TRICHODERMA SPECIES BIOSORPTION OF NICKEL AND CADMIUM FROM AQUEOUS SOLUTIONS

By

LOW PUI MAN

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
ANOVA	Analysis of Variance
BOD	Biochemical Oxygen Demand
CCD	Central Composite Design
COD	Chemical Oxygen Demand
EDX	Energy Dispersive X-ray Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
PPE	Personal Protective Equipment
RSM	Response Surface Methodology
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscope

LIST OF SYMBOLS

A	Code of pH
B	Code of biosorbent dosage
C	Code of contact time
C_e	Equilibrium concentration
C_i	Initial concentration
C_f	Final concentration
k_1	Rate constant of first-order biosorption
k_2	Rate constant of second-order biosorption
K_b	Langmuir equilibrium constant
K_f	Freundlich constant
k_{id}	Intraparticle diffusion constant
q	Metal ions adsorbed per g of biomass
q_{max}	Maximum specific uptake corresponding to the sites saturation
q_e	Amount of metal ions uptake at equilibrium
q_t	Amounts of adsorbed metal ions on the biosorbent at time t
R	Removal efficiency of adsorption
R^2	Correlation coefficient
V	Volume of metal solution in the flask
W	Weight of biosorbent

Y_1 Nickel (II) removal

Y_2 Cadmium (II) removal

PENJERAPAN BIO *TRICHODERMA* SPECIES TERHADAP NICKEL DAN KADMIUM DARIPADA LARUTAN AKUEUS

ABSTRAK

Kesan pH ke atas pertumbuhan *Trichoderma* sp. dalam kelalang koncang telah dikaji. Biojisim tertinggi diperoleh pada pH 5 iaitu sebanyak 1.45g dalam 100 mL media cecair. Dalam kajian ini, bahan penjerapan yang dicirikan oleh FTIR (Fourier Transform Infrared Spektroskopi) dan Mikroskop Imbasan Elektron (SEM) dan Tenaga Serakan X-Ray Spektrometer (EDX). Keadaan penjerapan optimum ditentukan sebagai fungsi pH, dos bahan penjerapan dan masa sentuhan. Data keseimbangan lebih sesuai dengan menggunakan model isoterma Langmuir berbanding dengan isoterma Freundlich ini. Kapasiti penjerapan maksimum nikel dan kadmium didapati masing-masing 5,456 mg / g dan 2,380 mg / g. Design Komposit Pusat (CCD) dalam kaedah permukaan tindak balas telah digunakan untuk mereka-bentuk eksperimen dan juga untuk anggaran permukaan tindak balas penuh. Keadaan optimum untuk penyingkiran maksimum nikel dan kadmium daripada larutan akueus 50 mg / L adalah seperti berikut: pH 4.92, dos bahan penjerapan (0.70 g), masa sentuhan (8.28 jam) bagi nikel dan pH 9.99, dos bahan penjerapan (0.60 g) , masa sentuhan (11,93 jam) untuk kadmium. Pekali korelasi yang tinggi (R^2 = 0,877 bagi nikel, R^2 = 0,959 untuk kadmium) di antara model dan data eksperimen menunjukkan bahawa model dapat meramalkan penyingkiran nikel dan kadmium daripada larutan akueus dengan menggunakan *Trichoderma* sp. Keputusan kajian FTIR menunjukkan

Trichoderma sp. boleh menjerap dan mengikat dengan beberapa logam berat kerana ia mempunyai amino dan hidroksil kumpulan.

TRICHODERMA SPECIES BIOSORPTION OF NICKEL AND CADMIUM FROM AQUEOUS SOLUTION

ABSTRACT

The effect of pH on growth of *Trichoderma* sp. in shake flask culture was studied. The highest biomass was obtained at pH 5 which is 1.45g in 100 mL of liquid media. In this research, the biosorbent was characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDX) and pH point of zero charge (pH_{pzc}). The optimum biosorption conditions were determined as a function of pH, biosorbent dosage, and contact time. The equilibrium data were better fit by the Langmuir isotherm model than by the Freundlich isotherm. The maximum adsorption capacity of nickel and cadmium were found to be 5.456 mg/g and 2.380 mg/g respectively. The Central Composite Design (CCD) in response surface methodology was used for designing the experiments as well as for full response surface estimation. The optimum condition for maximum removal of nickel and cadmium from an aqueous solution of 50 mg/L were as follows: pH 4.92, biosorbent dosage (0.70 g), contact time (8.28 hours) for nickel and pH 9.99, biosorbent dosage (0.60 g), contact time (11.93 hours) for cadmium. The high correlation coefficient (R^2 = 0.877 for nickel, R^2 = 0.959 for cadmium) between the models and experiment data showed that the models were able to predict the removal of nickel and cadmium from aqueous solution by using *Trichoderma* sp. efficiently. The results of FTIR study showed that *Trichoderma* sp. can adsorb and bind with some heavy metals due to it possesses amino and hydroxyl groups.

