CHAPTER 2

LITERATURE SURVEY

2.1 MERCURY

2.1.1 SOURCES

2.1.1.1 Biogenic sources

Mercury is a naturally occurring element found in rocks, soils, water and volcanic dust, and is ubiquitous in the environment (Stein, Cohen, and Winer, 1996). Cinnabar (HgS) is the only high mercury associated ore for mining or smelting for commercial uses, the world's major source of cinnabar is Amalden in Spain (Napt, 1996). In natural condition, concentrations between 5 and 100 μg/kg are common in rocks, and the level of mercury in the air above rocks and minerals high in mercury ranges from 1.6 to 16 μg/l. In surface waters $Hg(OH)_2$ and $HgCl_2$ are the most common species. Generally, level in unpolluted waters is less than 0.1 μg/l. HgS is the most common species of mercury in sediments due to the low redox potential (Gavis and Ferguson, 1972). Most atmospheric mercury exists as Hg(0) or methyimercury, whereas much lower levels of dimethylmercury are reported. Mercury released into the atmosphere at $2.5x10^4$ to $5.0x10^5$ tons/year, whereas total levels of mercury in the ocean are estimated at $2x10^8$ tons (Robinson and Tuovinen, 1984).

2.1.1.2 Anthropogenic sources

Anthropogenic sources of mercury include those associated with its use in the chlor-alkali, paint, agriculture, pharmaceutical, and paper and pulp industries as disinfectants, catalysts, and fungicidal agents. Consumption of more than 9 million tons world wide is estimated. Over 12,500 tons of mercury per year are released into the environment from industrial mining activity (D'Itri, 1972 cited in Robinson and Tuovinen, 1984).

Approximately 80% of the anthropogenic sources of mercury are the emissions of elemental mercury to the air, primarily from fossil fuel combustion, mining, smelting, and solid waste incineration. The burning of fossil fuels is believed to be a major source of mercury released into the environment. Although the content of mercury in fuels is relatively low (on the order of 180 ppb), over 3,000 tons of mercury per year are released into the environment through the burning of coal, and an additional 10,000 to 60,000 tons are released from crude oils (Joensuu, 1971 cited in Robinson and Tuovinen, 1984). Another 15% of anthropogenic mercury emissions occur to the land via direct application of fertilizers, fungicides and municipal solid waste (e.g., batteries and thermometers). An additional 5% of mercury emissions occur via direct discharge of commercial effluent to water bodies (Stein, Cohen, and Winer, 1996). Therefore, human activities are estimated to account for $2x10^4$ to $7x10^4$ tons of mercury per year being released into the atmosphere and water supply. Sewage treatment facilities constitute a widespread source of both inorganic and organic mercury compounds with values ranging from 0.5 to 105 ppb of Hg (Robinson and Tuovinen, 1984).

2.1.2 PHYSICAL AND CHEMICAL PROPERTIES

Mercury is known as the transition metals that are oxyphilic and sulfophilic. It occupies Group II B in the Periodic Table. Atomic number and atomic massof mercury are 80 and 200.59, repectively. It exists in three valance states (0, +1, and +2) as well as in various inorganic and organic complexes. The melting point, boiling point and density are -38.9 degree celsius (^{0}C), 356.58^o C, and 13.546 g/cu. cm (liquid) and 14.193 g/ cu. cm (solid), respectively.

However, mercury is unique among the metals. It is a dense, silver-colored liquid at room temperature with relatively high vapor pressure, Hg (0) vapor pressure is 0.001201 mm at 20^{o}C (Baldi, Parati, Semplici and Tandoi, 1993). Other characteristics of this elements are: they are more electropositive, softer, lower melting points, more volatile, and also they can form complexes with compounds such as ammonia, amines, halides, and cyanides make it widely distribute into the environment (Cheremisinoff and Schiff, 1985; Stein, Cohen and Winer, 1996; Pollution Control, Department, Thailand, 1993).

2.1.3 USES OF MERCURY

Mercury has been used in a variety of agricultural and industrial proposed. Depending on its remarkable characteristics, i.e., high surface tension, uniform volume expansion, and inability to wet and cling to glass make mercury useful in measuring devices such as barometers and thermometers. By it's low electrical sensitivity and high thermal conductivity mercury could be chosen as a useful coolant. In addition, its ability to form amalgams make it useful for metal recovery and dental fillings. Its brilliant hues have led to mercury use in dyes and paints. Also, the toxicity of mercury to biota has led to its widespread use as a

toxicity of mercury to biota has led to the its widespread use as a bacteriocide and fungicide. The main sources and uses of mercury are summarized in **Table 2.1**.

Table 2.1 Sources and uses of mercury

Name	Form	Source or use
Mercury	Metallic or element	Chlorine-alkali manufacturing, Mining Dental fillings, Electrical equipment (batteries, switches) Instruments (thermometers, barometers)
Mercuric mercury	Inorganic (Hg^{2+})	Electrical equipment (batteries, lamps) Skin care products (cosmetics) Medicinal products, Chemical reagents in labolatory
Mercurous mercury	Inorganic (Hg^{+})	Electrical equipment (batteries), Medical products
Methylmercury	Organic (CH_3Hg^{+})	Diet (e.g., contaminated fish), Polluted sediment.
Phenylmercury	Organic ($C_6H_5Hg^{2+}$)	Fungicides, Pigments (paints)
Arylmercurial salts		Polymerisation catalysts (solid elastomeric), Polyurethanes, Adhesives.

Source: Stein,Cohen and Winer, 1996.

In Thailand, consumption of mercury during 1995s-1999s are summarized in **Table 2.2**

Table 2.2 Importation of Inorganic and Organic mercurial compounds in Thailand

Year	Amount (Kg)	Value (million Baht)
1995	13,145	8.6
1996	30,405	7.3
1997	10,547	14.0
1998	12,519	16.8
1999	13,064	16.0

Source: Customs official 28 January 1999

2.1.4 TOXIC EFFECT ON ORGANISMS

Mercury being widly use in a highly toxic metal. It's toxicity affects various processes in various organisms including growth, respiration, photosynthesis, membrane transport and cellular metabolism.

