CHAPTER 1

INTRODUCTION

Nowadays, the problem of heavy metal pollution has received a great deal of attention due to their highly toxic nature and translocation through the food chain. Mercury (Hg)* is the one of heavy metals that causes highly toxic pollution due to its adverse effect on all living system. Mercury and mercurial compounds have been used on a variety of industries as catalysts, in hospitals as disinfectants and in agriculture as fungicides, insecticides and bacteriocides. Because of their high mobility, the polluted mercurial compounds disperse widely into the environment via physical, chemical, and biological pathways during which time they are potentially concentrated hundreds of times through the food chain (Chang and Law, 1998).

The problem of mercury pollution came into focus after the discovery of high levels of methylmercury in fish and shellfish in Minamata Bay, Japan, that resulted in 46 deaths (Robinson and Tuovinen, 1984). In Dortmund, Germany, the main source of mercury was from tap water and the air. Many estuaries are contaminated with mercury wastes from many sources such as antifouling treatments for boats, agricultural runoff, and industrial processes (Cheremisinoff and Schiff, 1985). In Sweden, fungicidal agents containing phenylmercuric acetate (PMM) and methylmercury were applied to seed dressings caused a significant decrease in the populations of seed-feeding birds (Gavis and John, 1972).

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*Abbreviate and symbol were shown in **ABBREVIATION** on page xiv

Although mercury pollution in Thailand is not a serious problem, contamination being reported. For example, Piyanart et al.(1997) presented that the mercury concentrations in biota of Bangsare coastal area of the eastern part of the Inner Gulf of Thailand were higher than the previous studies. Mercury in urine of fisherman were analyzed by Manoon et al. (1981) during to 1976s-1980s and it was found that 44.7% of the fisherman have Hg in high level value is 0.1 μg/L to 180 μg/L.

Mercury and their compounds are highly toxic. Methylmercury is 100 times more toxic than inorganic mercury and has been found to be mutagenic under experimental conditions (Robinson and Tuovinen, 1984). The solubility of inorganic and organomercurial compounds in lipids as well as their binding to sulfhydryl groups of proteins in membranes and enzymes account for their cytotoxicity.

Conventional mercury-removal processes from liquid wastes commonly involve precipitation with polysulfides (Findlay and McLean, 1981), thiourea or thioacetamide at pH 3.5-4.0 (Baldi,Parati, Semplici and Tandoi, 1993 cited in Nelson et al., 1987). Reduction of mercuric ions to elemental mercury with dithionite, hydrazine, hydroxylamine, zinc or sodium borohydride which is then recovered by filtration or with an inert gas at high temperature (Baldi et al., 1993). Physical processes, used in low content of mercury-contaminated water are the use of selective ion exchange/redox resins, exchange/co-ordinating resins, and starch xanthate (Baldi et al., 1993). The disadvantage of chemical methods have various problems which may limit the application of these procedures to industrial situations. For instance, high cost of sodium borohydride will be a significant factor. Sulfide precipitation procedure one of chemical

processes, often leave hazadous by-products or residual sludge. Additional treatments has been required after physical methods. Therefore, it is necessary to research for alternative methods, such as biological methods, a more natural and efficient cleanup of mercury waste at a relatively low cost. Microbiological methods for the extraction and recovery of metals have previously been proposed, i.e., involving metal uptake and/or binding to the microorganisms such as a green alga, a yeast, a fungi and a bacteria (Brunker and Bott, 1974; Crist, Oberholser, Shank and Nguyen, 1981; Yannai, Israela, and Lea, 1991; Fischer, Rapsomanikis and Andreae, 1995). The use of mercury-resistant bacteria performing Hg^{2+} to Hg^{0} transformation has been propose in this study because bacteria are easily to grow and improve their removal ability.

Mercury resistant bacteria both gram negative and gram positive can resist mercury due to their ability to volatilize soluble forms of mercury from the environment via a sequence of enzymatic reactions, which are recognized as mercury detoxification (Tonomura et al., 1968; Summer and Silver, 1972, 1978; Wood and Wang, 1983; Silver, Misra and Laddaga, 1989). The genetic basis and mechanisms of mercury resistance were found to be encoded in ***mer*** operons located on either plasmids or transposable elements (Summer and Silver, 1972, 1978; Silver et al., 1989; Brown et al., 1991; Summer, 1992; Silver and Phung, 1996). With the aid of organomercurial lyase originated from the ***mer*** B gene, mercury resistant microorganisms are able to cleave the carbon-mercury (C-Hg) bonds of organomercurial compounds; the resulting mercuric ions are enzymatically reduce to less toxic and more volatile elemental or metallic mercury, Hg(0), by the mer A product mercuric reductase (Robinson and Tuovinen, 1984; Summer, 1986;