CHAPTER ONE

INTRODUCTION

1.1 Heavy metal pollution

Rapid industrialization and economic development in Malaysia has gave rise to heavy metal pollution problems in Malaysia. In the past, little attention was paid to environmental issue in Malaysia due to abundant resources and inappreciable development pressure. Later, the government realized the problems and taken concrete steps by practicing Environmental Quality Act, 1974. Table 1.1 presents the parameter limits of effluents for standard A and standard B that ruled out by government. The upstream discharged effluent of a water supply intake should meet standard A, in the meanwhile effluent that discharged downstream should meet standard B.

Heavy metals are defined by its metallic element which has a relatively high density and it is poison although at low concentration (Lenntech, 2004). Heavy metals are included zinc (Zn), Silver (Ag), nickel (Ni), cadmium (Cd), arsenic (As), mercury (Hg), chromium (Cr), iron (Fe), lead (Pb), copper (Cu) and the platinum group elements. Anthropogenic activities have interrupted the natural distribution of heavy metals in the environment on land and in water bodies like lakes, rivers, and sea. Heavy metals in wastewater discharges by many kinds of industries, such as mining, metal finishing, plastics, color pigments, and electroplating, are threaten to the environment and human health severely (Kurniawan et al., 2006). In the case study of Cikijing River, West Java showed that the concentration of Cr and Cu were highly

increased after received the discharged effluent from a largest textile industry area (Roosmini *et al.*, 2010).

Manufacturing is one of the main industry that brought metal pollution to the environment. Metal finishing process like electroplating, etching, and metal components preparation for various of industries have been recognized as the main source of wastes with high concentration of Ni, Zn, Cr, Fe, Cd, Mn, Ni, Al, Sn, and Cu (Rahman and Surif, 1993). According to Yusof *et al.* (2001), mining of copper, iron, gold, and tin may cause pollution to the water bodies.

In most of the countries, the source of heavy metals includes mining industrial, smelting, hospital waste, agrochemicals, electroplating, sewage sludge, and thermal power plants. Elements of high concern are including Chromium, Thallium, Cobalt, Lead, Mercury, Copper, Arsenic, Nickel, Vanadium, Cadmium, and Zinc. Table 1.2 presents some of the sources of heavy metals.

Table 1.1: Environmental Quality Act 1974, Environmental Quality (Sewage and Industrial Effluents) Regulations 1979, 1999, 2000. Parameter Limits of Effluents of Standard A and B (DOE, 2007)

Parameter	Unit	Standard A	Standard B
Temperature	°C	40	40
pH value	--	6.0-9.0	5.5-9.0
BOD at 20°C	mg/L	20	50
COD	mg/L	50	100
Suspended Solids	mg/L	50	100
Mercury	mg/L	0.005	0.05
Cadmium	mg/L	0.01	0.02
Chromium Hexavalent	mg/L	0.05	0.05
Chromium Trivalent	mg/L	0.20	1.00
Arsenic	mg/L	0.05	0.10
Cyanide	mg/L	0.05	0.10
Lead	mg/L	0.10	0.50
Copper	mg/L	0.20	1.0
Manganese	mg/L	0.20	1.0
Nickel	mg/L	0.20	1.0
Tin	mg/L	0.20	1.0
Zinc	mg/L	1.0	1.0
Boron	mg/L	1.0	4.0
Iron (Fe)	mg/L	1.0	5.0
Silver	mg/L	0.1	1.0
Aluminum	mg/L	10	15
Selenium	mg/L	0.02	0.5
Barium	mg/L	1.0	2.0
Fluoride	mg/L	2.0	5.0
Formaldehyde	mg/L	1.0	2.0
Phenol	mg/L	0.001	1.0
Free Chlorine	mg/L	1.0	2.0
Sulphide	mg/L	0.50	0.50
Oil and Grease	mg/L	No detectable	10.0
Colour	AMDI*	100	200

*ADMI = American Dye Manufacturers Institute

Table 1.2: Sources of heavy metals

Heavy metals	Sources
Chromium	Mining industrial coolants, chromium salts manufacturing, leather tanning
Lead	Lead acid batteries, paints, e-waste, smelting operations, coal-based thermal power plants, ceramic, bangle industry
Mercury	Hospital waste (damaged thermometers, barometers, sphygmomanometers), thermal power plants, fluorescent lamps, electrical appliances
Copper	Mining, smelting operations, electroplating
Arsenic	Geogenic/natural processes, smelting operations, thermal power plants, fuel
Nickel	Smelting operations, thermal power plants, battery industry
Vanadium	Spent catalyst, sulphuric acid plant
Cadmium	Zinc smelting, waste batteries, e-waste, paint sludge, incinerations & fuel combustion
Zinc	Smelting, electroplating

1.1.1 Heavy metal pollution in sediments

The quality of sediment is a good indicator to detect metal pollution in water because it inclines to concentrate the heavy metals and other organic pollutants (Chang *et al.*, 1998). The heavy metals concentration in sediment from the offshore waters and coastal of Peninsular Malaysia have been vastly studied.