2.1.4.1 Human

Mercury toxicity in human is related to its affinity to form tight coordinate bonds with sulfhydryl groups diffusely disrupting enzyme systems in multiple organs such as brain, kidney, lung and developing

fetus. Acute or chronic effect is found after mercury exposure, depends on it chemical form. For example, elemental form is relatively inert and nontoxic but vapor mercury inhaled by and directly attacked brain and nervous system is highly toxic. Inorganic mercury in mercurial soluble salts form will severe injure the gastrointestinal tract, liver and kidney.

Organic mercury is the most toxic form , e.g., methylmercury, causes irreversible nerve and brain damage. This form is easily transported across biological membranes ($1.3x10^{-2}$ cm per sec was estimated by Gutknecht, 1981), stored in fat tissue and is able to be absorbed at small intestinal 90-95% (Cheevaporn, 1996). The half-lifes of mercury persistence in various organs of the human body ranged from 20 d to 80 d, depending on the species of mercury and human tissue being sampled (see **Table 2.3**).

Table 2.3 Half-life of mercury in Human Organs

Mercury species	Target organ	Half-life (d)
Methylmercury	Blood	52-65
Methylmercury	Kidney	70
Methylmercury	Whole body	71-79
Inorganic	Lung	2
Inorganic	Brain	30
Inorganic	Blood	3-30
Inorganic	Kidney	60
Inorganic	Whole body	42-60

Source: Stein, Cohen and Winer, 1996

2.1.4.2 Animals

Mercurial compound is the one of the most effective inhibitors of cellular metabolism in animal. For example, it will reduce efficiency of thyroid gland function, disturb metabolism of protein, affect endocrine system and reproduction system. Low concentrations of Hg(II) rapidly inhibit the uptake of glucose by rat muscle. (Pollution control, Department, Thailand, 1993).

2.1.4.3 Plants

Mercury toxicity affects various processes in plants including growth, photosynthesis, membrane transport and cellular metabolism. The effects on photosynthesis are inhibition of O_2 evolution and rate of chlorophyll synthesis. It causes a breakdown of membrane permeability resulted in a passive leaking of potassium ions from cells. And it has been reported that Hg(II) to inhibit acetylene reduction (N_2-fixation), growth and photosynthesis in ***Anabaena inequalis*** at concentrations of 6 to 10 ng/10^5 cells (Jeffries, 1982).

2.1.4.4 Microorganisms

All mercury compounds are cytotoxic to bacterial cells. Organic mercury is more toxic than inorganic form. Hg(II) can affect cell membrane permeability and inhibit enzymes. In gram-negative bacteria, the most frequently observed defect was an apparent loss of regulation in the cell wall synthesizing process, e.g., irregular cell wall contours, elongated pleomorphic as well as giant cells and spheroplasts, plasmolysis and irregular mesosomes. In gram-positive bacteria, the major defect occurred in cross-wall formation (Vaituzis, Nelson, Wan and Colwell, 1975). Exposure of ***Pseudomonas aeruginosa*** cells to $HgCl_2$ was shown

to cause swelling which could be reversed by addition of sulfhydryl compounds (Bernheim, 1971 cited in Vaituzis et al., 1975). Inhibition of cell-free protein synthesis in ***Clostridium cochlearium*** by inorganic mercury is more stronger than by methylmercury. ***E. coli*** given low concentrations of $HgCl_2$ caused an increase in ribonuclease 1 synthesis thus causing a degradation of RNA. Hg(II) and organomercurials can inhibit DNA mediated genetic transformation in ***Bacillus subtilis*** 168 (Cheremisinoff and Schiff, 1985).

2.2 METHODS OF WASTE MERCURY REMAVAL

Conventional mercury-removal processes from waters commonly involve precipitation with polysulfides (Findlay & Mclean, 1981) thiourea or thio acetamide at pH 3.5 to 4.0. The most common methodology involves treatment of wastewater with sodium sulfide, polysulfides or hydrosulfide to convert the mercury to mercury (II) sulfide which could be further precipitated. In some treatment plants, ion-exchange resins and activated carbon were used as polishing steps after sulfide precipitation to reduce mercury concentrations in effluents even further (Findlay and McLean, 1981).

2.2.1 PHYSICO-CHEMICAL METHODS

2.2.1.1 Chemical precipitation

Generally, inorganic mercury can be removed from aqueous solution by raising the pH to 10-12 with an appropriate alkali, or adding a soluble sulfide or dithionate.

Alkoxylalkyl mercury is readily converted to inorganic mercury by the addition of acids, then treated by above method.

Care should be taken to ensure as complete precipitation as possible takes place. Dry distillation (or retorting) is sometimes use to recover mercury from the precipitate.

Arylmercury is converted to an insoluble chloride, then add a flocculating agent such as a polyamide, and allow to settle, with the sludge being sent for recovery or disposal (Department of the Environment Waste Management, U.S.A., 1977; Habashi, 1978).

2.2.1.2 Chemical reduction

Many reducing agents including zinc, dithionite, aluminium, iron felt, formaldehyde, formic acid, hydroxylamine, hydrazine and sodium borohydride are use to produce mercury from wastes. Some reducing agents are hazards, therefore competent control is needed (Department of the Environment Waste Management, U.S.A., 1977; Grau and Bisang, 1995).

2.2.1.3 Filtration and adsorption

Sometimes processes are used to polish effluents after precipitation. For example montmorillonite, a hydrated silicate of magnesium is used.

Various activated or treated charcoals are known to be used as final effluent polishing processes. In fact, activated carbon itself is a relatively inefficient adsorbent for mercury and its use tends to be expensive. However, several treatments can enhance their performance such as porous cellulose carrier modified with polyethyleneimine (Navarro, Sumi, Fujii and Matsumura, 1996). Adsorption process of mercury vapor on

sulfur-impregnated activated carbon, active alumina and zeolite by using packed beds have been investigated by Otani, Emi, Kanaoka, Uchijima and Nishino (1988).

2.2.1.4 Ion exchange

Usually, ion exchange resins are used in the removal of mercury from aqueous streams, particularly at low concentration levels, 1 to 20 ppb, (McGarvey, 1993).

