca Raton, FL, 1997).Victims may experience a metallic taste, abdominal pain, diarrhea, nausea, and vomiting. If the damage is severe, death usually occurs within 10 days (Budavari, Ed., Merck Research Laboratories, Merck & Co, Rahway and NJ, 1996).Both genders may experience reproductive problems, and there is some limited evidence for spontaneous abortions in women whose husbands were exposed to metallic mercury (Ryan, R. P., Terry, C. E., Eds., Taylor and Francis, 1997).

Chronic mercury poisoning may develop gradually without conspicuous warning signs because mercury accumulates in body tissues (Dialog Corporation, 1998). Symptoms of repeated exposure include gray skin color, gum problems, tremors, memory and concentration problems, mood changes, and visual disturbances including clouding of the eyes. Rescuers are not directly at risk from individuals exposed to mercury vapor, although contaminated clothing can expose rescuers through direct contact or off gassing of mercury vapor.

2.1.8 Exposure Procedure

Exposure victims may exhibit any of the symptoms discussed in this section. It is important to move the mercury-exposed victims to fresh air, and call emergency medical services; inform medical responders that mercury is involved. A trained responder should wash the victim's exposed skin thoroughly with soap and water. If the victim's eyes have been in contact with the mercury, flush the eyes with water for at least 20 minutes. If the victim has ingested a large amount of mercury and is still conscious, give the victim a large amount of water to drink, then induce vomiting by having the victim touch the back of their throat with a finger (Foden, C. R., Weddell and J. L,1992). Keep the victim warm and at rest. Get immediate medical attention.

Victims or suspected victims of mercury exposure should remain under medical observation for 24 to 48 hours. Each victim should get a neurological exam (especially a handwriting test to detect tremors, an early sign of neurological damage). Kidney function and mercury levels in the urine should also be measured. The victims should get chest x-rays to detect signs of chemical pneumonia. An allergist should evaluate the victims' skin for reaction.

2.1.9 Exposure Limits

2.1.9.1 Mercury Limit in Wastewater

The Environmental Protection Agency (EPA) is the regulating body for Springs Utilities' IPP. In 1999, the EPA approved a maximum limit 25 parts per trillion (or 25 PPT) or 0.01 pounds per day for mercury, (known as the Maximum Allowable Headworks Loading or MAHL) based on acceptable levels entering the influent of the wastewater treatment plant, and limits on mercury in the Springs Utilities' Colorado Discharge Permit System.

Environmental legislation has been enacted in most countries and the standards have been made more stringent than ever including Malaysia. In our country DOE (Department of Environment) is empowered under the Environmental Quality Act 1974 (Amendment) 1985, to control and prevent pollution, as well as to protect and enhance the quality of the environment in Malaysia. Our government has initiated programs to control scheduled waste management to safeguard the environment and the safety of the people. In the Environmental quality (Scheduled Wastes) Regulations 1989, prescribed a listing of 107 categories of toxic and hazardous wastes defined as "scheduled wastes." And in 1993 the government introduced the legal control on import / export of scheduled wastes. DOE, Ministry of Science, Technology and Environment, Kuala Lumpur reported that in 1998, the generation of hazardous wastes was metal finishing, chemical, electronics and electrical, printing and packaging. The wastes were solids, liquids and semi-solids with difference chemical compositions. For mercury, the limit of concentration mercury in wastewater is 0.005mg/l.

