CHAPTER I

INTRODUCTION

The pollution of natural environment by heavy metal elements is a potential menace to the health and welfare of mankind. Attention has, therefore, been focused on this problem in relation to pollution of the food chain. It is necessary that heavy metals in industrial waste water must be appropriately removed before discharge into the environment. Conventional methods for removing dissolved heavy metals include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, and evaporative recovery (Schroeder, 1977). All of these methods are complicated and expensive. The use of microorganisms as bioadsorbents for heavy metals offers a potential alternative to existing methods for detoxification and for recovery of toxic or valuable metals from industrial discharge water (Norberg, 1984).

Microorganisms require certain heavy metals for their living by serving as cofactor and incorporating into structural components. Nickel depletion in Anabaena cylindrica cultivation results in a diversion of ammonia

from protein synthesis into cyanophycin, thereby delaying the de novo synthesis of proteins and enzymes required for the synthesis of active nitrogenase (Daday, Mackerras, and Smith, 1988). However, high concentration of some heavy metals is toxic in living cells. Photosynthetic microorganisms including blue green algae are highly sensitive to heavy metal ions. Metals like Cu^{2+}, Cd^{2+}, and Zn^{2+} have similar electronic characteristics, but different affinities for biological ligand. They inhibit photosynthetic electron transport in PS II. The possibility has been raised that Cd^{2+} and Zn^{2+} may share the same site of action on the oxidizing side of PS II. Singh and Singh (1987) studied in Anacystis nidulans and suggested that Cd^{2+} and Zn^{2+} are inhibitory to H_2O_2 degradation process and their site of action lies between the electron donation sites of H_2O_2 and ascorbic acid, while the Cu^{2+} action site seems to be located beyond the ascorbic acid donation site. Cd^{2+} induced inhibition of nitrate, ammonium and phosphate uptake in Anacystis nidulans (Singh and Yadava, 1983, 1984). In Nostoc calcicola, Hg^{2+} and Cu^{2+} inhibited glutamine synthetase and nitrogenase activities (Singh, Verma, and Singh, 1987).

The resistance of heavy metal by microorganisms such as yeast, fungi, algae and bacteria including blue green algae has been studied. The resistance depended on organisms (Malanchuk and Gruendling, 1973), environments

(Trevors, Stratton, and Gadd, 1986) and metal species (Laube, McKenzie, and Kushner, 1980). Many of these resistance mechanisms are encoded on plasmid or transposons (Silver and Misra, 1988). Microorganisms have different detoxification mechanisms. Some bacteria detoxify by oxidation process. For example, arsenicals as in the case of arsenite are oxidized to arsenate which is less toxic. Methylation of trimethyl leads to the more volatile tetramethyl form (Belliveau, Starodub, Cotter and Trevors, 1987). Some blue green algae such as Anabaena cylindrica, Anacystis nidulans, Lyngbya sp., Microcystis aeruginosa, Nostoc muscorum and Phormidium foveolarum excreted extracellular chelator (Lange, 1974). The chelators of Chlorella stimatophora are polysaccharides containing high contents of free carboxylic and uronic acid (Kaplan, Christian, and Arad, 1987). Dugan (1987) concluded that the chemical composition of extracellular polymer varies with bacterium species. All of polymers from Gram-negative bacteria isolated are polysaccharides but the polymer from the only floc-forming Gram-positive bacterium studied is a complex polypeptide. Uptake of heavy metals by extracellular polysaccharide and cells of Zoogloea ramigera are rapid, the processes are completed within 15 min or less. The metals were released from the biomass by hydrochloric acid (Norberg and Persson, 1984).