Most successful ion exchange processes for removing mercury from solution employ anion-exchange or chealating resins. A few cation exchangers have reported to be quite successful in extracting ionic mercury, brines mercury exists as the $HgCl^{=}_{4}$ anion (Department of the Environment Waste Management,U.S.A., 1977).

2.2.1.5 Solvent extraction

This method can be used to remove mercury from liquid or solid mercury-containing wastes and has involved high molecular weight organic amine extractants on a laboratory-scale (Department of the Environment Waste Management,U.S.A., 1977).

All of the treatment, precipitation with sodium sulfide was the most common methodology. In some treatment plants, ion-exchange resins and activated carbon were frequently used in polishing steps after sulfide precipitation to reduce mercury concentrations in effluents. Mercury-bearing wastes will be disposed further in many ways.

2.2.2 METHODS OF WASTE DISPOSAL

Currently disposal method used for mercury-bearing wastes include:

1) To rivers, canals and estuaries;
2) To sewers;
3) To rock salt cavities and mineshafts;
4) To lagoons and soakaways;
5) To land-by controlled landfill, or by spraying on land;
6) By chemical fixation (e.g., solidification processes) followed by the fifth method as appropriate and
7) By incineration, burning or retorting, followed by landfill for residues.

Disposal methods may be covered by considering individually industries. Mercurial wastes arising from firms within an industry are often of similar types (Department of the Environment Waste Management, U.S.A., 1977). Mercury can be removed from wastewaters by those methods easily; however, each methods have various problems which may limit the application to industrial situation. For instance, the cost of sodium borohydride, the cost of landfill will be a significant factor in the implementation of these methods, while chemical methods often leave hazardous by-products or residual sludge. Therefore, searching for alternative approaches may be necessary such as biological methods.

2.2.3 BIOLOGICAL METHODS

Biological processes of the removal of Hg from waste waters have been investigated. Most of them involve uptake or binding to the microorganisms as follow :

2.2.3.1 Algae

Chlorella sp., a green alga, has a high sorptive capacity for a variety of metal ions including mercury (Baldi et al, 1993) and was able to volatile mercury. The rate of volatilization observed from 1 μm $HgCl_2$ is 3.0-3.3 μmol/mg dry wt ⌣ h (Jeffries, 1982).

2.2.3.2. Yeast and fungi

A yeast of the genus ***Cryptococcus*** has been shown to be capable to reducing mercury to the elemental state (Brunker and Bott, 1974; Yannai, Berdicershy and Duck, 1991). A mutants yeast, ***Saccharomyces cerevisiae***, has increasing the Hg(II)-binding capacity of the cell wall (Ono, Ohue and I shihara, 1988). Macrofungus was able to transform mercury to methylmercury and/or bioaccumulate methylmercury (Fischer, Rapsomanikis and Andreae, 1995).

2.2.3.3 Plant

Azolla pinnata was able to accumulate mercury 2.7 ± 0.3 μg/l-14.3 ± 1.5 μg/l in 30 days (Mishra, Nanda and Misra, 1987). A research of transgenic plant, ***Arabidopsis thaliana***, receiving highly modified bacterial mercuric ion reductase qene, ***mer*** A 9, to detoxify ionic mercury (II), reducing it to Hg(0) was conducted by Rugh et al., 1996).

2.2.3.4 Bacteria

Hansen et al.(1984) reported that a continuous culture of a Hg-resistant ***Escherichia coli*** KP 245, harboring the cloned plasmid pRR 130, was maintained on raw sewage for 2 weeks at 37 C, during which time relatively high concentrations of mercury (70 mg/l) were removed Hg at a rate of 2.5 mg/l,h, with an efficiency of up to 98%.

A model for mercury ion reduction by a recombinant strain of ***Escherichia coli*** has been proposed that the overall Hg(II) reduction process can be considered as two sequential steps, i.e., the Hg(II) transport system and the enzymatic Hg(II) reduction reaction (Philippidis, Schotte and Hu, 1991). A continuous culture of a Hg-resistant ***Pseudomonas putida*** strain FB-1 was fed with a synthetic medium containing 1 mg Hg/l as $HgCl_2$. The removal efficiency at different dilution rates (from 0.1 to 3.0/day), ranged form 99.2% to 99.8%, and the residual Hg was maintained below 5 μg/l (Baldi, Parato, Semplici and Tandoi, 1993). Biosorption of mercury by the inactivated cells of ***Pseudomonas aeruginosa*** PU21 (Rip 64) was reported that the maximum adsorption capacity as approximately 180 mg Hg/g dry cell in deionized water and 400 mg Hg/g dry cell in sodium phosphate solution at pH 7.4 (Chang and Hong, 1994). Low-inoculum batch cultures of mercury-resistant bacteria have been used to determined the growth rate and the mercury detoxification rate of ***Pseudomonas aeruginosa*** PU21 (Rip 64). It was found that the specific growth decreased as mercury concentration increased and the specific mercury detoxification rate increased slightly between 0-2 μg Hg(II)/mL (Chang and Hong, 1995). Mercury volatilization by immobilized mercury-resistant bacterial cells and native cells of ***Azotobacter chroococcum*** were conducted by Ghosh et al. (1996

a, b). The result indicated that mercury volatilization efficiency of immobilized cells was much greater than native cells and immobilized cells can be reused. Developing bioprocesses of mercury-hyperresistant strain of ***Pseudomonas seruginosa*** PU21(Rip64) was investigated the kinetics of mercury detoxification to determine the parameters needed for bioprocesses design. The results showed that the lag phase cells exhibited the best specific mercury detoxification rate, approximately 1.1 x 10^{-6} μg Hg/cell/h and the rate was optimal at an initial mercury concentration of 8 mg/l (Chang and Law, 1997).