In the Juru River, Penang, the sediments in rivers had been polluted by industry, runoff, and sewage in area of high population have been found to be polluted by Zn, Cu, Pb, and Cd. Zn concentration was up to 71.30 ppb, concentration of Cu, Cd, and Pb were up to 17.00 ppb, 2.25 ppb and 10.81 ppb respectively (Idriss and Ahmad, 2012). The sources of these metals were came from a sewage treatment plant which discharge sewage into the river. Thus, the lead (Pb) enrichment was probably due to the emission of vehicles and zinc (Zn) from tire wear (Wood *et al.*, 1997). Zhang et al. (2012) discovered that the estuarine sediments in Hailing Bay, China

were mainly polluted by Cu (1.21–58.84 mg/kg, As (2.17-20.35 mg/kg), Zn (11.69–219.22 mg/kg), and Ni (1.37–42.50 mg/kg).

Sediment samples from the coast of River Orogodo in Agbor, Nigeria were analyzed for heavy metals and the results show that the water body is significantly contaminated by iron (Issa *et al.*, 2011). Another study of Seybouse River, Algeria showed that the average concentrations of Fe, Mn, Zn, Cr, Sn, Ni, Cu, and Pb have exceeded the acceptable standards for sediment pollution which means the Seybouse River are heavy polluted (Louhi *et al.*, 2012).

1.1.2 Heavy metal pollution in aquatic system

Problems of heavy metal pollution in aquatic environments are being concern because of its abundance, persistence, and toxicity in the environment, and accumulation in aquatic habitats. Heavy metals are intruded into water systems by volcanic activities, weathering of soils and rocks, and anthropogenic activities like color pigments, mining and processing. Heavy metal residues in contaminated habitats may accumulate in aquatic flora and fauna, and microorganisms and it may enter into the human food chain and cause to health problems (Cook *et al.*, 1993; Deniseger *et al.*, 1990).

Pollution of the aquatic systems by heavy metals has been considered as a major treat to the aquatic organisms including fishes. The drainage water from agriculture activities containing pesticides and fertilizers and effluent of industrial activities discharge large quantities of heavy metals into water bodies and sediment (ECDG, 2002).

As a result of rapid growth of human population, agriculture and economic activities on the west coast of Peninsular Malaysia, a lot of studies on heavy metals have been focused in this area (Abdullah *et al.*, 1999). As a major international shipping lane on the west coast of Malaysia, the Straits of Malacca also contribute to the marine environment pollution. However, in the states of Pahang and Terengganu, the oil and gas related industries are developing along the east coast may also cause the marine pollution.

A research had been done in the Suquia river, Argentina showed that Co, Ni, and Zn in surface water were significantly higher than 2006 (Harguinteguy *et al.*, 2014). The concentrations of Cu, Cd, Zn, and Pb in the Yangtze river basin are 7.91, 0.08, 18.7, and 15.7 μg/L, respectively (Zhang and Zhang, 1992).

1.2 Hazards of heavy metal pollution

Human may be uncovered to potentially harmful physical, chemical, and biological agents in food, air, water, or soil. Heavy metals bring harmful effects to human body when contacted exceed the bio-recommended limits. The common signs related with lead, cadmium, arsenic, mercury, aluminum, and copper poisoning are gastrointestinal disorders, ataxia, diarrhea stomatitis, tremor, paralysis, depression, vomiting, and pneumonia when inhaled volatile vapors and fumes (McCluggage, 1991). The nature of impacts could be toxic, carcinogenic, mutagenic, neurotoxic, or teratogenic.

Skeletal damage was first reported from Japan in 1950s which cause by long term exposure of cadmium, where the itai-itai disease (a combination of osteoporosis

and osteomalacia) was discovered. The reason of the exposure was due to the usage of cadmium-contaminated water for agricultural irrigation (Jarup *et al.*, 1998).

Long term exposure to cadmium in human may results in renal dysfunction, characterized by tubular proteinuria. Furthermore, dust and fumes contained cadmium which inhaled by human can result to obstructive lung disease and cadmium pneumonitis (Duruibe *et al.*, 2007). Cadmium is also related to bone defects, osteoporosis, and spontaneous fractures.

