REMOVAL OF HEAVY METAL IONS FROM AQUEOUS SOLUTIONS ONTO BIOMASS OF *ASPERGILLUS NIGER*.

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A project report submitted in partial fulfilment of the
requirements for the award of the degree of
Bachelor (Hons.) of Chemical Engineering

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for any other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled **“REMOVAL OF HEAVY METAL IONS FROM AQUEOUS SOLUTIONS ONTO BIOMASS OF *ASPERGILLUS NIGER*”** was prepared by **CHEONG HUI MIN** has met the required standard for submission in partial fulfilment of the requirements for the award of Bachelor of ENGINEERING (Hons.) CHEMICAL ENGINEERING at Universiti Tunku Abdul Rahman.

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Specially dedicated to
my beloved family.

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ABSTRACT

There is a need to develop cost-effective technologies that can remove toxic heavy metals ions such as lead from wastewater. In this study, the biosorption characteristic of *Aspergillus niger* (*A.niger*) fungal biomass on lead, Pb(II) removal was examined as a function of initial pH and metal ion concentrations. Dried biomass of *A.niger* was used in the study. The equilibrium data obtained were analysed using both Langmuir and Freundlich isotherm models and examined using non-linear regression analysis. The results showed that the equilibrium data fitted well to both Langmuir and Freundlich models except at pH 2 and pH 3, respectively. The pH of the metal ion solutions strongly affected the degree of heavy metal ions on the dried biomass. Biosorption of metal ions was inhibited at pH values below 3 whereas the adsorption capacity increased sharply when the pH values increased from 5 to 6. The maximum adsorption uptake was found to be 66.23 mg/g at pH 6. The percentage removal of lead ions by *A.niger* increased when the initial concentration of metal ions solution increased from 5 to 200ppm. The kinetic study of biosorption of heavy metal ions by *A.niger* presents the Ho and McKay' pseudo-second order adsorption with a correlation coefficient of 0.99 which also described a chemisorption happened during the metal ion uptake mechanism. The results of this study indicated that *A.niger* is a suitable biosorbent for the removal of Pb(II) ions from aqueous solution.

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LIST OF SYMBOLS / ABBREVIATIONS

M	molarity, mol/L
M	amount of dry biosorbent, g
T	temperature, ℃
C_e	equilibrium ion concentration, mg/L
C_i	initial ion concentration, mg/L
C_f	residual concentration, mg/L
k	rate constant of pseudo-first order
k_2	rate constant of pseudo-second order
K_F	Freundlich constant
K_L	Langmuir constant
q/Q	metal uptake rate, mg/g
Q_e	metal uptake at equilibrium, mg/g
Q_{max}	maximum adsorption capacity, mg/g
Q_t	metal uptake at time t, mg/g
V	reaction volume, L
Θ	surface sites
ICP	inductively coupled plasma

INTRODUCTION

1.1 Biosorption prospects

The history of research based on the biomass-metal interactions is dated back to the 1960s (Chojnacka, 2010). It was discovered that the metal's properties can be altered by biomass in an amazing way. The processes of biological metal binding were found to be useful for biohydrometallurgy and biogeochemistry.

In the next ten years after the discovery, the first description of bioaccumulation as the method of wastewater treatment was elaborated. It was reported by Chojnacka (2010) that in the lagoon in the vicinity of lead and zinc mining and milling works a dense mat-like growth of algae was observed. The observation also proved that the algae were capable of accumulating metal cations and eliminating other competing organisms.

According to Gupta et al. (2000), biosorption was regarded as an emergent technology at a meeting organized by the Solvent Engineering Extraction and Ion Exchange Group of the Society of Chemical Industry at UK in year 1986. Since then a number of research centre from all over the world have been engaged in the area ofbiosorption with precise goals of identifying potential biomass in the removal of heavy metal from industrial wastewater (Volesky & Holan, 1995).

The history of biosorption as a regular branch of science began in 1990's. A large contribution has been made by Prof. Bohumil Volesky from McGill University in Canada in providing many theoretical basis of the biosorption process. Biosorption can be regarded as an alternative cleanup process because it is cost-effective in terms of the source of biosorbents, feasible desorbing process for the reuse and regeneration of biomass and other properties (Volesky, 2007).

Many biological materials bind heavy metals; however, the implementation of a full-scale biosorption process needs only sorbents with sufficiently high metal binding capacity and selectivity. Thus, the first major challenge for the biosorption field is to select the most promising types of biomass from an extremely large pool of readily available and inexpensive biomaterials.

1.1.1 The achievement over the past decades

The researches throughout the years have allowed the biological materials with high metal binding capacity and selectivity for heavy metals to be utilized in the full-scale biosorption process (Kratochvil & Volesky, 1998).