In 1998 Chang, Chao and Law was demonstrated a wild-type mercury-resistant strain ***Pseudomonas aeruginosa*** PU21 (Rip64) and ***Escherichia coli*** PWS1 strain qenetically engineered to harbor mercury resistance for their capacity to detoxify soluble mercuric ions with repeated fed-batch operations. The results showed that the wild-type and the recombinant strains had an optimal specific activity of $5x10^{-7}$ and $8x10^{-8}$ μ g/ cell h, respectively. Mercury removal by ***Escherichia coli***, cells engineered to express an Hg(II) transport system and metallothionein accumulated Hg(II), effectively over a concentration range of 0.2-4 mg/l in batch systems was proposed (Chen and Wilson, 1997a, b; Chen, Kim, Shuler and Wilson, 1998) based on its kinetics and isotherm. A hollow fiber bioreactor was capable of removing and recovering Hg(II) effectively at low concentrations, reducing a 2 mg/l solution to about 5 μg/L.

A variety of chromatographic carriers activated were used as chemically immobilize mercuric reductase obtained from ***Pseudomonas putida*** KT2442 (***mer*** 73). Best results were achieved with tresyl chloride-activated carriers and the optimum binding conditions were found at pH 8.

They also constructed fixed-bed reactor with the immobilized enzyme to evaluate the performance of mercury reduction in a continuous process, space-time yields up to 510 nmol/min.mL (Anspach et al., 1994). Mercuric reductase from a recombinant strain, ***E coli*** PWS1, was immobilized on a diatomaceous earth supportt and was examined for mercury reduction in a continuous fixed-bed operations. Electron transfer efficiency of four dyes was determined as the alternative of NADPH for mercury detoxification. The result showed that immobilized enzyme exhibited maximum activity (1.2 nmol Hg/mg protein/s) of substrate-inhibition-type kinetics at initial Hg(II) concentration of 50 $\mu mol.dm^{-3}$. Mercury-reducing efficiency of using neutral red was only 30-40% of that obtained using NADPH (Chang, Hwang, Fong and Lin, 1999).

2.3 MICROBIAL TRANSFORMATIONS OF MERCURY

2.3.1 METHYLATION

The capacity to methylate mercury is fairly widespread among microorganisms, but rates are generally low. The rate of methylation is affected by various environmental factors including salinity, nutrients, pH, redox potential, H_2S concentration and oxygen (Gilmour and Henry, 1991; Compeau and Bartha, 1984; Gilmour, Henry and Mitchell, 1992; Steffan, Korthals and Winfrey, 1988). Methylcorrinoid derivatives are believed to be the cofactors for methylation (DeSimone et al., 1973; Imura et al., 1971; Bertilsson and Neujahr, 1971; Choi and Bartha, 1993).

2.3.1.1 IN SEDIMENTS AND AQUATIC SYSTEMS

Methylmercurywhich is the predominant form in fish can occur both aerobic and anaerobic conditions. Fagerström and Jernelöv (1971) showed

that it could be formed from pure HgS by organic sediments under aerobic conditions, but at a rate slower than that observed for formation from $HgCl_2$. Methylmercury and dimethylmercury have a high solubility in lipids and solvents and a high affinity for the sulfhydryl groups on proteins. It is a potent neurotoxin and may be accumulated in the food chain, making it a potential health problem and was found in low levels in environmental. Biological methylation of mercury by microorganisms is believed to play a role in the formation of methylmercury in aquatic organisms and sediments and may represent an important link in the mercury cycle.

2.3.1.2 RATES AND STIMULATORY EFFECTS

Imura et al.(1971) reported that the methylation proceeded at a remarkably high rate when methylcobalamin and inorganic mercury were mixed. Methylmercury is formed from $HgCl_2$, HgI_2, HgO, $Hg(NO_3)_2$, $Hg(SO_4)_2$ and $Hg(CH_3COO)_2$ but not from HgS by the anaerobic bacterium, ***Clostridium cochlearium***. The formation of methylmercury was confirmed by thin-layer chromatography and by the degradation of the product by the ***Pseudomonas*** sp. K62 soil strain capable of degrading methylmercury (Yamada and Tonomura, 1972; Baldi and Filippelii, 1991).

Regnell and Tunlid (1991) confirmed that the proportion of methylated ^{203}Hg was significantly higher, in both water and sediment, in the anaerobic systems than in the aerobic systems and found that episodes of anoxia in bottom waters and sediment cause an increase in net mercury methylation and, hence, an increase in bioavailable mercury.

Sulfate reducers of anoxic aquatic sediment were identified as the principal environmental methylators of mercury (Compeau and Bartha, 1985; Compeau and Bartha, 1987).

Addition of $CoCl_2$ and benzimidazole to fermentative cultures increased methylation activity (Choi and Bartha, 1993). Choi and Bartha (1994 a, b) proved that ***in vivo*** Hg(II) methylation is an enzymatically catalyzed process, the methyl group may originate from C-3 of serine or from formate via the acethyl-CoA synthase pathway, in ***Desulfovibrio desulfurican*** LS.

Methylmercury is also reportedly produced in aerobic sediments and by pure cultures of aerobic microorganisms (Hamdy and Noyes, 1975; Spangler et al., 1973a). A comparison of aerobic and anaerobic methylation of $HgCl_2$ in San Francisco Bay sediments indicated that methylmercury formation was faster and resulted in higher net levels under anaerobic conditions and in samples with the highest organic content (Olson and Cooper, 1976).

Yeast, ***Saccharomyces cerevisiae*** and ***Candida albicans***, were produce methylmercury in the growth media after 12 days of incubation (Yannai, Berdicevsky and Duek, 1991); furthermore, ***Coprinus comatus*** and ***Coprinus radians***, macrofungus, are able to methylate and accumulate mercury (Fischer, Rapsomanikis and Andreae, 1995).

2.3.1.3 MECHANISM OF METHYLATION OF MERCURY

The pathways involving the methylation of mercury are given below.

1). Abiotic or photochemical methylation of Hg(II), depend on existence of methyl donor and mercury in the environment and

methylmercury was produced with irradiation of sunlight or ultraviolet light (Hamasaki, Nagase, Yoshioka and Sato, 1995).

2) The methylation of Hg(II) in sediments by bacteria that excrete methylcobalamin which can act as a methyl donor.