Mercury poisoning has a latent period of 1 month or longer after acute exposure. Nervous system damage is one of the main symptoms (Weiss *et al.*, 2002). The first stage symptoms of mercury poisoning are numbness and parestesias in the hands and feet and high doses of exposure may lead to death. Increasing in risk of coronary heart disease has been hypothesized if human exposures to a high dietary intake of mercury from fish consumption (Salonen *et al.*, 1995).

Acute lead poisoning caused the symptoms of headache, irritability, and abdominal pain. Characteristics of Lead encephalopathy is restlessness and sleeplessness. Learning and concentration difficulties may face by children. Some people may suffer from acute psychosis, reduced consciousness, and confusion for severe cases. Long term low level lead exposure in children may lead to minified intellectual capacity. The results of research suggest for a 0.48 μmol/l increase in blood lead level decrease a weighted mean in IQ of 2 points (WHO, 1995).

1.3 Removal of heavy metal from aquatic system

Nowadays heavy metal water is commonly treated by using several technologies which includes ion exchange, chemical precipitation, electrodialysis, reverse osmosis, ultrafiltration, and phytoremediation (Ahalya *et al.*, 2003). Table 1.3 presents some of the frequently-used technologies for heavy metal removal and their advantages and disadvantages.

Dabrowski *et al.* (2004) reported the removal of Pb, Cd, Hg, Ni, Cr, V, Zn, and Cu from water and industrial wastewaters by ion exchange method. According to Wang and Chen (2006), remediation using electrochemical and chemical precipitation is ineffective when the metal ion concentration is 1 to 100 mg/L which produces huge amount of sludge. However, Volesky (2001) reported that chemical oxidation and reduction is slow in the process of biological system, as for reverse osmosis will cause scaling problems and high cost. Furthermore, Volesky (2001) also documented that using membrane technology and activated carbon to treat heavy metal wastewater are expensive and cause difficulties when frequently use especially when come to large scale treatment.

Table 1.3 Comparison of technologies of heavy metal removal from wastewater (Farooq *et al.*, 2010)

Method	Advantage	Disadvantage
Chemical Precipitation	• Simple • Inexpensive • Most of metals can removed • Dewatering	• Large amount of sludge produced • Disposal problems • High cost • Large consumption of chemicals
Chemical coagulation	• High generation of materials • Metal selective	• High cost • Less number of metal ions removed
Electrochemical methods	• Metal selective • No consumption of chemicals • Pure metals can be achieved	• High capital cost • High running cost • Initial solution pH and current density
Adsorption using activated carbon	• Most of the metals can be removed • High efficiency (99%)	• Cost of activated carbon • No generation • Performance depends upon adsorbent
Using natural zeolite	• Most of metals can be removed • Relatively less costly materials	• Low efficiency
Membrane process and ultrafilteration	• Less solid waste produced • Less chemical consumption • High efficiency (>95% for single metal)	• High initial and running cost • Low flow rates • Removal percentage decreases with the presence of other metals

1.3.1 Bioremediation

Bioremediation is a natural process which uses biomass like parasites, fungus, and vegetation to reduce contaminants as these organisms bring out their regular life features. Bioremediation has an important role in cleaning environment from contaminants and pollutants by using fungi and microorganisms. Removing heavy metals by using microbes can be achieved through bioaccumulation and biosorption process. Bioaccumulation is a process of taking up heavy metal by metabolism dependent processes while biosorption is a process of metal uptake by living or dead microbes through physiochemical mechanisms such as adsorption, ion exchange process (Gadd, 1993, Tobin *et al.*, 1984, Kapoor and Viraraghavan, 1997a).

1.3.1 (a) Bacteria

Bacteria present in great quantity and they are the most versatile microorganisms. Bacteria comprise a significant fraction of the entire living terrestrial (Mann *et al.*, 1990). Bacteria use as a sorbent in bioremediation process because they are small in sizes, able to grow under controlled conditions, ubiquity, and their elasticity in a wide range of environmental conditions (Urrutia, 1997). Bacteria species such as *Micrococcus*, *Pseudomonas*, *Bacillus*, *Escherichia*, *Streptomyces* and so on have been experimented for metals uptake (Vijayaraghavan and Yun, 2008; Nakajima and Tsuruta, 2004; Mameri *et al.*, 1999; Ahluwalia and Goyal, 2007; Savvaidis *et al.*, 2003).

1.3.1 (b) Algae

Algae different with other biomass or microbes because do not generate toxic substances. Algae produce large quantities of biomass in autotrophic conditions and have low nutrient requirements. Seaweeds from the oceans are another inexpensive biomass because they produced in voluminous quantities. Marine algae, like *Sargasso*

seaweed, was tested for heavy metal removal (Davis *et al.*, 2003). According to Brinza *et al.* (2007), brown algae has higher uptake capacity than the red and green algae.

