

**BIOSORPTION OF SELECTED HEAVY METALS BY FREE AND
IMMOBILIZED *PYCNOPORUS SANGUINEUS*:
BATCH AND COLUMN STUDIES**

by

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LIST OF SYMBOLS

A	Code of initial concentration	(mg/L)
a_{rp}	Redlich-Peterson isotherm constant	$(\text{dm}^3/\text{mg})^\beta$
B	Code of initial concentration	
β	Redlich-Peterson isotherm constant	
C	Code of biomass loading	(g/L)
C_e	Equilibrium concentration	(mg/L)
C_i	Initial concentration	(mg/L)
C_f	Final concentration	(mg/L)
E_a	Activation energy	(kJ/mol)
ΔG°	Gibbs free energy change	(J/mol)
ΔH°	Standard enthalpy	(kJ/mol)
k_1	Rate constant of first-order biosorption	(min^{-1})
k_2	Rate constant of second-order biosorption	$(\text{g}/\text{mg min})$
K_b	Langmuir equilibrium constant	(dm^3/mg)
K_f	Freundlich constant	
K_{rp}	Redlich-Peterson isotherm constant	(dm^3/mg)
K_s	Intraparticle diffusion constant	$(\text{mg}/(\text{g min}^{0.5}))$
MT	Metric ton	
n	Freundlich constant	
q	Metal ions biosorbed per g of biomass	(mg/g)
q_{max}	Maximum specific uptake corresponding to the sites saturation	(mg/g)
q_e	Amount of metal ions uptake at equilibrium	(mg/g)
q_t	Amounts of adsorbed Cu (II) ions on the	(mg/g)

	biosorbent at time t	
R	Gas law constant	(J/mol K)
R_L	Separation factors	
ΔS°	Standard entropy	(J/mol K)
μ	Specific growth rate	(h ⁻¹)
μ_{max}	Maximum specific growth rate	(h ⁻¹)
V	Volume of metal solution in the flask	(L)
X_i	Natural value of the i th independent variable	
X_i^x	Natural value of the i th independent variable at the centre point	
ΔX_i	Step change value.	
W	Weight of biosorbent	(g)
Y_1	Response for Cd (II) removal	%
Y_{21}	Response for Cu (II) removal	%
Y_{31}	Response for Pb (II) removal	%

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometer
ANOVA	Analysis of variance
ATSDR	Agency for Toxic Substance and Disease Registry
BET	Bruner, Emmet and Teller
CCD	Central Composite Design
CV	Coefficient of variance
DOE	Department of Environment
DoE	Design of Experiment
EDX	Energy Dispersive X-ray Spectroscopy
FTIR	Fourier transform infrared
RSM	Response surface methodology
SEM	Scanning electron micrographs

**BIOERAPAN LOGAM-LOGAM BERAT TERPILIH OLEH *PYCNOPORUS*
SANGUINEUS BEBAS DAN TERSEKAT GERAK:
KAJIAN KELOMPOK DAN TURUS**

ABSTRAK

Kemampuan kulat pereput putih, *Pycnoporus sanguineus* (*P. sanguineus*) untuk tumbuh di dalam kelalang kultur goncang telah dikaji. Sel biomassa yang paling banyak didapati adalah dalam media 1 (glukos, malt ekstrak dan yis ekstrak), pH 9 dan 150 putaran per minut. Sel biomassa yang digunakan sebagai biopenjerap untuk penyingkiran logam-logam berat (kadmium Cd (II), kuprum Cu (II) dan plumbum Pb (II)) telah dipilih dari kultur di pertengahan fasa pertumbuhan. Untuk penyingkiran logam berat (kadmium Cd (II), kuprum Cu (II) dan plumbum Pb (II)), kesan pH (2 - 6), kepekatan awal logam (50 - 300 mg/L), muatan biomassa (1 - 6 g/L) dan suhu (30°C - 40°C) ke atas sel *P. sanguineus* bebas dan tersekat gerak telah dikaji. Didapati pengambilan logam meningkat dengan peningkatan pH, kepekatan awal logam dan suhu. Bagaimanapun, keputusan sebaliknya diperhatikan apabila muatan biomassa bertambah. Sel tersekat gerak *P. sanguineus* menunjukkan keafinitian yang tinggi berbanding sel bebas untuk menyingkirkan ion-ion logam dan ianya berpadanan dengan model isoterma Langmuir, Redlich-Peterson diikuti oleh Freundlich.

Kajian kinetik untuk penyingkiran ion Cd (II), Cu (II) and Pb (II) ke atas sel bebas dan tersekat gerak telah dijalankan pada suhu yang berbeza (30 to 40°C) dalam sistem kelompok. Bioerapan ion Pb (II) ke atas sel tersekat gerak berpadanan dengan tertib pseudo pertama, tertib pseudo kedua dan model resapan intrapartikel berbanding dengan ion Cd (II) dan ion Cu (II). Pengoptimuman menggunakan metodologi permukaan sambutan (RSM) pula menunjukkan kepekatan awal logam, pH dan muatan biomassa memainkan peranan penting dalam bioerapan ion Cd (II),

Cu (II) dan Pb (II) ke atas sel tersekat gerak *P. sanguineus*. Keadaan optimum penyingkiran ion Pb (II) didapati pada pH 4, kepekatan awal ion Pb (II) 200 mg/L dan 10 g/L biopenjerap. Bagi penyingkiran Cd (II) and Cu (II), didapati pH optimumnya ialah pada pH 6 dan 5, dengan kepekatan awal logam 110 mg/L dan 20 g/L biopenjerap.