3) The methylation of mercury by bacterial flora of aquatic organisms also perhaps utilizing methylcobalamin (Summer and Silver, 1978). The methylation reaction is believed to proceed via electrophilic attack of the mercuric ion on the carbanion species which is stabilized by the cobalt atom (DeSimone et al., 1973). The overall reaction proceeds was shown as follow.

$$Hg^{2+} \xrightarrow[CH_3B_{12}]{\text{methylcobalamin}} (CH_3)Hg^{+} \xrightarrow[CH_3B_{12}]{\text{methylcobalamin}} (CH_3)_2Hg$$

4) Enzymatic catalysis of mercury methylation by ***Desulfovibrio desulfuricans*** LS which the methyl group may originate from C-3 of serine or from formate via the acethyl-CoA synthase pathway (Choi, Chase and Bartha, 1994).

2.3.2 DEGRADATION AND VOLATILIZATION

Degradation of organomercurials and volatilization of Hg (II) have been demonstrated in sediments, with mixed cultures, and by a wide variety of bacteria, algae and yeast. In the case of bacteria, abilities to degrade and volatilize are mediated by plasmids. The mechanisms for volatilization by algae and yeast are not as well known but might consist of both enzymatic and non enzymatic components (Jeffries, 1982).

2.3.2.1 IN NATURAL ENVIRONMENTS

It has long been investigated that organomercurial compounds are degraded and volatilized by mercury-resistant bacteria (Tonomura, 1968; Nelson et al., 1973; Shariat, Anderson and Mason, 1979; Nakamura, Sakamoto, Uchiyama and Yagi, 1990).

Methylmercury degradation also occurs in bacterial isolated from fish and sediment from Lake St. Clair, result indicated that demethylation is an aerobic process. However, the facultative organisms also degrade methylmercury under both aerobic and anaerobic condition (Spangler et al., 1973b). Furthermore the strain ***Desulfovibrio desulfuricans***, one known to synthesize monomethylmercury from ionic mercury, can produced insoluble dimethylmercury sulfide, which slowly decomposed under anaerobic conditions to metacinnabar and volatilized to dimethylmercury and methane (Baldi, Pepi and Filippelli, 1993).

2.3.2.1 RATES IN PURE CULTURES

A highly resistant pseudomonad (***Pseudomonas*** K62) degrades ethylmercuric phosphate and monomethylmercury to Hg(0) under aerobic conditions, ***Pseudomonas*** K62 has been reported to remove phenylmercuric acetate from a 100 mg/l solution at a rate of about 0.68 to 0.81 mg (2.0 to 2.4 μmol) phenylmercuric acetate /mg dry wt cells.h (Furukawa, Suzuki and Tonomura, 1969; Furukawa and Tonomura, 1972).

Strains of ***Pseudomonas aeruginosa*** bearing resistance plasmids R3108 and FP2 volatilized Hg(II) at maximal rates of about 600 and 680 nmol/mg dry wt. h and phenylmercuric acetate at rates of 42 and 29 nmol/mg dry wt. h (Clark, Weiss and Silver, 1977).

The rates of monomethylmercury degradation have been measured for strains of ***Enterobacter aerogenes*** and ***Serratia marcescens*** . The initial rates of demethylation are governed by methylmercury concentration and pH. Maximal rates were obtained at a concentration of 5 mg methylmercury (MM^+)/L. The maximum rate of demethylation reported for ***E. aerogenes*** was about 180 μg (0.73 μmol)/L.day at pH 6. The authors used suspensions containing 10^6 cells/ml, so this rate is equivalent to the degradation of about 6.5 nmol MM^+/mg dry wt of cells. h. For ***S. marcescens***, the maximak rate obtained was 3 nmol/mg dry wt. h at pH 8 (Mason, Anderson and Shariat, 1979).

Strain of ***Staphylocoocus aureus*** bearing mercury resistance plasmids RN23 (pI258) volatilized Hg(II) and degraded PMA at maximal rate 1.8 nmol/min. mg. cells and 0.7 nmol/min mg of cell, respectively (Weiss, Murphy and Silver, 1977).

In 1971 Komura and Tzaki reported that plasmid-bearing strains of ***E .coli*** volatilize and resist mercuric ions (Komura and Tsaki, 1971). The rate of volatilization by a plasmid-bearing strain of ***E. coil*** was depend on the concentration of the substrate and reached a maximum value of 4 to 5 nmol Hg (II)/min.10^8 cells or about 520 to 650 nmol Hg (II)/mg dry wt cells. h, at a concentration of about 30 μm (6 mg/l) mercuric ions (Summers and Silver, 1972). A yeast of the genus ***Cryptococcus*** has been isolated from a stream and was shown to be capable of reducing mercury to the elemental state (Brunker and Bott, 1974).

2.3.2.2 MECHNISM OF MERCURY DEGRADATION AND VOLATILIZATION.

Mechanisms of mercury degradation and volatilization depend on intracellular enzyme which produced by resistant gene in mercury resistant bacteria. The enzyme will cleavage the carbon-mercury bond in PMA or other orgnomerourial compound. Metallic mercury and benzene are detectable as reaction products. The details of these mechanism will be discuss in the next sections.

2.3.3 OXIDATION

Elemental mercury vapor, Hg (0), the majority of mercury emissions can arise from a number of natural and anthropogenic sources (Stein, Cohen and Winer, 1996). Hg (0) is oxidized to Hg (II) in the atmosphere by the interaction with ozone in the presence of water. It has also been known for over a decade that mammals and plants effectively oxidize Hg (0) vapor to Hg (II) using catalase and possibly other peroxidases. The two electron transfer from Hg (0) to Hg (II) occurs at the expense of hydrogen peroxide and is mediated by a high-spin Fe (IV) in the heme cofactor. This reaction is central to Hg(0) intoxication as it converts the relatively nonreactive qaseous form of mercury into the highly reactive and toxic water-soluble ionic form, Hg (II), which avidly combines with sulfhydryl and imino nitrogen ligands in preteins and other important biological molecules. Holm and Cox (1974) investigated method for introducing elemental mercury into biological growth system and in the next year (1975) they tested ability of bacteria to transform elemental mercury, found that the quantity of elemental mercury oxidized by bacteria ranged from small amounts for ***Psendomonas aeneginosa, P.fluorescens, Escherichi coil,*** and ***Citrobacter*** sp. to essentially all of

the added elemental mercury for ***Bacillus subtilis*** and ***B. meqaterium.*** The percentage of the total mercury in the system associated with bacterial cells ranged from 18.6 to 43.2 %. Wild-type ***Escherichia coli*** and several derivatives with altered catalase activity and common soil bacteria, ***Bacillus*** sp. and ***Streptomyces*** sp. Could effect Hg(0) transformation. The result found that ***E coli*** can oxidize Hg(0) to Hg(II) and two typical soil bacteria also oxideze Hg(0) to Hg(II) (Smith, Pitts, McGarvey and Summers, 1998). When mercury and their compounds enter the environment. The control of these substance are by biological or geological factors. Microorganisms can mobilize mercury in the aquatic environment and possibly recycle mercury through the environment by transformation then into various forms. The generalized cycling of mercury was shown in **Figure 2.1** on page 37.