Algal cell walls are mainly made from cellulose. The potential metal binding groups in this class of microbes are amines, imidazoles, carboxylates, phosphates, sulfhydryls, hydroxyls, and sulfates. Metal ion binding on algal surface depends on one different conditions like the algal species, chemical composition of the metal ion solution, and metal ion charge (Freire-nordi *et al.*, 2005; Gupta *et al.*, 2001; Sheng *et al.*, 2004). The ability of algae to remove heavy metals depends on algal group and morphology (Brinza *et al.*, 2007). Cossich *et al.* (2004) mentioned that pH is an important effect on chromium biosorption capacity. However, the size of biosorbent does not affect the chromium biosorption rate and capacity.

Chojnacka *et al.* (2005) studied the metal adsorption performance of cadmium, chromium, and copper ions by blue-green algae *Spirulina* sp. and Nayak *et al.* (2003) also reported the heavy metal biosorption and toxic radionuclides by three genera of algae from different taxonomic groups, including Hg-197, Tl-198, Tl-199, Tl-200, Tl-201, Bi-204, Po-204, Po-205, Pb-199, Pb-200, and Pb-201 radionuclides. Baran *et al.* (2005) studied the maximum biosorption capacity of *Sargassum vulgare, Halimeda tuna, Pterocladia capillacea, Laurencia papillosa, Hypnea musciformis,* for Cr^{6+} were 33.0, 2.3, 6.6, 5.3, and 4.7 mg g^{-1} respectively. The results showed that *Sargassum vulgare* is most effective for chromium removing from aqueous solution.

1.3.1 (c) Use of fungi

Fungi are omnipresent in natural environment and important in industrial processes. Their main roles are to decompose organic substances, as symbionts and pathogens of animals and plants, as spoilage organisms of natural, have concomitant nutrients cycling, and as synthetic materials such as paint, wood, leather, fabrics, and food. Fungi also used to produce economically important substances such as citric acid, ethanol, enzymes, polysaccharides, vitamins, and antibiotics (Gadd, 1993).

The application of fungi as a sorbent for heavy metal uptake from contaminated waters opens a novel area of biotechnology. Fungal cell walls contain chitosan and chitin which have been shown to sequester metal ions (Muzzarelli, 1972; Tsezos and Keller, 1983). The accumulation of heavy metal in the high parts per million (ppm) range of micronutrients such as Cu and Zn beyond normal cellular requirements and wholly non-nutrient metals (Hg, Sn, Ni, U) are common fungal capabilities (Strandberg *et al.*, 1981; Gadd, 1986). Fungi are better suited than other microbial biomass in treating industrial wastewater because of they have better tolerance towards metals and other environmental conditions such as high cell wall binding capacity, intracellular uptake potential, and low pH (Huang and Morehart, 1990).

A lot of researches had been conducted on the ability of heavy metals uptake of fungi on cadmium (Cd), copper (Cu), lead (Pb), and uranium (U) from aqueous solution in substantial quantity (Holan and Volesky, 1995). Tomko *et al.* (2006) conducted a research by using three kinds of fungi species, *Agaricus campester, Amanita muscaria,* and *Trametes gibbosa* for the removal of copper, aluminium, and antimony. Fungi such as *Aspergillus niger*, *Mucor rouxii*, *Rhizophus arrhizus* have

been found that to take up precious metals such as silver (Ag), and gold (Au) (Mullen *et al.*, 1992).

1.4 Objectives of study

The objectives which carried out by this study are

- To study the effect of pH, biosorbent dosage, and contact time of biomass on *Trichoderma* sp. biosorption.

- To study the biosorption of *Trichoderma* sp. on nickel and cadmium in the contaminated artificial aqueous solution.

- To evaluate the applicability of Freundlich and Langmuir adsorption isotherms models and kinetic study to determine biosorption capacity of *Trichoderma* sp.

- To obtain the optimal conditions for the treatment in order to achieve best efficiency by using *Trichoderma* sp.

1.5 Scope of study

In this study, *Trichoderma* sp. were chosen as a biosorbent to remove nickel and cadmium in a batch system. These metals were chosen in this study because they are used widely. Nickel is used in chemical, metallurgical, and food processing industries, especially as pigments and catalysts (Cempel and Nikel, 2006). On the other hand, cadmium metal mostly obtained as a by-product from smelting zinc, lead, or copper ores, and it also used widely in making batteries, pigments, and plastics, electroplating and coating (Llewellyn, 1994). Therefore, they cause serious pollution to the environment and bring harm to human health.

The difference of pH in growth of *Trichoderma* sp. experiments were carried out. Range of pH from 4 to 8 were tested to study its effect on growth of *Trichoderma* sp. Thus, the removal of nickel and cadmium in metal solution were studied by using *Trichoderma* sp. as a biosorbent in different time interval, different biomass dosage, and different pH of metal solution. The data of the biosorbent were analyzed by using Langmuir and Freundlich equilibrium models.