Some microorganisms are able to accumulate heavy metals by rapid adsorption on cell surfaces and

transportation into the cells. Zn^{2+} uptake by fungi Neocosmospora vasinfecta (Paton and Budd, 1972), yeast Candida utilis (Failla, Benedict, and Weinberg, 1976) and Saccharomyces cerevisiae (White and Gadd, 1987) were biphasic. The first phase was independent of metabolic energy, consisting of adsorption to the cells surfaces. The second phase was dependent on metabolic energy and consisted of uptake into the cells. Neocosmospora vasinfecta accumulated Zn^{2+} in the form $Zn_3(PO_4)_2$. Citrobacter sp. detoxified Cd^{2+} by forming insoluble cadmium phosphate (Macaskie and Dean, 1984, 1985b). The mechanism of Cd^{2+} uptake is dependent on the activity of a cell bound phosphatase which releases inorganic phosphate from organic phosphate to precipitate Cd^{2+} as cell-bound metal phosphate. The heavy metals are adsorbed by algae cell walls, form coordination bond with amino and carboxyl groups, and imidazole of histidine or electrostatic bond with unprotonated carboxyl oxygen and sulfate in the cell walls. Ion exchange studies in Vaucheria sp. showed a strength of adsorption in the following order $Cu^{2+} > Sr^{2+} > Zn^{2+} > Mg^{2+} > Na^{2+}$. (Crist, Oberholser, Shank, and Nguyen, 1981). Chlorella vulgaris (Khummongkol, Canterford, and Fryer, 1982) and Chlorella regularis (Sakaguchi, Tsuji, Nakajima, and Horikoshi, 1979) have rapid cadmium adsorption and approached equilibrium within 10 minutes and 30 minutes respectively . Copper, zinc and cadmium were bound rapidly by Chroococcus paris (Les and Walker, 1984). Approximately

90% of the total amount of the added metal was bound within 1 minute. Metal binding increased significantly when pH was increased from 4 to 7. Nearly all of the metal was found to be rapidly EDTA extractable. Zinc accumulation by Chlorella sp., had an adsorption constant 0.4 mg/ml (ratio of zinc concentration in solution and zinc accumulation by cells) (Suthep Mongkollertlop, 1987). Cyanidium caldarium precipitated heavy metals at their surface. The precipitation of insoluble metal complexes occurs through the biosynthesis of oxidizing agents such as oxygen or through the activities of sulfate reductase. These processes depend on the metabolic activity of the cells and are closely tied to oxidation and reduction process (Ahlf, 1988).

Heavy metals are transported into cells. The metals transportation depends on the membrane potential of the cells (Khummongkol et al., 1982; Campbell and Smith, 1986). Lead uptake of Anabaena sp. occurred during stationary phase and the level of uptake increased when the medium was supplemented with carbon dioxide (Bender and Ibeanusi, 1987). The energy dependent efflux of Cd^{2+} was encoded by a plasmid in Staphylococcus aureus 1781OR (Trevors et al., 1986). The complete efflux of Cd^{2+} was observed when cells preloaded with Cd^{2+} were transferred to Cd^{2+}-free medium. The Cd^{2+} efflux was blocked by 2,4 - dinitrophenol, N,N,- dicyclohexylcarbodiimide, and

incubation at 4°C. In this particular strain, the plasmid controls the reduced uptake of Cd^{2+} by means of the Mn^{2+} transport system. It is likely that an energy-dependent efflux system prevents the internal loading of cells with Cd^{2+}. Efflux of Cd^{2+} in 1781OR may be an antiporter that effluxes Cd^{2+} in exchange for protons (figure 1). This suggestion is based on indirect experimental evidence using inhibitors, uncouplers, and ionophores. Another investigation using membrane vesicles showed that Cd^{2+} uptake could occur by means of the Mn^{2+} transport system, which was energized by the membrane electrical potential. Cadmium chloride and Mn^{2+} were competitive inhibitors of each other's transport system, and displayed Km of 0.2 uM for Cd^{2+} and of 0.95 uM for Mn^{2+}. These uptake pumps operate in all bacterial strains. Moreover, it is still possible for bacteria to have non plasmid encoded resistance mechanisms.