2.4 BACTERIAL MERCURY RESISTANCE

2.4.1 INTRODUCTION

Mercury and organomercurial compounds are often released into the environment in biologically available from by geochemical processes and by human intervention. This evidence tends to select for bacteria which can resist to mercury in different bacterial genera and species.

Mechanism of resistance results from enzymatic detoxification of mercurials, intracellular enzyme, code by gene on plasmid or chromosome in both Gram-negative and Gram-positive bacteria. These enzyme are highly specific with mercuric and mercurous ions and no reduction and oxidation with other metal (Rinderie, Booth and Williams, 1983). Details of population, genetic and mechanism of resistance are discussed in the following sections.

2.4.2 POPULATIONS

Mercury and organomercurial-resistant bacteria were first isolated from mercury-contaminated sediment in Japan (Tonomura, 1968 ; Nakamura, Fujisaki ans Tamashiro, 1986, Nakamura, Fujisaki and Shibata, 1988 : Nakamura et al., 1990) and in other country (Austin, Allen, Mills and Colwell, 1977; Baldi, Filippelli and Olson, 1989 ; Nelson et al.,1973; Olson, Lester, Cayless and Ford, 1989; Spangler et al., 1973b) They have since been isolated from municipal sewage (Buelva, Kakii and Kuriyama, 1995), from fish (Sadhukhan et al., 1997)from Coral reef and mangroves (Garcia, Orta and Suarez, 1999), from Soil (Ray, Gachhui, Chaudhuri and Mandal, 1989 ; Kelly and Reanneys, 1984) and from Oral and Fecal flora among persons with amalgam fillings (Österblad et al., 1995 ; Summers et al., 1993).

All of these mercury resistant bacteria belong to qenera are categorized as follow.

1. Iron-and sulfur-oxidizing, acidophilic bacterium.

Thiobacillus ferroxicians.(Olson, Iverson and Brinckman, 1981)

2. Sulfate-reducing bacteria.

Desulfovibrio desulfurican. (Baldi, Pepi and Fillippelle, 1993)

3. General bacteria.

Bacillus sp. (Nakamura and Silver, 1994).

Pseudomonas sp.

Vibrio sp.

Corynebacterium sp.

Micrococcus sp.

Staphylococcus aureus.

Serratia sp.

Acinetobacter sp.

Flavobacterium sp.

Chromobacterium sp.

Shigella sp.

Escherichia coli.

Erwinia sp.

Klebsiella sp.

Enterobacter sp.

Salmonella sp.

4. Consortium bacteria

Xanthomonas maltophilia HGS1

Aeromonas hyolrephila HGS2

Alcaliqenes eutrophus HGS4

5. Nitregen fixing bacteria.

Azotobacter chroococcum.

Bacterial resistant to mercury and organomercurials is determined by plasmids, which in many instances also encode resistance to other heavy metals and antibiotics (Hermansson, Jones and Kjelleberrg, 1987;Ö sterblad et al., 1995; Summers et al., 1993; Timoney, Port, Giles and Spanier, 1978; Wireman, Liebert, Smith and Summers, 1997; Mahler, Levinson, Wang and Halvorson, 1986; Aiking, Govers ans Riet, 1985).

The relationship between resistance to mercury and other heavy metals and antibiotics in the hospital environment has been explored in numerous studies. There appears to be a strong correlation between antibiotic resistance and mercury resistance and several other metals (Nakahara et al., 1977a; Nakahara et al., 1977b; Spangler et al., 1973a). In most instances, the frequency of heavy metal resistance is the same as or higher than that of antibiotic resistance.

Of a total of 787 clinical isolates of ***Pseudomoans aeruginosa***, 99.8% were found to be metal resistant, with 99.5% exhibiting multiple resistance. The frequency of mercury resistance among these isolate was 75.1% only 53.2% of these metal resistant isolated were also multiply antibiotic resistant (Nakahara et al., 1977b). These results suggest that the frequency of resistance to metals is greater than resistance to antibiotics and that most of the metal-resistant strains are multi resistant.

An investigation of the frequency of drug and heavy-metal resistance in clinical isolates of ***Escherichia coli,*** ***Klebsiella*** sp., ***Pseudomonas aeruginosa,*** and ***Staphylococcus aureus*** revealed that metal ion resistance occurred at frequencies equal to or higher than resistance to antibiotics. The frequencies of mercury resistance were 57.3, 65.9, 75.1, and 36.3% for the organisms listed above. The mercury resistance determinant was transferred in bacterial mating 89.9% of the time and could be cured at a high frequency by treatment with acriflavin or growth at 48 ^{0}C (Nakahara et al., 1977 a).

Moreover mercury-resistant bacteria were have ability to utilize oil when the concentration of mercury was adjusted to 1-5 ppm mercury in the oil. Mercury-resistant strains of bacterial isolated from samples collected in the Chesapeade Bay, utilize petroleum in 7 days and tolerant to concentration of mercuric chloride at high level (60 mg/l), mostly belong to ***Pseudomonas*** sp.(Walker and Colwell, 1974, 1976).