The characterization of biosorbent was carried out by using Scanning Electron Micrographs (SEM), Energy Dispersive X-ray spectroscopy (EDX), and Fourier Infrared Spectrophotometer (FTIR).

Trichoderma sp. is recommended to use in heavy metal removal in variety of metals included nickel and cadmium biosorption because it is easily available in large quantities, easily grown in basic fermentation medium, and low cost (Kumar *et al.*, 2011; Sujatha *et al.*, 2013; Chew *et al.*, 2012).

1.6 Outline of Thesis

There are five chapters in this thesis and each chapter describe the sequence of this research.

Chapter 1 presents the heavy metal pollution in Malaysia and details about using *Trichoderma* sp. as a biosorbents in heavy metal adsorption process. The significance of studies and objectives are clearly outline throughout the flow of the research studies.

Chapter 2 describes an overview of related knowledge in bioremediation and biosorption process. Characteristic and uses of *Trichoderma* sp. in different industries are discussed in detail.

Chapter 3 refers to the experiment procedure in the research and analysis of sample and the characterization of biosorbents before and after treatment. Materials and chemicals which used in this study are showed in this chapter.

Chapter 4 presents the results and discussion of heavy metal biosorption by dead cells of *Trichoderma* sp. using heavy metal contaminated synthetic water in batch biosorption experiments. The results of effect of pH on growth of *Trichoderma* sp. were collected and discussed in this chapter. Freundlich and Langmuir adsorption isotherm were tested for nickel and cadmium. The Central Composite Design (CCD) was used for designing the experiments as well as for full response surface estimation to find out the optimum conditions for nickel and cadmium removal. Besides that, reports on the initial study of *Trichoderma* sp. by using Scanning Electron Microscope (SEM) also presented in this chapter. Furthermore, comparison of infra-red spectra of biomass before and after adsorption are showed in this chapter too.

Chapter 5 refers the overall conclusion that based on the findings obtained in the results and discussion in Chapter 4. Recommendations with valuable strategies and improvement in the next coming also given in this chapter.

CHAPTER TWO

LITERATURE REVIEW

2.1 Heavy metals and its harm to the environment

Heavy metals are any metallic elements with a specific density of 5.0 or more, and is toxic to organisms although at low concentration (Lenntech, 2004). There are three categories of heavy metals as described by Wang and Chen (2006) such as toxic metals (Zn, Pb, Hg, Cr, As, Ni, Cd, Co, Sn, Cu, etc.), precious metals (Ru, Ag, Au, Pt, Pd, etc.) and radionuclides Th, Ra, U, Am, etc.). Usually, the main heavy metals which related to water pollution include mercury, cadmium, zinc, lead, copper, chromium, and nickel (Abel, 1996). Some metals such as zinc and copper are basic trace elements for human and living organism at low concentration (less than 10 mg/L). However, they become toxic to living organism at a high concentration which more than 10 mg/L (Abel, 1996).

The development of technological and industrial activities poses a significant threat to the human, environment, and soil health (Cerbasi and Yetis, 2001). Mostly the heavy metal ions such as Cu, Hg, Cd, Zn, As, Ag, Fe, Cr, Ni, etc.) discharged from the industries are in simple cationic (+) forms (Volesky, 2007) and they are among the most that are common derived from electroplating, plastics manufacturing, rubber manufacturing, pesticides, fertilizers, pigments, pottery glazes, ink, dyes, mining and metallurgical processes (EPA, 1976).

Heavy metals are non-biodegradable inorganic chemicals that cannot be metabolized, and will not break down into nontoxic forms (Kromhout *et al.*, 1985).

They also tend to persist indefinitely, and eventually accumulating in the tissues of living organisms throughout the food chain (Gupta *et al.*, 2000; Aleem *et al.*, 2003). In fact, they can enter the environment wherever they are produced, used, and ultimately discarded as wastes and they may be easily absorbed by living organisms which can be bound to important cellular components such as structural proteins, enzymes, and nucleic acids. As a result, they will cause severe physiological and health effects even though in very small amount (Landis *et al.*, 2001). Parkinson's disease can be caused by chronic exposure to heavy metals such as Zn, Pb, and Cu and the metals might act alone or together to propagate the disease over time (Gorell *et al.*, 1997).

2.2 Heavy metal pollution

Heavy metal pollution in air, soil, and water is one of the biggest concern because it poses risks and hazards to our environment. There are thousands of sources of heavy metal pollution, including mining, metal smelting, coal combustion, natural gas, nickel-cadmium batteries, paper industry, combustion of fossil fuel, industrial effluents, solid waste disposal, phosphate fertilizers, and metal processing (Alloway, 1996 and McDonald and Grandt, 1981). Therefore, resource like metal is becoming a deficit and it also brings harm to our environment through pollution and contamination, thereby threatening ecosystem and human health. Currently toxic metals, precious metals, and radionuclides are in our human concern (Wang and Chen, 2006).