According to Volesky (2007), several proprietary biosorption processes were developed and commercialized early in this decade, such as AlgaSORB™ and AMT-Bioclaim™ (Goyal & Ahluwalia, 2007), however, a lack of better understanding of the mechanism underlying the metal sorption process has hindered adequate assessment of process performance and limitations and thus the expected wide-spread application of biosorption. Consequently, the selection of the industrial effluents for pilot testing has remained largely intuitive as has the task of scaling up the process.

1.2 Objectives

The main objective of the present project is to investigate the use of *A. niger* dry biomass as a biosorbent for the removal of lead, Pb(II) from aqueous solutions by using batch method. The optimum biosorption conditions are determined as a function of initial pH, initial metal ion concentration and time.

The specific objectives are as below:

i. to evaluate adsorption capacity of dry biomass of *A.niger* to remove lead ions from aqueous solutions.
i. to evaluate the effect of pH on the metal uptake by *A.niger.*
ii. to evaluate the effect of initial concentration on the adsorption capacity of *A.niger*.
iii. to determine the kinetics of the biosorption process for *A.niger*.

CHAPTER 2

LITERATURE REVIEW

2.1 Presence of heavy metals in environment

Metal compounds are released into the environment through industrial activities. Throughout the world there is a growing concern that the heavy metal contents in soils and water are increasing as the result of industrial, mining, agricultural and domestic activities.

The man’s exploitation of the world’s mineral resources and the technological activities involved along the process tend to unearth, dislodge, and disperse chemicals and particularly metallic elements, which have recently been brought into the environment in unprecedented quantities and concentrations and at extreme rates. The pollutant caused by man-made sources could lead to disastrous effects on animals and humans.

There are many sources of water pollution, but two main general categories exist: direct and indirect contaminant sources. Direct sources include effluent outfalls from industries, refineries and waste treatment plants; whereas, indirect sources include contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water. In general, contaminants come under two broad classes which are organic and inorganic. Some organic water pollutants include industrial solvents, volatile organic compounds, insecticides, pesticides and food processing wastes. The inorganic water pollutants on the other hand include metals, fertilizers and acidity caused by industrial discharges. The heavy metal pollutants can be caused by both direct and indirect sources.

According to Naja & Volesky (2009), heavy metals can be defined in several ways. One possible definition is the following: heavy metals form positive ions in solution and they have a density five times greater than that of water. They are of particular toxicological importance. Many metallic elements play an essential role in the function of living organisms; they constitute a nutritional requirement and fulfil a physiological role. However, over abundance of the essential trace elements and particularly their substitution by nonessential ones, such as the case may be for cadmium, nickel, or silver, can cause toxicity symptoms or death.

Apart from this, heavy metals are difficult to be removed from the environment due to their toxic effects. The toxic effects of heavy metals are mainly result from the interaction of metals with proteins (enzymes) and inhibition of metabolic processes in biosystems. The mobilized metals tend to persist indefinitely, circulating and eventually accumulating throughout the food chain. In other words, these toxic metals are not metabolically degradable and accumulate in living tissues which impose serious threats on environment, animals and humans at a significant severity.

Furthermore, when considering the environmental impact of mobilized metals, the “big three”: mercury, lead and cadmium are in the limelight (Volesky & Holan, 1995).The “Big Three” metals are known for their high toxicity and environmental impact. Lead is used very extensively in many industries such as production of electrical accumulations and batteries, gasoline industries as alkyl additives and has many other usages (Akar & Tunali, 2006). It is the most common of heavy elements (Naja & Volesky, 2009) in the league of heavy metals. It has also been cited as one of the three most toxic heavy metals that have latent long-term negative impacts on health, causing anaemia, encephalopathy, hepatitis and nephritic syndrome (Lesmana et al., 2009). Lead can be absorbed through inhalation, ingestion, dermal contact (mainly as a result of occupational exposure), or transfer via the placenta. It can severely affect the central nervous system depending on the severity measured in acute and chronic intoxication.

Despite of reducing the exposure to lead from the point sources, removal of lead containing compounds from the industrial effluents as well as drinking water are therefore mandatory to protect living organisms. According to the National Primary Drinking Water Regulations, the allowable lead concentration in drinking water is only 0.015mg/L (EPA). Therefore, the mobilized heavy metal ions must be removed from the environment to minimize the impact of its toxicity to the living organisms.

Removal of the excesses of heavy metal ions from wastewater is essential because water is the most valuable natural resource existing on our planet Earth as without water, the life on the Earth would be non-existent. Although this fact is widely recognized, pollution of water resources is a common occurrence. In particular, potable water has become greatly affected, and in many instances has lost its original purpose.

2.2 Conventional technologies in metal ion removal

Before the discovery of biosorption mechanism in the removal of metal ions, there are other various existing technological processes such as adsorption, precipitation, coagulation, ion-exchange, cementation, electro-dialysis, electro-winning, electro-coagulation and reverse osmosis have been developed and used to mitigate the heavy metal pollution problems.