2.4.3 Mechanisms of Resistance

The mechanism of resistance to mercuric ions and organomercurials, involves the elimination of the metals from the growth medium. Reported by several groups (Rajinirani and Mahadetan, 1989; Silver, Misra and Laddaga, 1989; Misra, 1992; Nies, !999) have shown that there are two processes involved in the resistance mechanism, i..e., transport of mercuric ions into the cell, and enzymatic reduction catalyzed by mercuric reductase, converting Hg(II) to Hg(O). To prevent toxic effects of Hg (II) in mercury-resistant bacteria, Hg(II) is transferred into cell via specific uptake systems (Figure 2.3 on page 35). Mercuric ions were bound by the periplasmid Hg(II)-bearing protein MerP as the first step of detoxification (Qian et al., 1998). MerP probably delivers the toxic cation to the mercury transporter MerTP, another uptake route exists which involves the MerC protein (Hamlett et al., 1992; Sahlman et al., 1997). Once inside the cell, Hg(II) is reduced with NADPH to Hg(0) by the MerA protein, which is related to glutathione reductase and other proteins (Schiering et al., 1991).

Organomercurials, which are more toxic than Hg(II), may also be detoxified if the ***mer*** resistance determinant encodes a MerB organomercurial lyase in addition to the other Mer proteins (Silver, 1996;

Silver and Phung, 1996). After cleavage by MerB, the resulting Hg (II) is reduced by MerA. In Gram-negative bacteria, the mechanism of mercury-resistance bacteria is the most widely found on plasmid-determined metal resistance (Olson et al., 1979; Misra, 1992). It occurs in two forms (**Figure 2.2** on page 37), i.e., Narrrow-spectrum and Broad-spectrum resistance. Narrrow-spectrum resistance allows bacteria to reduce inorganic mercury, Hg(II), to less toxic, volatile, metallic mercury, Hg(0). Such bacteria are also resistant to a few organomercurials such as merbromin and fluorescein mercuric acetate, Broad-spectrum resistance allows bacteria to degrade certain organomercurials (such as phenylmercuric acetate and methyl mercury) as well as to reduce inorganic mercury (Levy and Miller, 1989). In Gram-positive bacteria, the mercuric resistance system occurs on plasmid, e.g., ***Staphylococcus aureus*** plasmid pI258 which consists of a series of six or seven genes (Weiss, Murphy and Silver, 1977; Levy and Miller, 1989). The next Gram-positive ***mer*** sequence determined came from a soil ***Bacillus*** sp. strain and it occurs on the bacterial chromosome (Nakamura and Silver, 1994). The last one of the mercuric resistance operons sequenced comes from a ***Streptomyces lividans*** strain (Silver, Misra and Lalddaga, 1989).

Numerous strains of ***Escherichia coli***, ***Pseudomonas aeruginosa*** and ***Staphylococcus aureus***, as well as ***Pseudomonas putida*** and ***Thilbacillus ferroxidans***, have all been found to volatilize mercury from added mercuric ions (Tonomura et al., 1969; Izaki, 1971; Komura, Funaba and Izaki, 1971; Furukawa, Suzuki and Furukawa and Tonomura, 1972; Izaki, Tashiro and Funaba, 1974; Clark, Weiss and Dilver, 1977; Izaki, 1977; Komura and Olson, Iverson, Frederick

and Brinkman, 1981; Olson, Porter, Rubinstein andSilver, 1982; Baldi and Olson, 1987).

2.4.4 REGULATION OF MERCURY RESISTANCE

Molecular genetic and biochemical studies of mercury resistance bacteria, reveal that each is an operon consisting of several structural genes (**Figure 2.3** on page 38) under the Hg-inducible control of a regulatory protein which acts in both a positive and negative manner. In the absence of mercuric ion, the mer R regulatory protein acts as a repressor and prevents initiation of transcription . When the concentration of mercuric ions exceed 10^{-7} M, the transcription rate reaches its maximum (O'Halloran, 1993). The gene products, Mer P and Mer T, mediate the specific uptake of mercuric ion Mer P is a periplasmic mercury binding protein that acts to scavenge Hg(II) from the environment and Mer T receives Hg(II) from Mer P and then transfer them into the cytoplasm where the reduction takes place by mercury reductase (Misra, 1992).

As according to literature reviews above remark that mercury resistance mostly found in Gram-negative bacteria which carry mercury-resistant gene on plasmid. In this property cause easily to improve the ability of mercury resistance and can transfer mercury-resistant gene to other organism to use in mercury removal.

For instance, Transgenic plant that receiving highly modified bacterial mercuric ion reductase gene to detoxify mercury is between investigating (Rugh, et al., 1996). Genetic engineering of bacteria for bioremedation of mercuric ion-contaminated water in U.S.A. (Chen and Wilson, 1997 a, b; Chen et al., 1998). An investigation of mutagenisis of ***Pseudomonas putida*** which constitutively overexpress mercury resistance for biodetoxification of organomercurial pollutants in Germany (Horn, Brunke,

Deckwer and Timmis, 1994).Furthermore, the study about gene of mercury-resistant bacteria still investigate in many year (Kiyono, Omura, Fujimori and Pan-Hou, 1995a; Kiyono, Omura, Fujkmori and Pan-Hou, 1995 b; Kiyono et al., 1997; Uno, Kiyono, Tezuka and Panj-Hou, 1997; Kiyono and Pan-Hou, 1999; Kiyono, Uno, Omura and Pan-Hou, 2000). These investigation indicate that mercury pollution is importance problem because they wilding spread by biogeochemical cycle. If we can cut this cycle by recovery them as useful form. The cycle of mercury may be reduce. Therefore, mercury-resistant bacteria might suitable to bioremediation in the future.