Table 2.9: Parameter Limits of Effluent of Standards A and B

Parameter	Unit	Standard	
		A	B
Temperature	C	40	40
pH Value		6.0 - 9.0	5.5 - 9.0
BODs at 20ºC	mg/1	20	50
COD	mg/1	50	100
Suspended Solids	mg/1	50	100
Mercury	**mg/1**	**0.005**	**0.005**
Cadmium	mg/1	0.01	0.02
Chromium, Hexavaient	mg/1	0.05	0.05
Arsenic	mg/1	0.05	0.10
Cyanide	mg/1	0.05	0.10
Lead	mg/1	0.10	0.5
Chromium, Trivalent	mg/1	0.20	1.0
Copper	mg/1	0.20	1.0
Manganese	mg/1	0.20	1.0
Nickel	mg/1	0.20	1.0
Tin	mg/1	0.20	1.0
Zinc	mg/1	1.0	1.0
Borom	mg/1	1.0	4.0
Iron (Fe)	mg/1	1.0	5.0
Phenol	mg/1	0.001	1.0
Free Chlorine	mg/1	1.0	2.0
Sulphide	mg/1	0.50	0.50
Oil and Grease	mg/1	Not Detectable	10.0

2.1.9.2 Mercury vapor limit

Several government agencies have established limits for various types of mercury exposure. Many of these limits deal with the chronic exposure of workers in industries that use mercury or mercury-containing devices. Other limits deal with the effects of acute exposure, such as might result from a mercury spill (Table 2.9).

OSHA's legally enforceable ceiling limit for workplace exposure is set at 100 micrograms per cubic meter (μg/m^3). At no time should the mercury concentration exceed this level. The NIOSH Recommended Exposure Limit (REL) for mercury vapor is set at 50 μg/m^3 [as a time-weighted average (TWA)] with a skin designation.
The American Conference of Governmental Industrial Hygienists (ACGIH) set their Threshold3 Limit Value (TLV) at 25 μg/m^3 of mercury vapor (as averaged during and 8-hour workday). The U.S Environmental Protection Agency (USEPA) has set a reference concentration of 0.3 μg/m^3 for inhalation exposure. The ATSDR's Minimal Risk Level (MRL) is 0.2 μg/m^3 with an action level of 1.0 μg/m^3 that triggers remediation if exceeded in air.

Table 2.10: Environmental and Occupational Health Standards for Inhalation Exposure to Mercury Vapor

Environmental and Occupational Health Standards for Inhalation Exposure to Mercury Vapor	
AGENCY	MERCURY CONCENTRATION (μg/m)
OSHA Ceiling Limit[2]	100
NIOSH REL[3]	50
ACGIH TLV[4]	25
ATSDR MRL [5]	0.2
ATSDR Action Level for clean up	1
EPA Rfc[6]	0.3

[1]micrograms per cubic meter (μg/m)
[2]Ceiling Limit = the concentration of mercury vapor cannot exceed this limit at any time
[3]REL = Recommended Exposure Limit, a time-weighted average for an 8-hour day
[4]TLV = Threshold Limit Value, a time-weighted average for an 8-hour day
[5]MRL = Minimal Risk Level
[6]Rfc = Reference Concentration

2.1.9.3 Mercury Limit in Drinking Water

The EPA and Food and Drug Administration (FDA) limit for mercury in drinking water is 2 parts per billion (ppb). Ingesting 0.3 grams of mercury can be fatal to humans, and 75 milligrams per day (mg/day) in drinking water is fatal. The Resource Conservation and Recovery Act (RCRA) limit for mercury in leachate is 200 µg/L. A summary of Environmental and Occupational health standards is presented in Table 2.3.
There is a significant potential contribution to the overall exposure from contact with the skin, eyes, and mucous membranes. Dermal exposure can result in over exposure even though air levels are less than the specified limits.

2.1.9.4 Biological Monitoring

Biologic monitoring is the measurement of an chemical agents in the blood, urine, or other body tissue of exposed individuals to determine how much of the chemical has been absorbed into the body. It serves as a back-up to environmental exposure measurements, since air measurements cannot assess skin exposure or the effects of protective equipment and work practices. Since it measures the amount of an agent actually absorbed into the body, it is usually a better estimate of risk for adverse health effect than air monitoring.