Kebolehan sel tersekat gerak *P. sanguineus* menjerap ion-ion logam telah dikaji di dalam turus lapisan terpadat. Kesan ketinggian lapisan (5 - 13 cm), kadar aliran (0.24 – 0.72 L/hr) dan kepekatan awal logam (50 - 300 mg/L) terus diteliti. Data eksperimen yang didapati telah dibandingkan dengan data simulasi daripada model Thomas, Adam-Bohart dan Yoon-Nelson. Keputusan menunjukkan data eksperimen berpadanan dengan model Thomas dan Yoon-Nelson. Turus ini juga telah dinyahjerap menggunakan 0.1 M HCl dan diulang sebanyak dua kitaran bioerapan-nyahjerapan. Didapati pengurangan berat biopenjerap sangat ketara selepas dua kitaran bioerapan-nyahjerapan. Pencirian biopenjerap menggunakan SEM, FTIR dan EDX menunjukkan strukturnya berubah sebelum dan selepas bioerapan, yang disebabkan penglibatan kumpulan berfungsi dan mekanisma pertukaran ion semasa bioerapan logam.

**BIOSORPTION OF SELECTED HEAVY METALS BY FREE AND
IMMOBILIZED *PYCNOPORUS SANGUINEUS*:
BATCH AND COLUMN STUDIES**

ABSTRACT

The ability of *Pycnoporus sanguineus* (*P. sanguineus*), a white-rot macrofungi to grow in shake flask culture was studied. The highest mycelial biomass was obtained in media 1 (glucose, malt extract and yeast extract), pH 9 and 150 rpm. The mycelium biomass used as biosorbent to remove heavy metals (cadmium Cd (II), copper Cu (II) and lead Pb (II)) was chosen from culture at the middle of exponential growth phase. For heavy metals removal (cadmium Cd (II), copper Cu (II) and lead Pb (II)), the effect of pH (2 - 6), initial metal concentration (50 - 300 mg/L), biomass loading (1 - 6 g/L) and temperature (30°C - 40°C) over freely suspended and immobilized cells of *P. sanguineus* were investigated. It was found that the metal uptake increased with increasing of pH, initial metal concentration and temperature. However, a reverse trend was observed as the biomass loading increased. The immobilized cells of *P. sanguineus* showed higher affinity compared to freely suspended cells to adsorb the metal ions and fitted well the Langmuir, Redlich-Peterson followed by Freundlich isotherm models.

The kinetic studies of Cd (II), Cu (II) and Pb (II) ions removal onto freely suspended and immobilized cells of *P. sanguineus* were carried out at different temperature (30 to 40°C) in a batch system. Pb (II) biosorption onto immobilized cells of *P. sanguineus* fitted well the pseudo first order, pseudo second order and intraparticle diffusion models compared to Cd (II) and Cu (II). Optimization using response surface methodology (RSM) showed that an initial metal concentration, pH and biomass loading played an important role for the biosorption of Cd (II), Cu (II) and Pb (II) ions onto immobilized cells of *P. sanguineus*. The optimum condition for

Pb (II) ions removal was found at pH 4, 200 mg/L of initial Pb (II) concentration and 10 g/L of biosorbent. As for Cd (II) and Cu (II) removal, the optimum pH was observed at pH 6 and 5, with initial metal concentration 110 mg/L and 20 g/L of biosorbent.

The ability of immobilized cells of *P. sanguineus* to adsorb metal ions was also investigated in a packed bed column. The effect of bed height (5 - 13 cm), flow rate (0.24 – 0.72 L/hr) and initial metal concentration (50 - 300 mg/L) were looked at. The experimental breakthrough data were compared with simulated breakthrough profiles obtained from Thomas, Adam-Bohart and Yoon-Nelson models. The results showed that the experimental data were best described by Thomas and Yoon-Nelson models. The column was also regenerated using 0.1 M HCl and repeated up to two biosorption-desorption cycles. Significant biosorbent weight loss was observed after the two cycles. The characterization of biosorbent using SEM, FTIR and EDX shows that the structure changed before and after biosorption, due to the involvement of functional groups and ion exchange mechanism during metal biosorption.

CHAPTER ONE

INTRODUCTION

1.0 Heavy metals pollution in Malaysia

Malaysia has undergone a rapid economic, social and environmental change in the Asia-Pacific region (Danilo, 1998; Hezri and Nordin Hasan, 2006). In fact, the country's pace of industrialization and its economic achievements have been impressive (Hezri and Nordin Hasan, 2006). In early days of abundant resources and negligible development pressures, little attention was paid to environmental issue, although some environment related legislation pertaining to different sectors was authorized. Realizing this, the government has since as early as 1974 taken concrete steps by introducing an enabling legislation called the Environmental Quality Act, 1974 and shown it in Table 1.1. The table presents the parameter limits of effluents for standard A and B. The main objective of this act is to prevent, abate and control pollution, and further enhancing the quality of the environment in this country. The Department of Environment has been entrusted to administer this legislation to ensure that Malaysia will continue to enjoy both industrial growth and a healthy living environment.