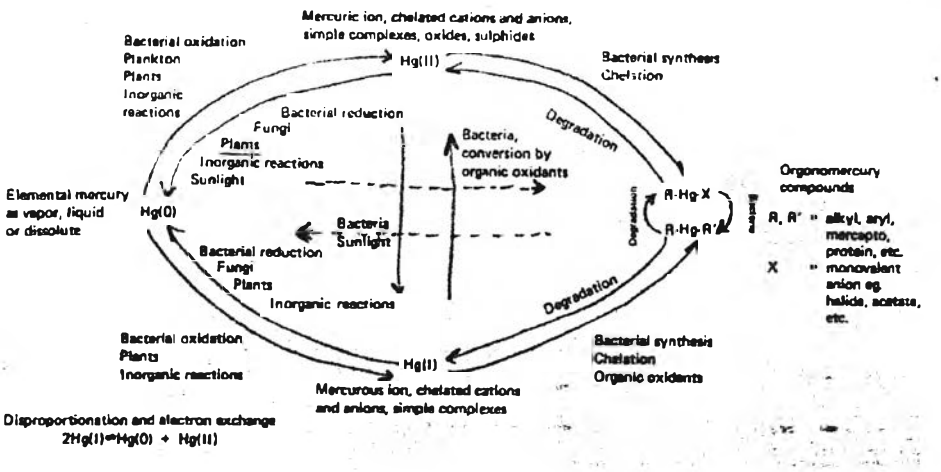

Figure 2.1 The biogeochemical cycling of mercury

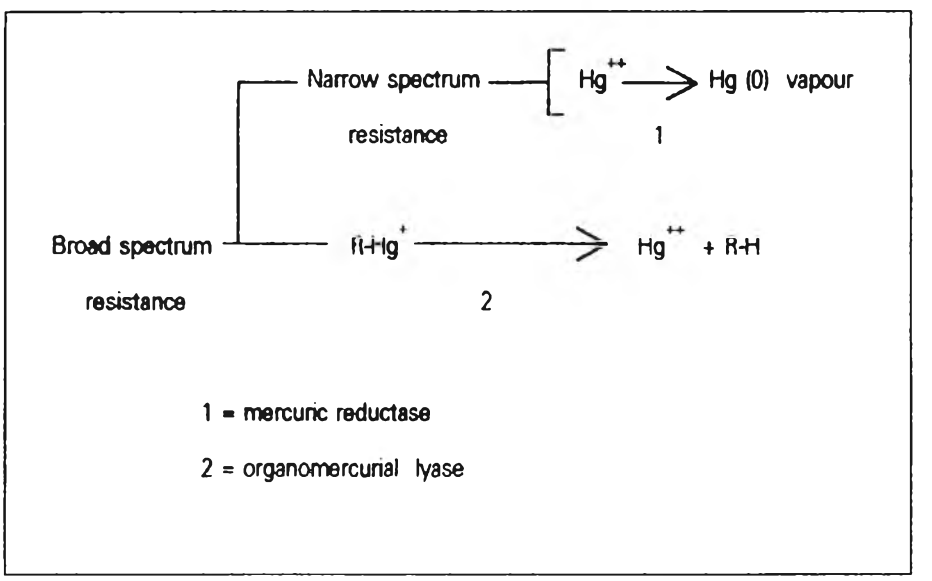

Figure 2.2 The classes of naturally occurring mercury resistance loci in gram- Positive and gram-negative bacteria

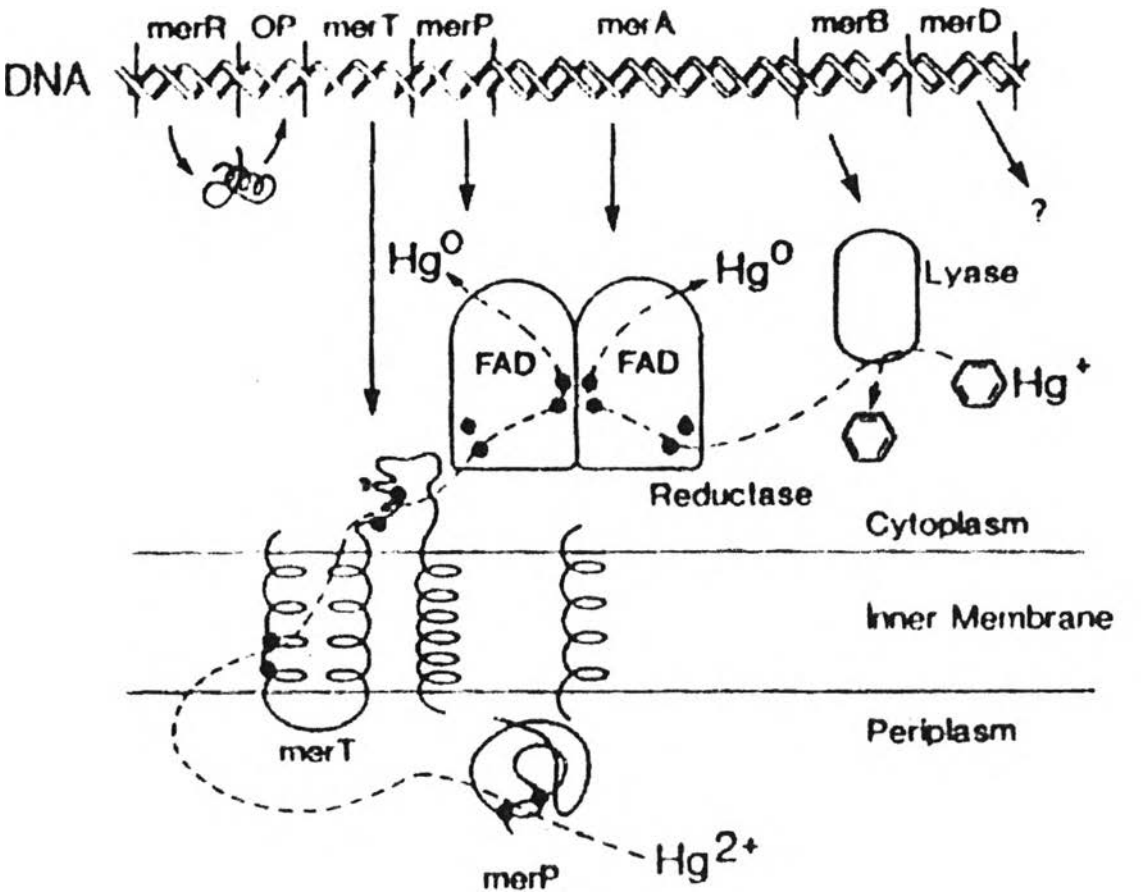

Figure 2.3 Model of the genetic determination of the system for detoxifying inorganic Hg^{2+}. Top line shows order of the genes on the DNA. The remainder of the figure shows the protein products of the genes and their known of postulated locations and functions.