However, the conventional methods such as chemical precipitation and electrochemical treatment are ineffective and expensive, especially when metal ion concentration in aqueous solution is in the range of 1 to 100 mg/L. Apart from the economic point of view, the major disadvantages of conventional cleanup methods is the production of sludge which lead to the problem of sludge disposal (Kapoor & Viraraghavan, 1995). The following table depicts the advantages and disadvantages of conventional methods and principles applied.

Table 2.1 Conventional metal removal technologies. (Source: Volesky, 2000)

Method	Disadvantages	Advantages
Chemical precipitation and filtration	Used for higher metal concentrations Resulting sludge	Simple Inexpensive
Chemical oxidation or reduction	Significant chemical input Slow mechanism rate Climate sensitive	Organic substance becomes impregnated by inorganic substances
Electrochemical treatment	Used for high metal concentrations Expensive	Metal recovery
Reverse osmosis	High energy input Plugging of membrane Expensive	Pure effluent (recycle)
Ion exchange	High dependence on particles' affinity Expensive resins	Effective Pure effluent (recycle)
Evaporation	High energy input Resulting sludge	Pure effluent (recycle)

Amongst the cleanup technologies listed in Table 2.1, the ion-exchange resin is the only option for remediation with least impact on the ecology system but it is not economically friendly as the resins used are expensive. Meanwhile, the increasing demand of eco-friendly technologies for waste-treatment, it has led to the search of low-cost alternatives to replace the conventional methods (Wang & Chen, 2006).

2.3 Biosorption technology

In recent years, environmental pollution has arisen due to the presence of heavy metals in aqueous waste streams from many industries such as metal plating, smelting, mining, pigment and metallurgical industries (Volesky, 1990). While environment and living organisms are at stake with severe exposure to metal toxic, numerous research have been done in many institutes for the discovery of new biosorbents in the heavy metal ions removal at principle virtues of low cost but good kinetics of biosorption mechanism (Romera et. al., 2008).

Biosorption is known as a property of certain types of inactive, non-living microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solution from 1 to 100 mg/L (Kapoor & Viraraghavan, 1995). Biosorption is the utilization of biomass that exhibits simple physicochemical process resembling conventional adsorption or ion exchange with the difference in the nature of sorbent which in this case is the material of biological origin (Chojnacka, 2010). Thus, microorganisms such as fungi, bacteria, yeast and algae with their metal-sequestering properties can be used to decrease the concentration of heavy metals ions in water stream from parts per million, ppm, to parts per billion, ppb level making them to be ideal candidates for the treatment of high volume and low concentration complex wastewaters (Kapoor & Viraraghavan, 1995) to serve as an alternative to conventional methods used for heavy metal removal.

Besides this, biosorption is also defined as a process that utilizes inexpensive dead biomass to sequester toxic heavy metals. Research in the area of biosorption suggests it as an ideal alternative for decontamination of metal containing effluents (Goyal & Ahluwalia, 2007). This is because the reported results are convincing and binding capacities of certain biomass is comparable with the commercial synthetic cation exchange resins (Goyal & Ahluwalia, 2007).

Biosorption is a relatively rapid process and it can be reversible. According to Dursun (2006), biosorption always happens in dual-stages where the first stage involves a physicochemical interaction between the metals and functional groups such as ketones, aldehydes, carboxyls present on the microorganism’s cell surface, based on physical adsorption, ion exchange, complexation and precipitation process. It is often followed by a slower metal binding process in which additional metal ions are bounded, often irreversibly. The slow phase of metal uptake can be due to a number of mechanisms, including covalent bonding, surface precipitation, redox reactions, crystallization on the cell surface or, most often, diffusion into the cell interior and binding to proteins and other intracellular sites. Table 2.2 tabulated some important facts on biosorption.

Table 2.2 Biosorption factors. (Source: Chojnacka, 2010)

Factor	Effect
Functional groups	Participation of functional groups in biosorption depends on: i. The concentration and the type of the group in the biomass ii. The accessibility of the group iii. The chemical state of the site (e.g. availability) iv. The affinity between site and metal (binding strength)
pH	i. Affects protonation (availability) of metal ions binding sites and ionic state of the sorbate in the solution ii. At low pH (high level of H^+), anionic sites become protonated iii. Metal cations can be eluted by acidic wash (e.g. regeneration, multiple reuse, better economy)

In the earlier studies of biosorption phenomenon, it has been found that the uptakes of heavy metal cations by most biomass types decrease dramatically as the pH of the metal solutions decreases from pH 6 to 2.5(Sag & Kutsal, 2001). A hypothesis of ion exchange between protons and heavy metals was formulated based on the experiments performed in a closed batch system without pH adjustment where the sorption of heavy metals onto acid washed biomass led to a decrease of the pH in the liquid were shown (Kratochvil & Volesky, 1998). Thus, the biosorption mechanism based on principle of ion exchange is well established.