Rapid economic changes have resulted in elevated level of toxic heavy metals and radionuclides entering the biosphere (Rani, 1995). The heavy metals such as lead, cadmium, copper, nickel and zinc are among the most common pollutants found in industrial effluents. Solid and/or liquid wastes containing toxic heavy metals may be generated in various industrial processes such as chemical manufacturing, electric power generating, coal and ore mining, smelting and metal refining, metal plating, and others (Yin *et al.*, 1999; Yalchinkaya *et al.*, 2002).

Table 1.1: Environmental Quality Act 1974, Environmental Quality (Sewage and Industrial Effluents), Regulations 1979 (Regulations 8(1), 8(2), 8(3)) Parameter Limits of Effluents of Standards A and B (DOE, 2006)

Parameter	Unit	Standard	
		A	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
Arsenic	mg/L	0.05	0.10
Cyanide	mg/L	0.05	0.10
Lead	mg/L	0.10	0.5
Chromium Trivalent	mg/L	0.20	1.0
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	2.0	2.0
Boron	mg/L	1.0	4.0
Iron (Fe)	mg/L	1.0	5.0
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	Not detectable	10.0

* Standard A is applied to industrial and development project which area located within the catchment area, otherwise Standard B generally apply.

Heavy metals pollution such as copper, cadmium, lead, mercury, arsenic and chromium has been classified as a priority pollutant by the Department of Environment Malaysia. In Malaysia, the Interim Marine Water Quality Standards (IMWQS) presents in Table 1.2 is used to monitor and analyze the marine quality water for 14 states in Malaysia. In 2006, a total of 1,035 samples from 229 monitoring stations have been analyze and the results are presented in Table 1.3 (DOE, 2006). Table 1.3 shows that lead was recorded the highest parameter exceeding interim standard in Kelantan (78 %) and Terengganu (86 %) while

mercury was exceeding in most of northern state of Malaysia (Perlis, Pulau Langkawi, Kedah and Pulau Pinang). Sources of these heavy metals pollution was from industrial development and land-based sources (DOE, 2006). The groundwater quality is also been monitored based on the National Guidelines for Raw Drinking Water Quality from the Ministry of Health (Revised December 2000) and the benchmark for the parameters limit is shown in Table 1.4. Continuous monitoring of heavy metals level in the environment is very important since it cannot be degraded and becoming public health problem when increased above acceptance level (Duruibe *et al.*, 2007). Health problem due to heavy metals pollution include nausea, vomiting, bone complications, nervous system impairments and even death become a major problem throughout many countries when metal ions concentration in the environment exceeded the admissible limits (Andrew and Henrique, 2006; Lodeiro *et al.*, 2006). Due to that, various treatment technologies had been searched to reduce the concentration of heavy metals in the environment.

Table 1.2: The Interim Marine Water Quality Standards (IMWQS) (DOE Report, 2006)

Parameter	Unit	Interim standards
<i>Escherichia coli</i> (<i>E. coli</i>)	MPN/100ml	100
Oil and Grease (O&G)	mg/L	0
Total suspended solids (TSS)	mg/L	50
Arsenic (As)	mg/L	0.1
Cadmium (Cd)	mg/L	0.1
Chromium (Cr) Total	mg/L	0.5
Cuprum (CU)	mg/L	0.1
Plumbum (Pb)	mg/L	0.1
Mercury (Hg)	mg/L	0.001

Table 1.3: Status of Marine Water Quality 2006 (DOE, 2006)

State	No of stations	No. of samples	Parameter Exceeding Interim Standard (%)								
			TSS	Oil & Grease	<i>E.Coli</i>	Cadmium	Chromium	Mercury	Lead	Arsenic	Copper
Perlis	2	24	64	43	100	7	0	50	0	0	0
Pulau Langkawi	7	35	94	17	43	0	0	23	0	0	9
Kedah	3	14	100	14	100	0	0	79	14	0	0
Pulau Pinang	25	191	74	10	78	0	2	26	9	0	4
Perak	13	52	100	12	64	0	0	-	56	0	0
Selangor	14	49	98	54	68	0	0	11	0	0	0
N. Sembilan	13	78	100	68	83	0	0	16	0	0	0
Melaka	9	28	92	20	66	0	-	-	0	-	0
Johor	51	122	60	11	39	0	0	10	13	1	9
Pahang	11	80	19	80	10	6	0	0	40	0	0
Terengganu	19	76	74	93	46	11	0	2	86	0	42
Kelantan	10	40	73	59	43	10	0	3	78	10	30
W.P.Labuan	5	20	60	0	25	0	25	-	0	-	0
Sabah	26	111	35	0	25	1	0	0	6	-	3
Sarawak	21	123	78	49	25	0	0	0	2	0	0
Malaysia (Sum)	22	1035									
Average (%)						2	2	18	20	1	6