Misra, 1992). Some mercurial resistance determinants (***mer***) lack the gene for organomercueial lyase and do not confer resistance against most organomercurials. Hence they are referred to as narrow spectrum mercury resistance systems, while broad spectrum resistance allows bacteria to degrade certain organomercurials as well as to reduce inoganic mercury because it has the gene for organomercurial lyase. The ***mer*** P and ***mer*** T genes in ***mer*** operons also express cysteine rich proteins located on the periplasma space and inner membrane, respectively, for the specific delivery of ambient mercuric ions toward mercuric reductase located in the cytoplasm where Hg(II) is reduced to volatile Hg(0) (Misra, 1992). The constitutive ***mer*** operon is induced by the subtoxic level of mercuric ions (Clark, Weiss and Silver, 1977; O'Halloran, 1993).

There are many mercury-resistant, i.e., ***Pseudomonas***K-62, ***Escherichia coli***, ***P. aeruginosa, Staphylococcus aureus, Arthrobacter*** sp., ***Bacillus*** sp., ***Citrobacter*** sp., ***Enterbacter*** sp., ***Flavobacterium*** sp., ***Vibrio*** sp., ***Moraxella*** sp., ***Chromobacterium*** sp., ***Erwinia*** sp., ***Corrynebacterium*** sp., ***and Micrococus*** sp. (Trevoes, 1987; Nakamuea, Salata and Nakahara, 1988; Nakamura et al., 1990). Recently, removal of Hg from wastewaters by biological process have been investigated, for example, Ghosh et al. (1996 a,b) demonstrated their studies on volatilization of mercury using resting or immobilized cell systems. Direct utilization of mercuric reductase to remediate mercury was also attempted with immobilization of the enzyme by activated supports (Anspach et al., 1994). The evidence showed that mercury-hyperresistant ***Pseudomonas aeruginosa*** PU21 strain, which contains plasmid Rip 64 that encodes for the ***mer*** operon, was able to remove mercuric ions effectively from the contaminated water (Chang, 1993; Chang and Hong, 1995). Chang and

Law (1998) reported that investigation of the dependence of detoxification kinetics on the bacterial growth phases and mercury concentrations. Baldi et al. (1993) presented their investigation of using mercury-resistant ***Pseudomonas putida*** strain FB-1 removing of inorganic Hg(II) as gaseous elemental Hg(0) by continuous culture. A process based on bioaccumulation of Hg by genetically-modified mercury-resistant ***Pseudomonas putida***, ***Aeromonas hydrophila*** and consortia has been developed on a bench-scale column.

This research began with the isolation, screening and selection of the mercury resistant bacterial strain that can reduced Hg(II) to volatile Hg (0) and the effect of temperature, pH and mercury concentrations on the growth of selected bacterial strain. A tentative mercury vapor recovery device was also designed to prevent the resulting product of mercury detoxification, Hg(0), from being released into the atmosphere. Experimental results obtained from this study were evaluated to justify the feasibility of the bioprocess for mercury remediation.

1.1 OBJECTIVES

1) To isolate mercury resistant bacteria that can reduced Hg(II) to volatile Hg(0).

2) To determination of minimal inhibitory concentration (MIC) of HgC1 against isolated bacterial strain.

3) To investigate the effect of temperature, pH and mercury concentrations on the growth of the bacterial isolates.

1.2 SCOPE OF THE STUDY

In this thesis, mercury-resistant bacterial strain were isolated from at least 61 samples, i.e., soil, water, sediment and sludge collected from different sites. They were tested for the maximum mercury-resistance concentration and volatilization of mercuric chloride ($HgCl_2$). Two mercury-resistant bacterial strains were further studied for the effects of pH, the temperature, concentration of mercury on growth and the capacity of mercury volatilization by living cells in differential conditions.

1.3 PLACES

Laboratories, especially, Rm 305 and 306, the Department of General Science, the Faculty os Science, Chulalongkorn University.

Scientific and Technological Research Equipment Centre, Chulalongkorn University for electron microscopy.

1.4 ANTICIPATED BENEFITS

Received a mercury resistant bacteria that can reduce Hg(II) to volatile Hg(0) in high concentration of $HgC1_2$. And expect that the experimental results obtained from this study were evaluated to justify the feasibility of the bioprocess for mercury remediation in the future.

1.5 COMPONENT OF THE THESIS

This thesis comprises five chapters including this introduction. Chapter 2 given literature survey concerning mercury, physico-chemical methods of mercury removal, mechanism of microorganisms for mercury resistance and detoxification, novel mechanism of respire mercury in bacteria, alternative methods for mercury removal and literature summary. In Chapter 3, material and methods was shown. The results could be found in Chapter 4 and the Chapter 5 is the discussion.