CHAPTER IV

DISCUSSION

Aphanothece halophytica was able to accumulate lead rapidly and became saturated at 90 ug/mg dry weight within 1 hour. Most of accumulated lead by *A. halophytica* could be removed by EDTA washing which indicated that *A. halophytica* accumulated lead at the cell surface. The accumulation of lead by living cells was not dependent on metabolic process as confirmed by experiments employing three metabolic inhibitors. The accumulation of lead by *A. halophytica* was similar to a passive adsorption of ion described by Crist et al. (1981). It is likely that a number of different functional groups are involved in this process. The rate of lead accumulation by *A. halophytica* was highest at pH 6.5. It seems that at pH 6.5, the cell walls possess negatively charged suitable functional groups capable of interacting with lead ion.

The pattern of zinc accumulation by *A. halophytica* was similar to lead accumulation. However, it still increased at a slower rate of 7.8 ug/hr. mg dry weight after a rapid rate in the first 10 minutes. This non-saturation might result from denaturation of surface proteins by zinc ion causing conformational changes. The conformational changes of the proteins in turn might expose more functional groups that could bind with zinc ion. This phenomenon was similar to the observed increase of metal accumulation by heat-killed cell of Chlorella sp. (Sakaguchi et al.,1979; Horikoshi, Nakajima, and Sakaguchi, 1981; Suthep Mongkollertlop, 1987). The zinc accumulation sharply increased above pH 6.0.

For S. platensis, the accumulation of lead was a biphasic one. There was a rapid rate in the first 10 minutes and the rate was slow down at 15.6 ug/hr. mg dry weight. About 30-50% of the accumulated lead could be washed out by EDTA. It was therefore likely that parts of lead were transported into the cells. When the incubation time was 40 minutes, the amount of accumulated lead in cells was limited at about 45 ug/mg dry weight whereas total accumulated lead still increased at the rate of 15.6 ug/hr. mg dry weight. It is possible that S. platensis was able to adsorb lead at the cell surface and transport it into the cell at the same time. Hence, the rapid rate occurred in the first phase. This non-saturated pattern is similar to the pattern of zinc accumulation by A. halophytica. It is possible that same phenomenon might occur.

It was difficult to conclude whether the accumulation of lead by S. platensis depended on metabolic process. This was because most of S. platensis appeared to die after the incubation with lead for 1 hour. The accumulation of lead was probably due to both adsorption and transportation into the cells before its death. Apart from covalent and ionic bonding at the cell surface, accumulation of lead might result from the precipitation in the form of insoluble metal complexes that occurs through the activities of sulfate reductase (Ahlf, 1988) or phosphatase (Dean et al., 1984). Another characteristic of S. platensis in the accumulation of lead was that it was more efficient when the pH was above 6.5.

Zinc accumulation by S. platensis occurred at the cell surface which could be washed out by EDTA. The accumulation increased linearly in the first hour with the rate of 15.6 ug/hr. mg dry weight and became saturated at 19 ug/mg dry weight within 5 hours. Metabolic inhibitors, 1.0 mM NaN_3 and 40 mM DCCD could partially inhibit the adsorption. This indicated that parts of zinc accumulation required energy from metabolic process. Zinc might be precipitated in the form of insoluble metal complexes that occurred through the activities of sulfate reductase (Ahlf, 1988) or phosphatase (Dean et al., 1984). The zinc accumulation sharply increased above pH 6.0.

In this experiment it was found that during 5 hours incubation the maximum capacities of A. halophytica to accumulate lead and zinc were 90 and 45 ug/mg dry weight respectively. For S. platensis the values for lead and zinc during the same period of incubation were 105 and 19 ug/mg dry weight respectively. Aphanothece halophytica and S. platensis are much more efficient to accumulate lead than Micrococcus luteus (Belliveau et al., 1987) and Water Hyacinth (Eichhormia crassipes) (Pleonjit Thomthichong, Sonti Kochawat, and Saksit Tredetch, 1987). Micrococcus luteus could accumulate lead at 4.9 ug/mg dry weight during 2 days incubation whereas the value for Water Hyacinth was only 0.43 ug/mg dry weight when grown in waste water containing 1 ppm lead for three weeks. However, the accumulation of lead by A. halophytica and S. platensis was lower than that of Azotobacter sp. with the reported value of 3.1×10^2 ug/mg dry weight during 7 days incubation (Tornabene, and Edwards, 1972).

The efficiency of zinc accumulation by A. halophytica and S. platensis was higher than that by Chlorella sp., the value of which was 1.5 ug/mg dry weight (Suthep Mongkollertlop, 1987) and by Chlorella vulgaris with 7 ug/mg dry weight (Ting, Lawson, and Prince, 1989) when incubated with 1 mg/l and 1.3 mg/l of zinc respectively. However, Chlorella vulgaris grown in 35 ug/ml zinc for three weeks was able to accumulate a higher quantity of zinc, i.e., at 68 ug/mg cell (Coleman, Coleman, and Rice, 1971). In the same condition, Coleman and his colleagues found that Pediastrum tetras and Euglena viridis were able to accumulate zinc at 62 and 78 ug/mg cell respectively. Although A. halophytica and S. platensis have less capacity of zinc accumulation than P. tetras and E. viridis, it is worth noting that A. halophytica and S. platensis used a much shorter incubation time. On the other hand, Chroococcus paris was found to be the most efficient to accumulate zinc. It exhibited 65 ug/mg dry weight maximum zinc binding capacity within 15 min (Les and Walker, 1984).

