# CHAPTER I

# INTRODUCTION

Nowaday, heavy metals are widely used in industries and agriculture purposes. These are chromium (Cr)*, manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), molybdenum (Mo), silver (Ag), mercury (Hg), nickel (Ni) and cadmium (Cd). Other metals from group IIIB , IVB and VB of the Periodic Table, in particular, aluminum (Al), tin (Sn), antimony (Sb), lead (Pb) and bismuth (Bi) and the metalloids, arsenic (As) and selenium (Se) are included (Fergusson ,1990 ; Brown and Lester, 1979). They are contaminated into the environment including soil, water and the atmosphere. Cadmium, one of those metals, is extensively used in different industrial products, e.g., protective plating on steel, various alloys, pigments (for plastics, enamels and glazes), Ni-Cd dry cell batteries, stabilizer for plastic and others (Fergusson,1990 ; Law, 1981 ; McCutcheon,1954).

When the consumption of Cd is rising, the concentration of Cd in the environment is also increasing. Furthermore, the problem of Cd contamination occurs when aqueous effluents from many industrial processes that contain dissolved heavy metals without treatment are disposed. They may have an adverse impact on the environment (Trevors, Stratton, and Gadd, 1986). Cd contaminated in the environment, can be accumulated and transferred into the sequence of food chains. Finally, it may cause the Itai-Itai disease in human being (Alloway, 1995 ; Atlas and Bartha, 1981 ; Sitting, 1976).

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* Abbreviation was shown in **ABBREVIATION,** page xiv.

There are different conventional methods for the removal of dissolved heavy metals, e.g., chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment and evaporation recovery (Norberg and Persson, 1984). These processes rarely meet regulatory requirement and create sludge disposal problem. The biological treatment has received increased attention for its advantages compared to traditional wastewater treatment processes. When residual heavy metal concentration is in the range of 1 to over 100 mg/l, it can effectively and economically be extracted by biosorption processes. (Yong and Macaskie, 1997 ; Carvalho, Chong and Valeskey, 1994).

Microorganisms, such as, algae, fungi, yeast, bacteria and actinomycetes, are biosorbents for heavy metals and offer potential alternatives to existing methods for both detoxification and recovery of toxic chemicals in industrial discharged water. Because they are easy to operate, rapid growth and high resistant to several metals. In addition, the microorganism have more detailed knowledge of each strain that can be easy to genetic adaptation ( Doyle, Marshall and Pfander, 1975 ; Gadd, 1990a), high Cd-resistant microorganisms, especially, bacterial strains are selected.

There are several mechanisms of heavy metal resistance in bacteria, e.g., i) efflux pumps which specially capture and release undesired divalent cation through cell membrane (Silver, Misra and Laddaga, 1989 ; Tynecka, Gos and Zajac, 1981 ; Higham, Sadler and Scawen, 1985 ; Laddaga, Bessen and Silver, 1985) ; ii) adsorption to the cell wall or by binding to detoxified ligands, e.g., proteins or polymers (Flemming, Schmitt and Marshall, 1996 ; Scott and Palmer, 1988 ; Kefala, Ekateriniadou, Zouboulis, Matis and Kyriakidis, 1994 ; Gehrke, Telegdi, Thierry and Sand, 1998) ; iii) precipitation of heavy metals as insoluble sulfides, carbonates, phosphates or hydroxides (Holmes and others, 1997 ; Wang and others,1997 ; Webb, McGinness and Lappin-Scott, 1998) and iv) conversions of heavy metal ions into organometallic compounds by detoxifying or volatilizing the metals (Ghosh, Sadhukhan, Chaudhuri, Ghosh and Mandal, 1996).

Biosorption or metal binding by exopolysaccharides (EPS) which is produced by certain strains of bacteria is the main interest in this study. Exopolysaccharide is an extracellular polysacchride or external structure of the outer surface of the cell walls of bacteria. There are three structures that can occur singly or in combination. First, capsule may extend 0.1-2 micrometers (μm) or more from the cell surface. It is composed of amorphous, loosely arranged homopolysaccharide, heteropolysaccharide or polypeptide. The components are chemically linked to the cell wall so that they cannot be easily washed off. The net change on the surface of all bacteria is usually negative because of the carboxylate or phosphate groups. Second, slime layer does not extend from the wall as wide as capsule. The last part, sheaths are less frequently encountered. There are two types. The first one is an amorphous tube composed of a loose arrangement of homopolymer or heteropolymer. This type is usually found in filamentous cyanobacteria and microorganisms in genera ***Biggiatoa, Sphaerotilus*** and ***Leptothrix***. The second type is much more ordered than the first one and is represented by the sheaths of the archaebacteria, e.g., Methanospirillum and Methanothrix species (Flemming, Schmitt and Marshall , 1996 ; Catley, 1992 ; Sutherland, 1990 ; Beveridge, 1989).

Many bacteria are able to produce EPS for Cd immobilization or accumulation. For examples, ***Klebsiella aerogenes*** produced exopolysaccharide more than its cell weight which is about 2 times and maximum adsorption of cadmium was 50-100 milligram/decimeter$^3$ (mg/dm$^3$; Scott and Palmer, 1990); ***Zoogloea remigera***, the common bacteria in sewage plants, produced substantial amount of EPS when it was cultivated on an enrich medium. When ***Z. ramigera*** cells were immobilized into the beads of calcium alginate, they were able to adsorb 99.9 % of Cd from approximately 100 mg/L Cd in three bubble columns (Pfister and Kuhn, 1989 ; Norberg and Persson, 1984). Also, immobilized cells of a strain of ***Citrobacter*** sp. effectively removed Cd, Pb and Cu about 83, 87, 85%, respectively. The efficiency was increased to nearly 100% by the incorporation of other additional immobilized cell column units (Macaskie and Dean, 1982, 1984a and 1984b).

According to their chelating property of bacteria exopolysaccharide and also research demand in the country, the suitable method(s) to remove Cd from wastewater containing heavy metals should be developed. Certain strains of Cd-resistant bacteria that are able to produce high quantities of high Cd-affinity polysaccharides should be selected. The researches should be done to develop the appropriate technology of Cd removal to be used in Thailand.

## 1.1 OBJECTIVES

i) To screen and select cadmium-resistant bacterial strains;

ii) To investigate the effects of some environmental factor on growth and exopolysaccharide production of the selected bacterial strains;

iii) To extract the exopolysaccharide from the selected strains and compare the Cd adsorption capability between the extracted exopolysaccharide and the living cells; and

iv) To investigate the Cd and other metals adsorption capability of bacteria isolates.

## 1.2 SCOPE OF STUDY

In this thesis, cadmium-resistant bacteria were isolated from at least 30 samples collected from different sites. They were tested for the maximum cadmium-resistance concentration. Five cadmium-resistant bacterial strains were further studied for the exopolysaccharide production, the effects of pH, the temperature on growth, the capability of cadmium adsorption by living cells, extracted exopolysaccharides and immobilized bacterial strains.

## 1.3 ANTICIPATED BENEFITS

This study may provide results for some applications as following:

i) The primary data about the capability of soluble cadmium removal by bacterial isolates;

ii) The application guideline of the alternative method of using the bacterial isolates for the removal of cadmium in waste or drinking waters;

iii) The advanced researches may be done to develop higher yields and affinity to cadmium of exopolysaccharides by genetic engineering or others; and

iv) The research to develop new technology which is suitable to be used in Thailand.