In many species of blue green algae, a large number of intracellular cyanophycin granules are produced in response to an overload of nickel or copper. The cyanophycin granules seem, therefore, able to remove transition metal ions, lowering their intracellular concentration below toxic levels, and so the synthesis of these granules may be regarded as a detoxification step (Piccinni, Coppellotti, and Guidolin, 1985). Copper chelating compounds from Euglena gracilis have two main

binding molecules which are present in the soluble phase of the cytoplasm, and that contribute to the detoxification and regulation of metal content. The two peptides from Euglena gracilis have a different molecular weight and a very dissimilar amino acid composition. Peptide No. 1 has a peculiar composition with a high content of aspartic acid and arginine. Peptide No. 2 has a lower molecular weight

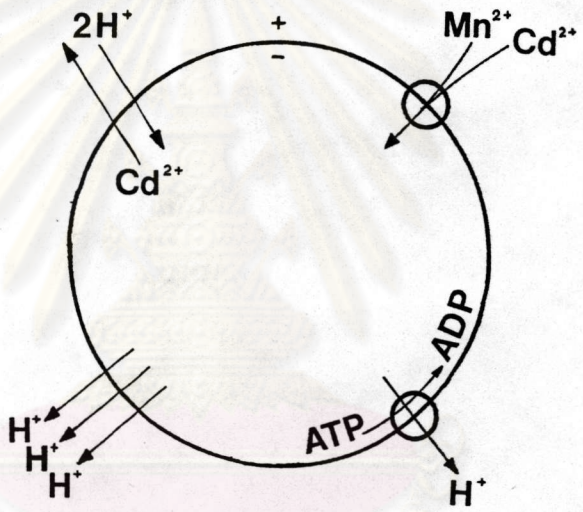

Figure 1 Cadmium uptake and efflux in Staphylococcus aureus 1781OR. The Cd^{2+} resistance plasmid may control the efflux of Cd^{2+} by exchanging Cd^{2+} for protons (Trevors et al., 1986).

and similar to only compound isolated from Ochromonas, in molecular weight and amino acid composition (Piccinni et al., 1985). Olafson (1986) reported that cadmium and zinc induce Synechococcus sp. to synthesize metallothioneins

whose regulation is at the level of transcription. These proteins have many aromatic and aliphatic residues and no apparent association of hydroxylated or basic amino acids with cysteines. Although the characteristic Cys-x-Cys sequences were present, an apparent amino acid sequence homology with the eukaryotic metallothioneins was found in the first 42 residues. Toxic resistance of metal mechanism of Pseudomonas putida involved polyphosphate and three low molecular weight (6700, 6900, 3600) cysteine-rich Cd-binding proteins ($CdBP_1$, $CdBP_2$, $CdBP_3$ respectively) (Trevors et al., 1986).

Although many microorganisms detoxify metals by binding the metals with proteins in cells but some microorganism do not. Klebsiella aerogenes NCTC418 detoxify Pb^{2+} by forming PbS compound as suggested by the presence of electron-dense granules in the cells (Belliveau et al., 1987).

In this study, blue green algae were selected for use in heavy metal removal from waste water. Blue green algae (cyanobacteria) belong to the great subclass of gram-negative prokaryotes. They are defined by the special structure and chemical composition of the cell wall (Stanier and Cohen-Bazire, 1977). Cell walls of cyanobacteria are composed of a peptidoglycan layer and an outer membrane. The peptidoglycan layer is considerably

thicker in many of these organisms than in other gram-negative bacteria. The outer membrane layer contains both proteins and lipopolysaccharides. The lipopolysaccharide components of the outer layer have been isolated from a considerable number of cyanobacteria and show a general chemical resemblance to those of other gram-negative bacteria; they contain a variety of sugars and amide-linked β-hydroxy-fatty acids (Stanier and Cohen-Bazire, 1977). The blue green algae, having a complete photosynthesis system, can be easily maintained. They have a strong potential for further immobilization process.

The blue green algae used in this study are Aphanothece halophytica and Spirulina platensis. Aphanothece halophytica was classified into Chroococcales order, chroococcacean cyanobacteria subgroup. It is a unicellular cocci and reproduces by binary fission. Spirulina platensis was classified into oscillatorian cyanobacteria subgroup. It's characteristic is a helical trichome.

Objectives

1. To study properties of and factors affecting heavy metal accumulation by blue green algae.

2. To study the potential of blue green algae to remove some heavy metals (zinc and lead) from waste water in an appropriate condition.