Advantages and disadvantages of biosorption

Recent studies on metal biosorption have largely confirmed the sorption potential of a vast variety of fungal and other biomass types as biosorbents. To date, biosorption has been regarded as an effective technology for the removal of soluble heavy metals from aqueous solution (Yu & Ya-Juan, 2008). However, there are usually no absolute advantages without some drawbacks exist in a process or mechanism of biosorption.

Goyal and Ahluwalia (2007) states that non-living biomass is not subjected to toxicity limitation of cells thus the process is not governed by the physiological constraint of living microbial cells. Meanwhile, there will be no requirement of costly nutrients to supplement for the growth of cells in feed solutions. Apart from the issue of supplying nutrients, dead biomass is also possible to be operated under wider range of operating condition such as pH, temperature and metal concentration. Besides, biomass can be procured from the existing fermentation industries, which in turn minimize the cost of cultivation of certain strain of microbes.

Biosorption process of non-living biomass is metabolism independent, thus the process is usually rapid and equilibrium can be reached between few minutes to hours so the metal uptake, q is usually efficient due to higher metal loading sites. The advantages of biosorption by non-living biomass can be compared with the bioaccumulation as compiled in Table 2.3. However, one significant drawback of using dead biomass is due to the constraint of improving the biological process through cell engineer because the cells are not metabolizing.

2.4 Biosorption versus bioaccumulation

There are typically two biological processes which has both similarities and differences: biosorption and bioaccumulation in the removal of heavy metal ions. Both of these mechanisms have several distinguished characteristics on its capabilities of removing heavy metal ions from aqueous solutions.

According to Chojnacka (2010), biosorption and bioaccumulation mechanisms involve interactions and concentration of toxic metals or organic pollutants in the biomass, either living (bioaccumulation) or non-living (biosorption). In other words, bioaccumulation mechanism happens with living microorganisms while biosorption is associated with non-living microorganisms. Therefore, it is ultimately important to differentiate the characteristics of biosorption and bioaccumulation.

Table 2.3 The comparison between biosorption and bioaccumulation. (Source: Chojnacka, 2010)

Biosorption	**Bioaccumulation**
Passive process	Active process
Biomass is not alive	Biomass is alive
Metals are bound with cellular surface	Metals are bound with cellular surface and interior
Adsorption	Absorption
Reversible process	Partially reversible process
Nutrients are not required	Nutrients are required
The rate is quick	The rate is slow
Not controlled by metabolism	Controlled by metabolism
No danger of toxic effect	Danger of toxic effects caused by contaminants
No cellular growth	Cellular growth occurs
Intermediate equilibrium concentration of metal ions	Very low equilibrium concentration of metal ions

Table 2.3 shows that both bioaccumulation and biosorption exhibit different properties. However, the use of dead fungal biomass is more advantageous than the use of live biomass because there are no toxicity concerns, no requirements of growth media or nutrients and techniques to desorb metal ions from the biomass for the regeneration purpose are stated (Jianlong et. al., 2001).

2.5 Mechanism of metal uptake

The understanding of the mechanisms by which microorganisms accumulate metals is crucial to the development of microbial processes for concentration, removal and recovery of metals from aqueous solution. According to Kapoor and Viraraghavan (1995), the uptake of heavy metals by biomass can take place by an active mode (dependent on the metabolic activity) known as bioaccumulation or by a passive mode. This passive mode is independent of metabolic activity and thus this type of mechanism only happens to cell walls and external surfaces in the case of non-living biomass. Metabolism-independent uptake essentially involves chemisorptions such as ionic exchange, complexation and/or chelation, physical adsorption, micro-precipitation and oxidation or reduction (biosorption).

Apart from that, the concentration gradient and diffusion through cell walls and membranes also causes ion entrapment in inter- and intra-fibrillar capillaries and spaces of the structural polysaccharide network (Volesky & Holan, 1995). Thus, the identification of microorganisms' functional groups involved in the biosorption process is important upon exploring the mechanism of metal uptake.

The cell wall structure of certain algae, fungi and bacteria was found to be particularly responsible for biosorption mechanism. The several chemical groups that could attract and sequester the metals in biomass are: acetamido groups of chitin, structural polysaccharide of fungi, amino and phosphate groups in nucleic acids, amino, amido, sulfhydryl, and carboxyl groups in proteins, hydroxyls in polysaccharides, and mainly carboxyls and sulphates in the polysaccharides of marine algae (Volesky & Holan, 1995). Volesky (2007) also stated that the amine, NH_2 group that is active and ubiquitous in fungal cell walls are also capable of sequestering anions.