The effects of cations on lead and zinc accumulation were different for the two algae. Adsorption of metal by covalent or ionic bond could act as ion exchanger (Crist et al., 1981). In general, adsorbed metal by the cell could be displaced with other cations that have higher strength of adsorption. Aphanothece halophytica shows a high strength of lead adsorption as indicated in figure 12 in which no other cations could inhibit lead adsorption and this was further confirmed in figure 28 in which lead completely inhibited zinc adsorption. For S. platensis the adsorption of lead was not as strong as that in A. halophytica. It could be displaced by a number of cations (figure 13).

The adsorption of zinc for both algae was not as strong as that of lead. This information can be beneficial in terms of the selection of the organism for the treatment of waste water containing different heavy metals. Another phenomenon with regard to the effect of cation was the stimulation of lead adsorption by zinc (figure 12). At present the mechanism underlying this phenomenon is not clear. However, the stimulation effect has been previously reported by White and Gadd (1987). Ni^{2+} was inhibitory at low concentrations but stimulated Zn^{2+} uptake by Saccharomyces cerevisiae at higher concentrations.

The rates of lead and zinc accumulation by both algae depended on metal concentration in solution but were saturated when the metals were in excess. The total lead and zinc accumulation by both algae increased with increasing cell density. However, the rate of lead accumulation per hr per mg dry weight of A. halophytica was stable and zinc accumulation per hr per mg dry weight decreased with increasing cell density and became stable after the cell density reached 0.5 mg dry weight/ml. The rate of lead and zinc accumulation per hr per mg dry weight of S. platensis decreased after the cell density reached 1 and 0.6 mg dry weight/ml respectively. These characteristics of metal accumulation were similar to those in Chlorella regularis (Sakaguchi et al., 1979).

The lead and zinc accumulations of A. halophytica were not dependent on the age of cells except for the younger cells (below 14 days) which showed a small increase. For S. platensis the ability of cells to accumulate lead increased up to 8 days and stayed relatively unchanged afterwards whereas the ability of cells to accumulate zinc was independent of the cell age up to 20 days after which the accumulation of zinc slightly decreased.

The ability to accumulate metal of cells was utilized to remove lead and zinc from waste water. It was not successful for the utilization of both algae to remove lead from the waste water sample obtained from the Battery Organization. Aphanothece halophytica and S. platensis exhibited only 21.7 % and 34.6% efficiency for lead removal respectively. These results might be due to the effect of acid pH, other cations competition and low concentration of lead in the waste water sample. Another waste water was obtained from the Thai Battery Industrial Co. Ltd.. It had a pH value of 10.7 and contained 149.1 ppm lead. Most of the lead existed as an insoluble precipitate which could be removed by filtration through a Whatman No.1 filter paper and was lost in the experimental process. However, it was successful to use A. halophytica to remove zinc from waste water sample with alkaline pH. It exhibited 85% efficiency of zinc removal when using the cell with the density of 0.87 mg dry weight/ml (figure 39). On the other hand, A. halophytica exhibited low efficiency of zinc removal for waste water with acidic pH. The effect of pH was further studied and it was found that percent efficiency of zinc removal by _A. halophytica_ was increased when the pH was increased (figure 38). When the pH is alkaline, there exists a condition which induces unprotonated carboxyl oxygen and sulfate group resulting in greater possibility for electrostatic bond formation between these groups at the cell wall and metal ions in the form of Zn^{2+} species. At pH 9.0, zinc is in the form of $Zn(OH)_2$ species with neutral property (Hahne and Kroontje, 1973). Therefore electrostatic bond cannot be formed and the consequence is the drop of percent efficiency of zinc removal by both algae. From the microscopic observation, it was found that parts of _A. halophytica_ were lysed and other intact cells were aggregated to form clusters at low and high pH conditions (figure 42 at the appendix). At pH 12.5, zinc might bind with both of inner and outer membranes of lysed cells. This together with the changing of structures and conformations at the cell surface may account for high efficiency of zinc removal at this pH. These results indicated that _A. halophytica_ exhibited higher efficiency for zinc removal than _S. platensis_ in both acidic and alkaline waste water. However, the waste water from each source had contaminations of other metal ions. Apart from Zn^{2+}, the waste water from Waste Water Eradication Service also contained Cu^{2+}, Cr^{3+}, Cr^{6+} and Ni^{2+} and that from the Samart Engineering Co. Ltd. also contained Cr^{3+} and Cr^{6+}

(Information received from analytical data of the companies). Therefore the total zinc accumulation could be affected by the presence of other cations.

Aphanothece halophytica absolutely lost the ability to accumulate zinc after washing by EDTA. Consequently, it was not available for repeated use. In terms of economy, the enhancement of the ability to accumulate metals was another interesting aspect. It had been reported that heat-killed cells had a greater ability to accumulate metal than living ones (Sakaguchi et al., 1979; Horikoshi et al., 1981; Suthep Mongkollertlop, 1987). Tseng, Weng and Sun (1986) used Spirulina sp. in the form of spray dry as adsorbent for the treatment of low-level liquid radwaste. The adsorption efficiency of Spirulina sp. for lead and zinc at pH 4 was high accounting for 73.3 and 91.8 % respectively.

In the present experiment, both algae were used to trap the metals and both could coagulate and sediment except for S. platensis which floated and formed a floc in the zinc-containing waste water. Indeed, $CuSO_4$ addition in the reservoir water is a conventional method for destruction of algae (Fair, Geyer, and Okun, 1985). There is a report that alum treatment works out to be cheaper than electro-flocculation. Completion of flocculation occurs in 10-15 minutes, and settling occurs in 2-3 hours

(Sridhar, Namasivayam, and Prabhakaran, 1988). In this experiment, the metal concentration was low, so sedimentation and flocculation occurred rather slowly and was not complete. If waste water contained suitable metal concentration, the flocculation might occur more rapidly and complete. However, other methods to remove *A. halophytica* and *S. platensis* were centrifugation and filtration with 20 micron plankton net respectively.