Table 1.4: Malaysia: National Guidelines for Raw Drinking Water Quality [Revised December 2000] (DOE, 2005)

Parameter	Symbol	Benchmark
Sulphate	SO ₄	250 mg/l
Hardness	CaCO ₃	500 mg/l
Nitrate	NO ₃	10 mg/l
Coliform	-	Must not be detected in any 100 ml sample
Manganese	Mn	0.1 mg/l
Chromium	Cr	0.05 mg/l
Zinc	Zn	3 mg/l
Arsenic	As	0.01 mg/l
Selenium	Se	0.01 mg/l
Chloride	Cl	250 mg/l
Phenolics	-	0.002 mg/l
Total Dissolved Solids	-	1000 mg/l
Iron	Fe	0.3 mg/l
Copper	Cu	1.0 mg/l
Lead	Pb	0.01 mg/l
Cadmium	Cd	0.003 mg/l
Mercury	Hg	0.001 mg/l

1.1 Treatment Technologies for Heavy Metals Removal

Heavy metals contamination is becoming a great concern to the environmental awareness and government policies. Several heavy metals removal technologies including chemical precipitation, ion exchange, reverse osmosis, electrodialysis, ultrafiltration and phyto remediation are commonly used in industries (Ahalya *et al.*, 2003). However, these technologies are becoming uneconomical and unfavourable to remove heavy metals from industrial wastewaters. Description and disadvantages of these treatment technologies are presented in Table 1.5.

Table 1.5: Treatment methods used in heavy metals removal (Rakhshae *et al.*, 2006; Sannasi *et al.*, 2006; Ahalya *et al.*, 2003; Chong *et al.*, 2000)

Treatments	Process details	Disadvantages
Chemical precipitation	Precipitation of metal ions were achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers	Large amount of sludge produced during the process will cause a disposal problem
Ion exchange	Metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin	High cost, Partial removal for certain ions
Reverse osmosis	Metal ions are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by the dissolved solids	Expensive
Electrodialysis	Metal ions are separated through the use of semi-permeable ion selective membranes. An electrical potential between the two electrodes causes a separation of cations and anions thus cells of concentrated and dilute salts are formed.	Metal hydroxides formed clogged the membrane
Ultrafiltration	Pressure driven membranes are used for the removal of metal ions	Generation of sludge cause disposal problem
Phytoremediation	Uses of certain plants to clean up soil, sediment and contaminated water with metal ions	The process takes a long time to remove metal ions, Regeneration of the plant is difficult

With increasing environmental attention and legal constraint on discharge effluents, a need of cost effective technologies are essential (Alluri *et al.*, 2007). Recently, focused of using microbial biomass as a biosorbent to sequester metal ions from contaminated effluent has emerged (Akar *et al.*, 2007; Alluri *et al.*, 2007).

1.2 Problem statement

The development of new treatment method to remove heavy metal ions from wastewater which could be cost effective and more efficient has spurred to overcome the conventional method. Biosorption treatment technology has received much attention as it offered low cost biosorbent and non-hazardous biomaterials. Since Malaysia is a tropical country, great diversity of microbes was established as they could exploit a wide range of substrate, having different behaviours and generally easily to adapt to changes in environmental condition. Previous works reported that some microorganisms such as bacteria, algae, yeast, fungi and cellulosic materials are well known capable to adsorb a large amount of metal ions (Zulfadhly, 1999; Ahalya *et al.*, 2003). Fungi may be suitable for the removal of metals from wastewater than other microbes because of their great tolerance towards heavy metals and other adverse conditions such as low pH, high cell wall binding capacity and high intracellular metal uptake capacity (Gadd, 1986; Rome and Gadd, 1987).

Although it is well understood what some macro-fungi does, white rot fungal biosorption pattern and physiological functions for freely suspended or immobilized cells in batch and packed-bed column is lessly reported. In fact, biosorption of Pb (II), Cu (II) and Cd (II) ions by freely suspended and immobilized cells system let alone to be exploited. Thus, this study was carried out to determine the potential of

freely suspended and immobilized living cells of *Pycnoporus sanguineus* to adsorb Pb (II), Cu (II) and Cd (II) ions in batch and packed bed studies.

1.3 Research Objectives

In view of the above observations, this study was carried out with the following objectives:

- To study the effect of different parameters on growth of *P. sanguineus* in shake flask culture.
- To study the equilibrium and kinetics of heavy metals (Pb^{2+} , Cu^{2+} and Cd^{2+}) removal by freely suspended and immobilized cells of *P. sanguineus* in batch system.
- To optimize the biosorption capacity of heavy metal removal by immobilized cells of *P. sanguineus* in batch system using statistical Design of Experiments (DoE).
- To study the desorption/regeneration of metal ions from the immobilized biosorbent.
- To determine the optimum parameters of metal ions (Pb^{2+} , Cu^{2+} and Cd^{2+}) biosorption by immobilized cells of *P. sanguineus* in a packed bed column.
- To study the characterization of *P. sanguineus* before and after biosorption process.