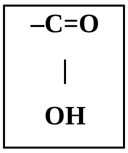
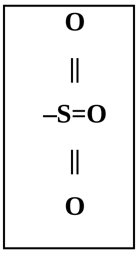
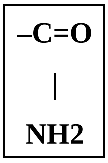
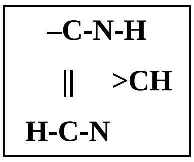
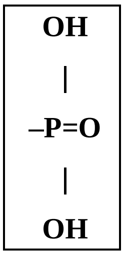
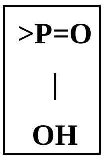
Apart from the functional groups of fungal and algae mentioned above, bacteria have quantities of peptidoglycan (Gram "+") and teichoic acid (Gram "-") in their cell walls. All of these components featuring an important ion exchange active groups in the structure (Treen-Sears, Volesky, & Neufeld, 1984). Thus, these constituents stated have contributed to the discovery of the active chemical groups are involved in the metal binding mechanism.

Both living and dead fungi are able to remove heavy metal ions from aqueous solutions. The metal uptake by dead cells occurs as a result of reaction with the functional groups present in cells specifically in the cell wall. According to (Kapoor & Viraraghavan, 1995), the metal uptake by the cell wall has been broadly based on two mechanisms: uptake directed by functional groups like phosphate, carboxyl, amine and phosphate diesther species of these compounds. The second up mechanism results from physicochemical inorganic interactions directed by adsorption phenomena.

The mechanism of uptake depends on the type of removal: radionuclides results from the combination of the above two processes. However, the removal of heavy metal ions is mainly affected by one of the processes; where the uptake is directed by functional groups which agree with literatures stated above. Meanwhile, the efficiency depends on the capacity and affinity including physico-chemical nature (Goyal & Ahluwalia, 2007) which is contributed by the environmental factors such as the solution pH, temperature and initial metal ions concentration (Vijayaraghavan & Yun, 2008).

These functional groups mainly consist of several types of ionisable sites including carboxyls, amines, hydroxyls, phosphates and sulfhydryls (Viraraghavan & John Peter, 2008).The major binding groups for biosorption are shown in Table 2.4. The table also depicted that the functional groups with different values of pK_a that are indicative of the binding properties of a given group.

Table 2.4 **Functional groups of biosorbents.** (Source: Volesky, 2007)

Binding group	Structural formula	pK_a	Ligand atom	Occurrence in selected biomolecules
Hydroxyl	–OH	9.5-13	O	Polysaccharides, Uronic acid, Sulfated PS
Carbonyl (ketone)		-	O	Peptide bond
Carbonyl	 $\begin{array}{c}\mathbf{-C=O}\\ \vert\\ \mathbf{OH}\end{array}$	1.7-4.7	O	Uronic acids, Amino acid
Sulfhydryl (thiol)	–SH	8.3-10.8	S	Amino acid
Sulfonate	 $\begin{array}{c}\mathbf{O}\\ \Vert\\ \mathbf{-S=O}\\ \Vert\\ \mathbf{O}\end{array}$	1.3	O	Sulphated PS
Thioether	>S	-	S	Amino acid
Amine	$-NH_2$	8-11	N	Chitosan, Amino acid
Secondary anime	>NH	13	N	Cti, PG, Peptide bond
Amide	 $\begin{array}{c}\mathbf{-C=O}\\ \vert\\ \mathbf{NH2}\end{array}$	-	N	Amino acid
Imine	= NH	11.6-	N	
Imidazole	 $\begin{array}{ll}\mathbf{-C\text{-}N\text{-}H} & \\ \quad\Vert & \mathbf{>CH}\\ \mathbf{H\text{-}C\text{-}N} & \end{array}$	12.6 6.0	N	Amino acid
Phosphonate	 $\begin{array}{c}\mathbf{OH}\\ \vert\\ \mathbf{-P=O}\\ \vert\\ \mathbf{OH}\end{array}$	0.9-2.1 6.1-6.8	O	Phospholipids
Phosphodiester	 $\begin{array}{c}\mathbf{>P=O}\\ \vert\\ \mathbf{OH}\end{array}$	1.5	O	Teichoic acid LipoPS

The mechanism of metal binding is not well understood yet due to the complex nature of microbial biomass, which is not readily amenable to instrumental analysis. However, there is a breakthrough of using spectroscopic techniques such as Infrared Spectroscopy (IR), Raman, Electron Dispersive Spectroscopy (EDS), X-ray Photoelectron Spectroscopy (XPS), Electron Microscopy (Scanning, Transmission), Nuclear Magnetic Resonance (NMR) and X-ray Diffraction Analysis (XRD) to enable identification of biosorptive sites which are also known as functional groups. According to Gupta et. al., (2000), instrumental analysis is a relatively new technique for determination of binding energy of electrons in atoms/molecules which depends on the distribution of valence charges and thus gives information about the oxidation state of an atom/ion.

Fourest et. al., (1996) stated that the properties of a biosorbent can be obtained by using simpler techniques such as titration which enables to determine acidic disassociation constants and also metal affinity constants. These properties play a major role in describing the selectivity of the biomass for different metal ion (Chojnacka, 2010).