There is no ideal biologic monitor for evaluating the risks of mercury intoxication from metallic or inorganic mercury. Mercury can be measured in both blood and urine. Individual levels may vary greatly from day to day and even within a given day. While proper interpretation of the results can be difficult, the measurements can nevertheless provide information on potential overexposure. Measurements should be carried out regularly (several times per year) in chronically exposed workers, and individual as well as group results should be evaluated. Baseline levels should be obtained before exposure begins for comparison purposes.

2.1.9.4.1 Mercury in Urine

Measurement of mercury in urine is the recommended biologic monitor for workers exposed to metallic and inorganic mercury. Ideally, the collection should be over 24-hours, but this is seldom feasible. Spot urine samples may also be taken, but care must be taken to always collect them at the same time of day near the end of the work week after several months of steady exposure. Overnight samples may also be collected; this collection extends from the time the employee goes to bed through the first urination of the morning. Samples must be collected in containers provided by the laboratory, since a preservative must be added. At least 25 cc of urine must be collected. Great care must be taken to prevent contamination of the sample containers or the urine with mercury from the skin or workplace air.

When results are interpreted, the urine values should be corrected for grams of creatinine in the sample, and should be expressed as ug Hg/gram creatinine. In persons not occupationally exposed to mercury, urine levels rarely exceed 5 ug/g creatinine.
While many laboratories indicate that only levels above 150 ug/L should be considered toxic, there is strong evidence that early signs of mercury intoxication can be seen in workers excreting more than 50 ug Hg/L of urine (standardized for a urinary creatinine of 1 gram/L). This value of 50 ug/g creatinine is proposed by many experts as a biological threshold limit value for chronic exposure to mercury vapor, and in 1980 this was endorsed by a World Health Organization study group. Exposed individuals with levels above 50 ug/g creatinine should be placed in a non-exposed job until the reason for their overexposure has been identified and corrected and their urine levels have fallen below the biologic threshold limit value.

2.1.9.4.2 Mercury in blood

The concentration of mercury in blood reflects exposure to organic mercury as well as metallic and inorganic mercury; thus it can be influenced by the consumption of fish containing methylmercury.

Samples should always be taken at the same time of day near the end of the work week after several months of steady exposure. The blood should be collected in mercury-free heparinized tubes after careful skin cleansing.

In unexposed individuals, the amount of mercury in blood is usually less than 2 ug/100 ml. According to some experts, an average airborne concentration of 50 ug/m3 corresponds to a mercury concentration in blood of about 3-3.5 mg/100 ml. Early effects of mercury toxicity have been found when the blood concentration exceeds 3 ug/100 ml. Any worker exceeding this level should be placed in a non-exposed job until dietary and workplace exposures have been evaluated and blood levels have returned to baseline.

2.2 Bacteria Overview

Life is very tenacious and can exist in very extreme environment. Living cells can be found almost anywhere like on the tops of mountains, the bottom of the deepest oceans, in the guts of animals, and even in the frozen rocks. The right temperature, pH, and moisture levels vary from one organism to another. Some cells can grow at -20˚ C (in the brine to prevent freezing), while others can be grow at 110˚ C (in brine under high pressure to prevent boiling).Cells that grows at low temperature are usually called *psychrophiles*, while those optimum temperature in range of 20˚ C to 50˚ C are *mesophiles*. Organism that grows best at temperature greater than 50˚ C is called *thermophiles*.

Many organisms have optimum Ph far from neutrality; some prefer ph values down to 1 or 2, while other may grow well at ph 9. Some organisms combine the ability to grow at low ph values and high temperature.Some cells require oxygen for growth and metabolism. Such organism can be termed aerobics. Other organisms are inhibited by the presence of oxygen and grow only anaerobically.Some organism can switch the metabolic pathways to allow them to grow under either circumstance. Such organisms are facultative.