1.4 Scope of Study

In this study, developments of biosorbent for heavy metals removal from living *Pycnopus sanguineus* (*P. sanguineus*) were considered. Two types of biosorbent; namely freely suspended and immobilized cells of *P. sanguineus* were chosen for equilibrium, kinetics, thermodynamic studies in batch system. The metals, Cd (II), Cu (II) and Pb (II) were used as a tested heavy metals element for biosorption. These metals were chosen as they were widely used in electroplating industries and exist in effluents from industrial processes (Lodeiro *et al.*, 2006; Vijayaraghavan and Prabu, 2006). Besides that, it causes serious water pollution and harmful to human health (Ilhan *et al.*, 2004). The equilibrium data for both biosorbents were analyzed using different equilibrium models such as Langmuir, Freundlich and Redlich Peterson. The kinetics models were also proposed and the differences between the two biosorbents were compared. Biosorbent that absorbed the highest metals uptake was used for further studies.

Response surface methodology, as one of a statistical method was used to optimize heavy metals removal in batch sorption process. The input factors that were considered for metals optimization were initial metals concentration, pH and biomass loading. The response of the metals optimization is the percentage of heavy metals removal. Regeneration of biosorbent was also conducted using three different concentrations of hydrochloric acid and nitric acids.

Cd (II), Cu (II) and Pb (II) biosorption onto living *P. sanguineus* in packed bed column was carried out. The immobilized cells of *P. sanguineus* were used as a biosorbent as it is easy to separate from operation process compared to free cells. The experiments were carried out to study the effect of bed height, flow rates and initial metals concentration. Three different models namely, Thomas, Adam-Bohart and

Yoon-Nelson were used to analyze the compatibility of experimental data of the tested metals. Regeneration of the column was carried out using eluent with highest elution efficiency tested in batch studies.

The characterization of biosorbent was carried out using Gas sorption analysis, Scanning Electron Micrographs (SEM), Energy Dispersive X-ray Spectroscopy (EDX) and Fourier Transform Infrared Spectrophotometer (FTIR), respectively.

White-rot fungi, *Pycnoporous sanguineus* is recommended as a biosorbent for Cd (II), Cu (II) and Pb (II) biosorption as it is easily available in extensive quantities, easily grown in basic fermentation medium and low cost (Tunali *et al.*, 2006). Possible regeneration of the biosorbent will overcome sludge disposal problem normally exist in conventional method. Therefore metals biosorption using these biosorbent will be more economical as the biosorbent can be used several times.

1.5 Organization of the thesis

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

Chapter 1 presents the heavy metal pollution in Malaysia and existing technologies used to remove metal ions from wastewater. This chapter also presents the problem statement, research objectives, scope of research and thesis organization.

Chapter 2 covers an overview of related knowledge of biosorption process. The biosorption isotherms, kinetic and modeling for heavy metals biosorption are discussed in detail.

Chapter 3 refers to the material and methods describing the experimental procedure in the research for batch and packed bed column system. This chapter also covers the analysis of sample and the characterization of biosorbent before and after treatment.

Chapter 4 presents the results and discussion covering heavy metals biosorption by freely suspended and immobilized cells of *Pycnoporus sanguineus* in batch and packed bed column. The adsorption equilibrium and kinetics models for each of the metal ions in batch and continuous system were also presented. Langmuir, Freundlich and Redlich-Peterson were tested in batch equilibrium studies for Cd (II), Cu (II) and Pb (II) biosorption for both freely and immobilized cells of *Pycnoporus sanguineus*. For kinetics studies, the pseudo first, pseudo second and intraparticle diffusion equations were applied to the experimental data. The optimization of heavy metals biosorption by *Pycnoporus sanguineus* in batch system were then obtained from the Design of Experiment. In column studies, the experimental data were examined using Thomas, Adam Bohart and Yoon Nelson models. The important parameters that influence the performance of the packed bed

column for each models were also been determined. Besides that, this chapter also covers the regeneration and characterization of the biosorbent used.

Chapter 5 refers to overall conclusions that are based on the findings obtained in the results and discussion (Chapter 4). Recommendations for future research were also given in the chapter.

CHAPTER TWO

LITERATURE REVIEW

2.0 Heavy Metals

Heavy metals are defined as those elements with a specific density at least five times the specific gravity of water (Jarup, 2003). Heavy metals include cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr), iron (Fe), and the platinum group elements (Duruibe *et al.*, 2007). The important heavy metals from water pollution view include mercury, cadmium, lead, zinc, copper, nickel and chromium (Abel, 1996). Copper and zinc are essential trace elements for living organism at low concentration (< 10 mg/L), however it become toxic at high concentration (>10 mg/L) (Abel, 1996). Most of these metal ions (Cd, Cu, Zn, Hg, As, Ag, Cr, Fe etc) release from the industries are in simple cationic (+) forms (Volesky, 2007). Table 2.1 listed the uses of several heavy metals and it's health effect on human. The characteristics of heavy metals are described as (Wang and Chen, 2006):