The accumulation of heavy metals in aquatic habitats is dramatically increase, especially in the industrial countries. Rivers are a dominant pathway of heavy metal

pollution (Harikumar *et al.*, 2009). Heavy metals have become significant pollutants to the riverine systems (Dassenakis *et. al.*, 1998). When the heavy metals transport in the river, they go through several changes in their speciation by cause of precipitation, sorption, dissolution, and complexation phenomena (Akcay *et al.*, 2003, Dassenakis *et al.*, 1998) which alter their behavior and bioavailability (Nouri *et al.*, 2011). Therefore, heavy metals are sensitive indicator to monitor changes in water systems.

Mining activities can release toxic metals to the environment. Metal smelting and mining activities are noted as dominant sources of heavy metals in the environment. Moreover, textile industries are indicated as a major source of heavy metal contaminants in water due to the dyeing process. The compounds applied for these dyeing process involve chromium, lead, copper, and nickel which is very poison and carcinogenic to human and living organisms. Furthermore, nuclear generating facilities are also described as source of heavy metal pollution because plenty of water is applied for operation and the effluent with heavy metals infiltrated into groundwater and surface water bodies (Hagberg and Lofgren, 2007; Begum *et al.*, 2011). Petroleum refinery industries discharged effluent which composed of different chemicals which include oil and greases, sulphides, phenols, ammonia, and heavy metals like iron, chromium, nickel, copper, selenium, vanadium, and zinc into coastal and marine environment (Wake, 2005).

Heavy metals exist as natural elements of the earth crust, or as persistent pollutants because they cannot be degraded or destroyed. Heavy metals can intrude the human body system through air, water, and food, then bioaccumulate in our body for some period of time (Lenntech, 2004; UNEP/GPA, 2004).

2.3 Techniques of removal heavy metal

The conventional technologies for treating heavy metal are precipitation, reverse osmosis, oxidation/reduction, ion exchange, activated carbon, physical adsorption, electrolysis and filtration (Veglio and Beolchini, 1997; Beolchini *et al.*, 2005). However, these methods are said to be expensive, high energy consumption, and might not be very efficient (Rhadika *et al.*, 2006, Kapoor and Viraraghavan, 1995, and Tripathi *et al.*, 2007). Chemical oxidation and reduction, where the chemical required is not universal and slow in the process of biological system (reaction with structure of biomass) as for reverse osmosis, will cause scaling problems and they are also expensive in usage (Volesky, 2001).

According to Attia *et al.*, (2010), the major drawbacks of activated carbon are expensive cost, incomplete metal removal, and toxic waste generation. Furthermore, remediation by using chemical precipitation and electrochemical treatment is said to be ineffective when the metal ion concentration is low (1 - 100 mg/L) while producing a huge amount of sludge (Wang and Chen, 2006; Veglio and Beolchini, 1997; Volesky, 1990c).

2.3.1 Precipitation

Precipitation is the technique of choice for the dissolved heavy metals removal in conventional treatment. This process can reduce the concentrations of all heavy metals. The vital key to make this method success is the solubility of precipitated metal compounds due to the heavy metal can be removed via clarification and filtration if it can form an insoluble compound.

Among the precipitation methods, hydroxide precipitation and sulfide precipitation are the two main methods that currently used. As hydroxide precipitation

method is inexpensive and simple, it is the most widely used method by far (Mohan and Pittman, 2006). This process is as simple as increasing the pH of the effluent using lime (CaO), caustic soda (NaOH) or magnesia (MgO) as precipitator and then immobilizes the heavy metals as their respective hydroxides (Esmaeili *et al.*, 2005). At low pH, magnesium oxide was found as a good adsorbent to remove heavy metal ions from their aqueous solutions (Brbooti *et al.*, 2011).

Pang *et al.*, (2009) applied the hydroxide precipitation methods to treat wastewater containing lead, copper, zinc, and iron. The results presented that the removal efficiency of heavy metals by hydroxide precipitation increased with the increased of metal concentration. The optimal removal of metals by hydroxide precipitation was attained at a fixed pH, which different with the type of heavy metals.

2.3.2 Ion exchange

Ion exchange can be defined as the exchange of ions process between the substrate and surrounding medium. The most practical reaction of ion exchange is convertible because the ion exchanger can be repeatedly. Basically resins are produced in the pressure, spherical, and strain freeway against physical deterioration. Resins can be used for a wide range of pH and they are stable at high temperature. In most aqueous and organic solutions, ion exchange resins are completely insoluble. They are consisting of a cross-linked polymer matrix to which charged functional groups are binding with covalent bonding (Sherrington, 1998).