Organisms come in a range of sizes and shapes. Spherical, cylindrical, ellipsoidal, spiral, and pleomorphic cell exist. Special names are used to describe the shape of bacteria. A cell with a spherical or elliptical is called a coccus; cylindrical cells is called rods or bacillus; spiral-shaped cells are called sprillum.Some cells may change shape in response to changes in their environment (Jun Miyake,Renewable Energy System by Biological Solar Energy Conversion, Elsevier 2004)

There are two primary cell types: eukaryotic and prokaryotic. The primary difference between these two types of cells is the presence or absence of a membrane around the cell's genetic information.Procaryotes has a simple structure with a single chromosome.Procaryotic cells have no nuclear membrane and no organelles, such as the mitochondria and endoplasmic reticulum.Eucaryotes have a more complex internal structure, with more than one chromosome in the nucleus. Eucaryote cells have a true nuclear membrane and contain mitochondria, endoplasmic reticulum, golgi apparatus and a variety of specialized organelles.The sizes of most procaryates vary from 0.5 to 3 micrometers (km) in equivalent radius. Different species have different shapes such as spherical or coccus (for example, (Stanphylococci), cylindrical or bacilluss (E.coli) and spiral or sprilium (Rhodospirillum).

Procaryotic cells grow rapidly and can utilize a variety of nutrients as carbon sources, including carbohydrates, hydrocarbons, protein and CO_2.These prokaryotic cells can be divided into two group which is Eubacteria and Archaebacteria.

The Eubacteria can be divided into several different groups. One distinction is based on the gram stain (develop by Hans Christan Gram in 1884).The gram stain is divided to gram-negative cells and gram-positive cells. A typical gram negative cell is E.Coli.It has an outer membrane supported by a thin peptidoglycan layer. Peptidoglycan is a complex polysaccaride with amino acids. A second membrane exists and separated from the outer membrane. The cells envelope serves to retain important cellular compounds and to preferentially exclude undesirable compounds in the environment. Loss of membrane integrity leads to cell lysis (cells breaking open) and cell death. A typical gram-positive cell is Bacillus subtilis. Gram-positive cell do not have an outer membrane. Rather they have a very thick, rigid cells wall multiple layers of peptidoglycan. Gram-positive cells also contain acids covalently bonded to peptidoglycan. Other distinctions within the eubacteria can be made based on cellular nutrion and energy metabolism.

Archaebacteria appear under the microscope to be nearly identical to many of the eubacteria. However, these cells differ greatly at the molecular level. In many ways, the archaebacteria are as similar to the eukaryotes as they are to eubacteria. One example of differences between archaebacteria and eubacteria is Archaebacteria have no peptidoglycan.

Meanwhile, Eucaryotes are five to ten times larger than prokaryotes in diameter. Fungi (yeast and molds), algae, protozoa, and animal and palnt cells constitute the eukaryotes. Eucaryotes have a true nucleus and a number of cellular organelles inside the cytoplasma. Cell wall and cell membrane structure of eukaryotes are similar to prokaryotes.

2.2.1 History of *Pseudomonas putida*

Pseudomonas putida was isolated from a polluted creek in Urbana, IL by enrichment culture with ethylbenzene as the sole source of carbon and energy. *P. putida* is one of the most well-studied aromatic hydrocarbon degrading bacterial strains. Well over 200 articles have been written about various aspects of *P. putida* physiology, enzymology, and genetics by microbiologists and biochemists, in addition to more applied studies by chemists and environmental engineers utilizing *P. putida* and its enzymes for green chemistry applications and bioremediation. Strain grows well with benzene, toluene, ethylbenzene, and *p* -cymene. Mutants of strain that are capable of growth with *n* -propylbenzene, *n* -butylbenzene, isopropylbenzene and biphenyl are easily obtained. In addition to aromatic hydrocarbons, the broad substrate toluene dioxygenase in strain can oxidize trichloroethylene (TCE), indole, nitrotoluenes, chlorobenzenes, chlorophenols and many other aromatic substrates. Although *P. putida* cannot use TCE as a source of