

CHAPTER 5

DISCUSSION AND CONCLUSION

The present studies document the removal of soluble mercury by mercury-resistance bacterial strains isolated from polluted and non-polluted sources. Mercury removal by ability of volatilization from those selected bacterial isolates was demonstrated and originated in Thailand.

Two of 272 strains of mercury-resistant bacterial isolates were selected namely, HgR-11 and HgR-14 which resisted to 250 ppm of mercury, and also they were able to volatilized soluble mercury in sodium-phosphate buffer containing 50 ppm of mercuric ions as mercuric chloride. They were characterized and chosen as the test organisms. All were Gram-negative, coccobacilli-shaped and identified by some biochemical tests, indicated that they should be classified in family *Pseudomonadaceae* genus *Acinetobacter* sp.

All of 272 strain isolated were tested to resist to mercury and other metal by multi-inoculation method which has an advantages that are low-cost, facilitate testing of a large volume of culture. In the procedure the quantity of cell density in each strain should be use 10^4 - 10^5 cells/ml (Nieto et al, 1989) to avoid spreading of colonies and different volume of media in each plate can cause differential of metal concentration. The component of media was an important factor in mercury-resistance test due to strong interactions with mercuric ions, thiols and other organic compounds were found to protect microbes from the toxic effects of Hg

(II) and the chelation of mercury by SH groups has been proposed as a mechanism of mercury with tryptone and yeast extract with 140 hr. Initial rate of mercury disappearance in solution were 0.70, 0.59 and 0.67%/h in tryptone yeast extract and LB, respectively. The complexed Hg(II) could not be reduced by mercuric reductase on *P. aeruginosa* (Chang, Hong, Ogunseitan and Olson, 1993).

Effects of pH and temperature on growth in the presented of 5 ppm Hg by the two selected strains were similar. Optimum pH was found to be slightly alkaline (pH 8-9), and optimum temperature was found to be mesophilic range (35°C). The result of pH also similar to previously work that the bacteria can growth at pH 6.0 and increased at pH 7 and at pH 8.0 toxicity of mercuric chloride was reduce (Farrell, Germida and Huang, 1990).

In addition, effects of mercury concentration on growth of the selected bacterial isolates were found that no effect on growth at low level of mercury (4 µg/ml and 8 µg/ml) and slightly effect at 50 µg/ml of Hg. The most effect was found at 150 µg/ml in HgR-11 strain, no growth was observed, in HgR-14 strain growth in medium containing mercury in this concentration cause the longer lag phase (4 hr) and the number of cell decrease rapidly from 10^7 to $<10^2$ cells/ml after inoculation, but the end of experiment (24 hr) the HgR-11 strain, the cell number of strain was increased upto 10^8 cells/ml.

Increasing in the inoculum size was recommended. The media containing 50, 100 and 150 µg/ml, respectively found that initial cell decreased as the mercury concentration increased similar to the study of

Chang and Hong (1995). Although cell number of all were reduced, they can grow in normal rate at the end of incubation because survive cells reduce mercuric ions in the medium to less amount. Then the residual cell start to grow (Hansen et al., 1984).

Effects of pH and temperature on volatilization of mercury were study at 24 hour incubating culture. The results indicated both strains can grew in the medium at pH6 containing high concentration of mercury. In other pH the mercury has remained in the same amount. The percentage of mercury removal were different and the mechanism was not clear but it's seem that they can grow even in the slightly alkaline condition.

The result of effect of temperature on volatilization of the selected bacterial isolated found in same value (~99.44, ~99.64) in each of temperature. It might be concluded that the bacterial isolates have high ability in mercury removal according to the results that showed in the same both pH and temperature. But in control medium containing 50 µg/ml of mercury similar to experimental sample found, the mercury was found in high level too. Therefore mercury in the loss medium was loss by binding to the complex compound in the medium and spontaneous volatilization. Then percentage of removal were quite high.

The reduction of Mercury concentration in the medium were reduce quite rapidly in the first 2 hours similar to the previous report, mature inoculum decreased the amount of mercury very rapidly and no growth was observed during that time. After removal of mercury (within 1.5 h) the growth of the organisms had recovered (Hansen et al, 1984).

Recovery of metals by trap solution was indicated that two selected bacterial strains reduced mercury concentration in the medium to volatile mercury. Then, it was oxidized by oxidizing agent in trap solution to mercuric ion (aq). The percentage of removal in trap solution by HgR-11 and HgR-14 were 95.38 and 98 %, respectively.