1. Toxicity that can last for a long time in nature.
2. Transformation of low toxic heavy metals to more toxic form in a certain environment, such as mercury.
3. Bioaccumulation and bioaugmentation of heavy metals by food chain that could damage normal physiological activity and endanger human life.
4. Heavy metals cannot be degraded including biotreatment.
5. Heavy metals are very toxic even at low concentration (1.0- 10 mg/L). Metal ions such as cadmium and mercury have been reported very toxic even in lower concentration range from 0.001 to 0.1 mg/L (Volesky, 1990; Wang, 2002; Alkorta *et al.*, 2004; Wang and Chen, 2006)

Table 2.1: Heavy metals uses and health effects on human

Heavy metals	Uses	Health effects	References
Arsenic (As)	Metal processing plants, burning of fossil fuels, mining of arsenic containing ores and use of arsenical pesticides.	Internal cancer, skin lesions and death.	(Fergusson, 1989; Anawar <i>et al.</i> , 2002 ; Cappuyns <i>et al.</i> , 2002).
Cadmium (Cd)	Electroplating, fertilizers, mineral processing and battery manufacturing	Cancer, lung insufficiency, disturbances in cardiovascular system, liver and kidney damage	(Yin and Blanch, 1989; Sharma, 1995; Arica <i>et al.</i> , 2003; Cruz <i>et al.</i> , 2004; Malkoc and Nuhoglu, 2005; Mashitah <i>et al.</i> , 2008)
Copper (Cu)	Copper and brass plating, mining, metal industries and copper-ammonium rayon industries	Normocytic, hypochromic anemia, leukopenia, and osteoporosis; copper deficiency	(Aksu and Kutsal, 1997; ATSDR, 2004)
Chromium (Cr)	Metal plating, electroplating, leather, mining, galvanometry, dye production	Ulcer, skin irritation, liver and kidney damage	(Landis and Yo, 2003; ATSDR, 2004; Kumar <i>et al.</i> , 2007; Fiol <i>et al.</i> , 2008)
Lead (Pb)	Metal plating, textile, battery manufacturer, automotive and petroleum industries	Spontaneous abortion, damage nervous system, kidney and brain damage	(Tunali <i>et al.</i> , 2006; ATSDR, 2007)
Mercury (Hg)	Metallurgy industries, chemical manufacturing and metal finishing	Memory problems, increased heart rate, tremors, kidney and brain damage	(Igwe and Abia, 2005; Igwe and Abia, 2006; ATSDR, 2007)

2.1 Biosorption process

Biosorption of metal ions using biological materials such as algae, bacteria, fungal and yeast have received greater attention due to its advantages over conventional method (Arica *et al.*, 2001). It has been defined as the property of biomass such as algae, bacteria, fungal and yeast to bind with metal ions from aqueous solutions (Dursun, 2006; Wang and Chen, 2006; Volesky, 2007). Biosorption process could involve several mechanisms such as ion-exchange, physical adsorption, complexation and precipitation (Veglio and Beolchini, 1997; Beolchini *et al.*, 2005). According to Ahalya *et al* (2003) and Sag *et al* (1998), biosorption mechanisms can be divided into metabolism dependent and non-metabolism dependent. Metabolism dependent is a slow process include of transport across cell membrane and precipitation. While non-metabolism dependent is a rapid process include of precipitation, physical adsorption, ion exchange and complexation (Sannasi *et al.*, 2006). The process is classified as i) extracellular accumulation/precipitation ii) cell surface sorption/precipitation and iii) intracellular accumulation (Ahalya *et al.*, 2003; Sag *et al.*, 1998). The major advantages of biosorption process over conventional technologies include (Kratochvil and Volesky, 1998; Ahalya *et al.*, 2003):

- Low cost
- High efficiency
- Minimization of sludge production
- Biosorbent can be regenerated and
- Possible of metals recovery

There were several factors that influence the biosorption process as reported by few researchers as listed in Table 2.2.

Table 2.2: Factors that influence the biosorption process

Factors	Description	
pH	Most important parameter in the biosorption process.	(Friis and Myers-Keith, 1986; Galun <i>et al.</i> , 1987)
Temperature	The biosorption performances does not influence by the temperature in the range of 20-35°C	(Aksu <i>et al.</i> , 1992)
Biomass loading	Low biomass loading resulting in an increase of metals uptake. However, increase in biomass loading cause interference between active binding sites thus decrease the metals uptake.	(Gadd <i>et al.</i> , 1988)
Presence of other metal ions	Existence of metals competition for the binding sites occurred by the presence of other metal ions	(Ahalya <i>et al.</i> , 2003)

2.2 Biosorbents

Both living and nonliving microorganisms such as algae, bacteria, fungal and yeast were used as biosorbent materials for heavy metals biosorption (Terry and Stone, 2002; Wang and Chen, 2006). Focus using these microorganisms as a biosorbent for metals removal was searched as it is cheap and abundant (Kapoor and Viraraghavan, 1997; Yan and Viraraghavan, 2003; Kim *et al.*, 2003; Say *et al.*, 2001). In the literature, some microorganisms are capable to remove heavy metals even at low concentration, in the range 1-100 mg/L (Chong and Volesky, 1995; Fagundes-Klen *et al.*, 2007). The advantage of using living cell over dead cells as a biosorbent is that living cells work similar as dead cells at low metals concentration and living cells were able to generate new cells through growth which allowed more space for biosorption mechanism to occur (Axtell *et al.*, 2003). Dushenkov *et al*

(1995) reported that living cells could adsorb metal ions rapidly and provide high degree of separation.