2.6 Fungi

2.6.1 Classification and general characteristics

Microscopic fungi include yeasts with spherical budding cells and molds with elongated filamentous hyphae in mycelia. The molds are filamentous fungi, such as *Penicillium*, *Aspergillus* and *Candida*. The body or vegetative structure of a fungus is called thallus, which varies in complexity and size from single cell microscopic yeasts to multicellular molds. A single filament is called a hypha. Hyphae usually grow together, collectively called a mycelium (Prescott, Harley & Klein, 2002). The classification of fungi is shown in Table 2.5.

Table 2.5 The classification of fungi. (Source: Wang & Chen, 2006)

Group	Common Name	Hyphae	Typical Representative
Ascomycetes	Sac fungi	Septate	*Neurospora* *Saccharomyces* *Morchella*
Basidiomycetes	Club fungi, mushroom	Septate	*Amanita* *Agaricus*
Zygomycetes	Bread molds	Coenocytic	*Mucor* *Rhizopus*
Oomycetes	Water molds	Coenocytic	*Allomyces*
Deuteromycetes	Fungi imperfecti	Septate	*Penicillium* *Aspergillus* *Candida*

Most fungi are filamentous. The hyphae are typically 5-10 μm wide but may vary from 0.5 μm to 1.00 *mm*, depending on the species (Lester & Birkettn, 1999). The mycelium is composed of a complex mass of filaments or hyphae while the hyphae have walls which are composed of cellulose and/or chitin as depicted in Figure 2.1. A common cytoplasm exists throughout the hyphae. Thus fungi cellular organization has three types. The types of fungi cellular organization is tabulated in Table 2.6. Generally, the principle of classification for fungi is mainly based on the type of sexual spores.

Table 2.6 Cellular organization in filamentous fungi.
(Source: Lester & Birkettn, 1999)

Cellular organization	Description
Coenocytic	Hypha contains a mass of multi-nucleate cytoplasm which also known as aseptate.
Septate with uni-nucleate protoplasts	Hypha is divided by crosswalls or septa, each compartment containing a single nucleus.
Septate with multi-nucleate protoplasts between septa	Central pore in the septum connecting the cytoplasm of neighbouring cells and permitting the migration of both cytoplasm and nuclei.

2.6.2 Cell wall and its main composite

Various polysaccharides are the main constituents of the fungal cell wall, typically in the range of 80-90% constituent. The polysaccharides are often complexes with proteins, lipids, polyphosphates, and inorganic ions, making up the wall-cementing matrix (Sag & Kutsal, 2001).

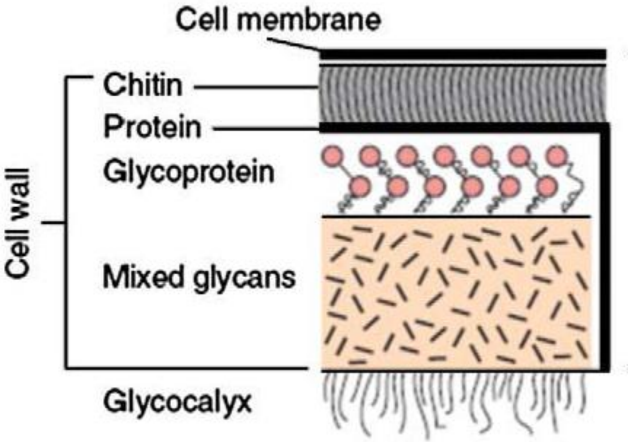


Figure 2.1 The cell wall composition of fungi.
Source: (Kapoor & Viraraghavan, 1995)

Figure 2.1 shows the general structure of cell wall and other features of the cross section of a fungal cell. It can be seen that the fungal cell wall is rigid and structurally composed of complex layers of polysaccharides and protein. In general, the fungal cell wall can be regarded as a two-phase system consisting of the chitin skeleton framework embedded in an amorphous polysaccharide matrix (Wang & Chen, 2009).

2.6.3 Fungal biomass as biosorbent

Gadd (1986) mentioned that fungi and yeasts able to accumulate micronutrients such as Cu, Zn and Mn, and non-nutrient metals like U, Ni, Cd, Sn and Hg in amounts higher than the nutritional requirement. Besides, the potential of fungal biomass asadsorbents for the removal of heavy metals and radio nuclides from polluted waters were regonized by Shumate et. al., (1978) and Jilek et. al., (1975). Azab and Peterson (1989) have shown in their studies that fungi outperformed activated carbon and ion exchange resins for cadmium removal from aqueous solutions (Viraraghavan et. al., 2008).

Meanwhile, fungi are also recognized for their ability to produce a large variety of extra cellular proteins, organic acids, enzymes and other metabolites, and their used biomass may be used as effective biosorbent material for removal, reduction and detoxification of industrial effluent composition (Tsekova et. al., 2010).