The technique of ion exchange can clear out metallic ion from process streams and water bodies to produce a product with desired quality. Metals like zinc, nickel, uranium, gold, silver, thorium, platinum, cobalt, and some rare earth elements can be recovered and purified by ion exchange process for industrial purpose. Ion exchange

are widely applied in the water treatment and pollution control process and also in purification and separation of radioisotopes, antibiotics, hydrometallurgy, and analytical chemistry (Clifford, 1999; Luca et al. 2009).

Gaikwad *et al.,* (2010) discussed the ion exchange system design to remove copper from acid mine drainage wastewater. A pilot plant for the treatment of acid mine drainage wastewater has been designed for copper removal using the dimensions. Approximately of 0.05m in diameter of 2-column design system is determined as the optimal size of the ion exchange column.

2.3.3 Activated carbon adsorption

Activated carbon has small and low-volume pores which can increase the surface area available for chemical or adsorption reactions. Adsorption on granular activated carbon is a widely-used method to remove organic pollutants and heavy metals. Its ability to remove metallic ions is undeniably diffused, although the removal efficiency for these substances is usually lower than for the organic compounds (Di Natale *et al.*, 2006).

Activated carbon can be profitably used because of their ability to contemporary remove different substances. In fact, they are less selective than ion-exchange resins and usually more cost-effective. For this reason, in order to minimize the amount of adsorbent required, the knowledge of the optimal conditions to maximize the adsorption capacity is needed (Clifford, 1999; Mohan and Pittman, 2006).

The value of parameters during the process such as pH, concentration of solution, temperature, composition of solution, and salinity seriously affect the

equilibrium adsorption capacity and playing a critical role in the industrial scale up of adsorption units (Mohan and Pittman, 2006).

In developing countries, the locally generated wastes such as sugar cane bagasse, cotton stalks, rice straw, and so on have been experimented in the activated carbon production to reduce cost (Logan and Rosemarie, 2002). The previous studies have showed that the use of these raw materials are available at inexpensive cost, contain high carbon content, and may be effective in the pollutants and heavy metals removal.

Zayat and Smith, (2010) applied activated carbon which produced by cotton stalks for lead, copper, and cadmium removal from water and wastewater. The study showed the highest adsorption capacity was for lead and followed by copper and cadmium.

2.4 Biosorbent

Living microorganisms and non-living microorganisms such as bacteria, fungi, yeast, and algae were applied as biosorbent materials for removal of heavy metal (Wang and Chen, 2006). It can effectively isolate the dissolved metal ions from dilute complex solutions with high efficiency and fast, in addition, it is an ideal biosorbent for the high volume and low concentration of wastewater treatment (Wang and Chen, 2006).

However, dead biomass is more preferable than living biomass because there are no poisonous concerns, can grow in media or nutrients with low requirements, easier to detach pollutants from the biomass and to reuse them. Besides, they also do not

contaminate the environment by releasing toxins or propagules (Aksu, 2005 and Mathialagan and Viraraghavan, 2009). In spite of that, treatments with living cells allow the bioremediation process more efficient because they can self-replenish and remove metals through different mechanisms (Malik, 2004). Golab *et al.*, (1995) mentioned that living cells of *Streptomyces pilosus* adsorb more heavy metals than dead cells.

Only biological materials with high metal-binding capacity and selectivity for heavy metals can adsorb heavy metals are suitable to be used in a full-scale biosorption process. Fungi are chosen as a biosorbent because of their special physiology and adsorbing capacity. Chitin and chitosan present in fungi are good ion adsorbers because of the presence of both carboxyl and amine groups (Ashkenazy *et al.*, 1997).

A variety of materials tested as metal adsorbents include crushed coconut shell, Girdish coal, bark, peat, straw, waste tyre rubber, corn cobs, polymerized orange skin, apple residues, wool, and human hair (Bailey *et al.*, 1999; Srivastava *et al.*, 1986; Lakatos *et al.*, 2002; Doyurum and Celik, 2006). Recent development in the field of the biotechnology include the use of microorganisms as sorbents for heavy metals (Kapoor and Viraraghavan, 1995). There are various statements which have been widely reported with regard to microorganisms being able to accumulate metal ions from aqueous solutions (Lopez-Errasquin and Vazquez, 2003; Al-Garni, 2005; Tangaromsuk *et al.*, 2002; Svecova *et al.*, 2006; Zamil *et al.*, 2009; El-Sherif *et al.*, 2008).

To study the heavy metals removal for zinc, nickel, and copper ions, including their removal efficiency in three different stages (biosorption, sedimentation, and desorption), Bakkaloglu *et al.* (1998) compared the different types of waste biomass