Lists of various microorganisms include algae, bacteria, fungal and yeast used as a biosorbent for metals removal were presented in Table 2.3 (a - d). These tables showed that both active (living) and inactive (dead) cells of each microorganisms have been tested to adsorb Cd (II), Cu (II) and Pb (II) from aqueous solution. Biosorbent used in these tables were focused only on Cd (II), Cu (II) and Pb (II) ions removal as these are the metals used in this study.

Table 2.3 (a): Cadmium (II), copper (II) and lead (II) uptake by algae species

Biosorbent	Type	Metal ions	Reference
<i>Durvillaea</i>			
<i>Ecklonia</i>	Inactive	Cd ²⁺	Figueira <i>et al</i> (2000)
<i>Homosira</i>			
<i>Laminaria</i>			
<i>Chlorella vulgaris</i>	Inactive	Cd ²⁺	Aksu, (2001)
<i>Chlorella vulgaris</i>	Active	Cu ²⁺	Mehta and Gaur, (2001)
<i>Scenedesmus abundans</i>	Active Inactive	Cu ²⁺ Cd ²⁺	Terry and Stone, (2002)
<i>Padina</i> sp	Inactive	Cu ²⁺	Kaewsarn, (2002)
<i>Ulothrix zonata</i>	Inactive	Cu ²⁺	Nuhoglu <i>et al</i> (2002)
<i>Microspora</i>	Inactive	Pb ²⁺	Axtell <i>et al</i> (2003)
<i>Sargassum</i> sp	Inactive	Cd ²⁺	Cruz <i>et al.</i> (2004)
<i>Gracillaria</i> sp			
<i>Padina</i> sp	Inactive	Cu ²⁺ Cd ²⁺ Pb ²⁺	Sheng <i>et al</i> (2004)
<i>Sargassum</i> sp			
<i>Ulva</i> sp			
<i>Caulerpa lentillifera</i>	Inactive	Cu ²⁺ Cd ²⁺ Pb ²⁺	Pavasant <i>et al</i> (2006)
<i>Fucus</i> sp	Inactive	Cd ²⁺	Herrero <i>et al</i> (2006)
<i>Spirogyra</i> sp	Inactive	Cu ²⁺	Gupta <i>et al</i> (2006)

Table 2.3 (b): Cadmium (II), copper (II) and lead (II) uptake by bacteria species

Biosorbent	Type	Metal ions	Reference
Indigenous iron-oxidizing bacteria	Active	Cu ²⁺ Pb ²⁺	Xiang <i>et al</i> (2000)
<i>Bacillus subtilis</i>	Active	Cd ²⁺ Cu ²⁺	Costa <i>et al</i> (2001)
<i>Acinetobacter</i> sp.	Active	Cd ²⁺ Pb ²⁺	Degiorgi <i>et al</i> (2002)
<i>Flavobacterium</i> sp			
<i>Escherichia coli</i>			
<i>Escherichia coli</i>			
<i>Ochrobactrum Anthropi</i>	Inactive	Cd ²⁺ Cu ²⁺	Ozdemir <i>et al</i> (2003)
Sulphate-reducing bacteria	Active	Cu ²⁺	Jong and Parry, (2003)
<i>Bacillus laterosporus</i>	Active	Cd ²⁺	Zouboulis <i>et al</i> (2004)
<i>Bacillus licheniformis</i>	Inactive		
<i>Escherichia coli</i> JM109	Active	Cd ²⁺	Deng <i>et al</i> (2007)
<i>B. lactis</i> Bb12			
<i>B. longum</i> 2C			
<i>B. longum</i> 46	Inactive	Cd ²⁺ Pb ²⁺	Halttunen <i>et al</i> (2007)
<i>Lactobacillus casei</i> Shirota			
<i>Lactobacillus fermentum</i> ME3			
<i>Lactobacillus rhamnosus</i> GG			

Table 2.3 (c): Cadmium (II), copper (II) and lead (II) uptake by fungal species

Biosorbent	Type	Metal ions	Reference
<i>Agaricus macrosporus</i>	Active Inactive	Cu ²⁺ Cd ²⁺ Pb ²⁺	Melgar <i>et al</i> (2000)
<i>Aspergillus terreus</i>			
<i>Cladosporium cladosporioides</i>			
<i>Fusarium oxysporum</i>	Active	Cd ²⁺	Massaccesi <i>et al</i> (2002)
<i>Gliocladium roseum</i>			
<i>Penicillium</i> sp			
<i>Talaromyces helicus</i>			
<i>Trichoderma koningii</i>			
<i>Lentinus sajor-caju</i>	Active Inactive	Cd ²⁺	Bayramoglu <i>et al</i> (2002)
<i>Mucor rouxii</i>	Active Inactive	Cd ²⁺ Pb ²⁺	Yan and Viraraghavan (2003)
<i>Aspergillus niger</i>	Active	Cu ²⁺ Pb ²⁺	Dursun <i>et al</i> (2006)
<i>Phanerochaete chrysosporium</i>	Active	Cu ²⁺ Pb ²⁺	Iqbal and Edyvean, (2004)
<i>Pycnoporus sanguineus</i>	Inactive	Cu ²⁺ Pb ²⁺ Cd ²⁺	Mashitah <i>et al</i> (1999a) Mashitah <i>et al</i> (1999b) Zulfadhly <i>et al</i> (2001)
	Active	Cd ²⁺	Mashitah <i>et al</i> (2008)