Various fungal species under the genus *Aspergillus*, *Penicillium* and *Rhizopus* have been shown to be effective in biosorption of heavy metals from polluted effluents both as immobilized cells and in the mobilized state (Tsekova et. al., 2010). There are reports that fungi belonging to the *Rhizopus* and *Penicillium* have already been studied as potential biomass for removal of heavy metals from aqueous solution. Meanwhile, fungus of *Aspergillus* species has also been studied with different influential parameters vastly in the research since decades (Akar & Tunali, 2006; Dursun, 2006; Tsekova et. al., 2010).

2.6.4 *Aspergillus niger*

In this study, *A. niger* is the biosorbent of interest. It is a filamentous ascomycete fungus and it is one of the most common species in the genus *Aspergillus* (Lesmana et al., 2009). It is an important microorganism in biotechnology applications (Bapat, Kundu & Wangikar, 2003).

A.niger has been extensively used in processes of organic acid production such as citric acid and oxalic acid due to its ability of organic acids excretion in abundant amount. Citric acid and several enzymes produced by *A.niger* are considered GRAS (generally regarded as safe) by the United States Food and Drug Administration (FDA).

About 30% of the *A. niger* biomass is comprised of an association of chitin and glucan. Chitin and chitosan components of the cell wall are considered to be important in metal uptake. In a review of fungal biosorption done by Kapoor and Viraraghavan (1995) the chitin and chitosan contents of the fungal cell wall can change during growth of mycelia and this can account for the variations in the metal-uptake capacity with the cell age. The interaction of metals with protein is well known and can also be involved in biosorption of metals while *Aspergillus* species have been shown to absorb heavy metals from aqueous solutions (Viraraghavan & John Peter, 2008).

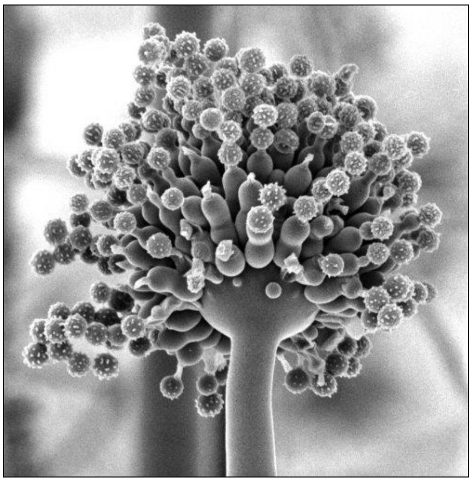


Figure 2.2 Scanning electron micrograph, SEM of the asexual reproductive apparatus of *Aspergillus niger*. Source: (Read, 2007)

Efforts have been made to determine the sorption capacity of fungal biomass of species *A.niger* Table 2.7 summarizes some of the biosorption capacity of *A.niger* in different type of conditions on various metal ions

Table 2.7 Biosorption capacity of *A. niger* (mg/g) on metal ions.

Condition	Metal ions	Biosorption capacity (mg/g)	Reference(s)
Pretreated with NaOH	Cu	28.7	Dursun (2006)
	Pb	32.6	Dursun (2006)
	Cu	25.5	Dursun (2003)
	Pb	28.9	Dursun (2003)
	Pb	7.24	Kapoor et al. (1999)
	Cd	3.43	Kapoor et al. (1999)
	Cu	2.66	Kapoor et al. (1999)
	Ni	0.96	Kapoor et al. (1999)
Growing	Cu	15.6	Dursun et al.(2003a)
	Pb	34.4	Dursun et al.(2003a)
Live	Pb	2.25	Kapoor et al. (1999)
	Cd	1.31	Kapoor et al. (1999)
	Cu	0.75	Kapoor et al. (1999)
	Ni	1.75	Kapoor et al. (1999)
Attached to wheat bran	Cu	-	Modak et al. (1996)
	Zn	-	Modak et al. (1996)

There is an obvious phenomena observed from the above tabulation of the biosorption capacity. Alkali treatment (pretreated with NaOH) of fungal have shown to increase significantly the metal uptake capacity compared to the rest of the *A. niger*.

CHAPTER 3

METHODOLOGY

The first stage of the project is the cultivation of fungi, *A.niger* and dried the obtained biomass of the cultivation. Then the following step is the preparation of synthetic lead solution with 0.15M of blank solution and 0.1M of stock solution, adjusted to the initial concentration and pH of interest. Then, the studies began with batch isotherm and kinetics studies for the investigation of pH and initial metal ion concentration on biosorption. Lastly, the results obtained from the studies were evaluated and analyzed by using isotherm and kinetic models.