Table 2.3 (d): Cadmium (II), copper (II) and lead (II) uptake by yeast species

Biosorbent	Type	Metal ions	Reference
<i>Rhodotorula rubra</i>	Active Inavtice	Cd ²⁺ Pb ²⁺	Salinas <i>et al</i> (2000)
<i>Candida</i> sp	Active	Cu ²⁺	Donmez and Aksu, (2001)
<i>Sacchaomyces cerevisiae</i> SN41	Active	Cu ²⁺	Brandolini <i>et al</i> (2002)
Baker's yeast	Active	Pb ²⁺	Skountzou <i>et al</i> (2003)
Baker's yeast biomass	Inactive	Cd ²⁺ Pb ²⁺	Goksungur <i>et al</i> (2005)
<i>Sacchaomyces Cerevisiae</i>	Inactive	Pb ²⁺	Ozer and Ozer, (2003); Wang and Chen, (2006)

2.3 Mechanism of biosorption process

Biosorption of metal ions onto microorganisms involve a combination of the following metal-binding mechanisms including physical adsorption, ion exchange, complexation and precipitation (Wang and Chen, 2006; Ahalya *et al.*, 2003). Each mechanism is described by Ahalya *et al* (2003) as follows:

Physical adsorption: Van der Waal's forces (electrostatic interaction) were observed to take place between metal ions in the solution and cell wall of the microbial. These interactions are reported to be responsible in copper biosorption using *Zoogloea ramigera* and *Chlorella vulgaris* (Aksu *et al.*, 1992)

Complexation: Metal ions removals from aqueous solution also take place by complex formation on the cell surface after the interaction between

metal ions and active groups. Metal ions can be biosorbed or complexed by carboxyl groups found in the microbial polysaccharides or other polymers. Aksu *et al* (1992) reported that copper biosorption onto *Zoogloea ramigera* and *Chlorella vulgaris* involve both adsorption and formation of coordination bonds between metals and carboxyl and amino groups of the cell wall. The active groups responsible in the metals biosorption were listed in Table 2.4

Table 2.4: Functional groups that are responsible in metals biosorption (Birch and Bachofen, 1990; Le Cloirec *et al.*, 2003)

Formula	Basic groups	Formula	Acidic groups
-NH ₂	Amino	-COOH	Carboxylic
=NH	Immino	-SO ₃ H	Sulphonic
-N=	Cyclic or non cyclic nitrogen	-PO(OH) ₂	Phosponic
=CO	Carbonyl	-OH	Enolic, phenolic
-O-	Ether	=N-OH	Oxime
-OH	Alcohol	-SH	Mercaptan
-S-	Thio ether		

Ion exchange: Polysaccharides existed on cell wall of microorganisms consist of counter ions such as K⁺, Na⁺, Ca²⁺ and Mg²⁺. These ions can exchange with metal ions resulting in metal ions uptake (Kuyucak and Volesky, 1988; Muraleedharan and Venkobachr, 1990).

Precipitation: This mechanism is dependent or independent on cellular metabolism.

Metal ions removal from aqueous solution often associates with active defence system of microorganisms. This active system is a system that produces compounds favoring the precipitation process (Veglio *et al.*, 1997).

2.4 Biosorption by free cells

The term free cells signify non-immobilization microorganisms, which is free in aqueous solution (Veglio and Beolchini, 1997). Biosorption of heavy metals using free cells is important as it provides information about equilibrium of the process, needed for the industrial scale up and design of biosorption process (Ross and Townsley, 1986; Golab *et al.*, 1991; Veglio and Beolchini, 1997). Both live and dead cells were reported capable to sequester metal ions from aqueous solution (Yan and Viraraghavan, 2001). Biosorption capacity of dead cells has been widely studied compared to living cells (Kapoor and Viraraghavan, 1995). The metals uptake by living free cells were dependent on pH, initial metal concentration, contact time, culture condition and biosorbent loading (Kurek *et al.*, 1982; Kiff and Little, 1986; Galun *et al.*, 1987; Siegel *et al.*, 1987; Gadd *et al.*, 1988; Kapoor and Viraraghavan, 1995). Metals accumulation using growing cells varied with the cell age (Kapoor and Viraraghavan, 1995). Kapoor and Viraraghavan (1995) reported that maximum metals uptakes generally take place during early stages of growth and lag phase.

However, the application of dead cells offers several advantages over living cells due to the sensitivity of living cells in adverse condition such as toxicity effect of metals concentration, pH and temperature and continuity in nutrient supply (Kapoor and Viraraghavan, 1995). It can be regenerated and reused for number of