3.1 Preparation of adsorbent

The fungus used in this project was *A. niger*. The biomass was cultivated in a liquid medium using the shake flask method. The culture medium contained the following composition: 10g/L peptone (R&M Chemicals); 20g/L sucrose (R&M Chemicals) and 3g/L yeast extract (Bacto$^{TM)}$. The medium was transferred to several 250ml Erlenmeyer flasks and autoclaved at 121 ℃ for 15mins and cooled to room temperature.

The *A.niger* spores obtained from the Department of Science in University of Tunku Abdul Rahman were inoculated into the medium and allowed to grow for a period of 7 days on an orbital shaker at 225 rpm. After seven days the harvested cells are washed several times with deionised distilled water. The biomass was then dried in oven at 85°C for four days, grounded and sieved to millimetre size of particles. The prepared dry biomass was used in all adsorption experiment of this study.

3.2 Preparation of metal ion solutions

The stock solution can be prepared using Plumbum Nitrate, (R&M Chemicals with M= 331.02 g/mol), where adequate amount of plumbum nitrate, $Pb(NO_3)_2$ is dissolved into 500ml of deionized water to obtain 0.1M of stock solution.

3.3 Preparation of blank solution

The blank solution can be prepared using Sodium Nitrate, $NaNO_3$(Merck). Blank solution is prepared by dissolving adequate amount of sodium nitrate, $NaNO_3$ into deionized water to obtain 0.15M of blank solution.

3.4 Metal ions solution

The metal ions solution of different working concentrations is prepared by diluting the 0.1M stock solution in accordance to the investigated range of concentration. The ranges of concentrations of both metal ions prepared from stock and blank solutions varied between 5 and 200ppm.

3.5 Batch biosorption studies

The effect of initial metal ions and pH on the biosorption of Pb(II) on fungal biomass was investigated in the pH range of 1 - 6 and metal ion concentration in the range of 5 to 200 mg/L. By using the 0.1M stock solution and 0.15M blank solution, 100 ml of metal ion solutions is prepared into several Erlenmeyer flask in accordance to the initial concentration of interest. The initial pH of each metal ion solution was adjusted to the required pH value by using either 0.1M of HCl or NaOH or 1M of HCl.

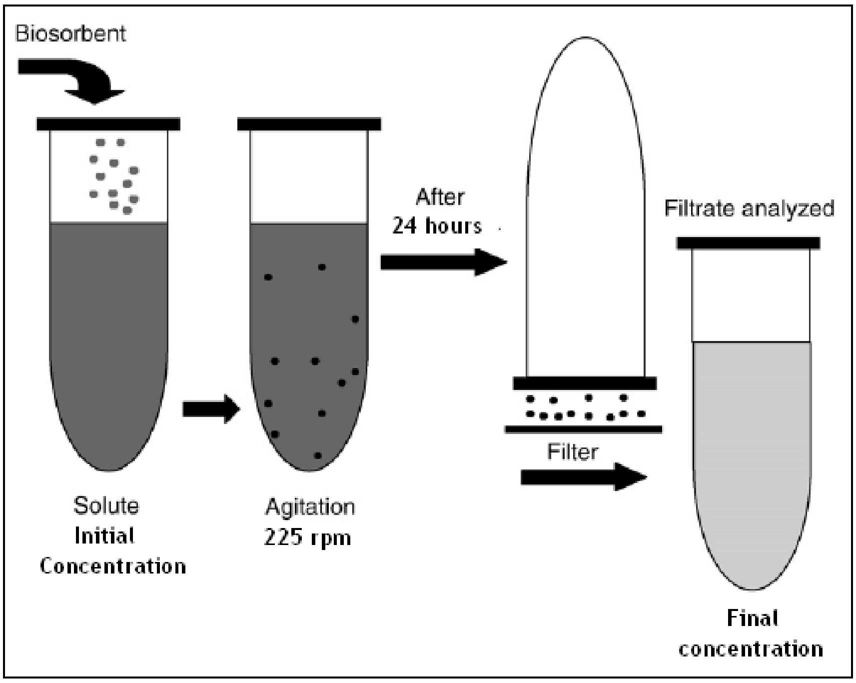


Figure 3.1 Schematic diagram of bath biosorption equilibrium study.

Figure 3.1 depicts the procedure of the batch biosorption study. Firstly, 100mg of *A. niger* dried biomass was added to each of the metal ion solution and sealed with aluminium foil. The reaction mixtures were shaken in incubator shaker at 225rpm, 25 and up to 24 hours. The content of the flasks was separated by filtration using filter paper and collected in centrifugal tubes. The final concentration, C_e of the metal ion solutions were then measured with ICP (Optical Emission Spectrometer, Optima 7000DV).

3.6 Equipments

There are several equipments used throughout the biosorption of *A. niger* from the preparation of fungal biomass, biosorption and kinetics studies and the analysis of metal ions concentrations. The figures are arranged chronologically based on the experimental steps.

Figure 3.2 Orbital shaker (SSL1;Stuart®)

Figure 3.3 Oven (Beschickung-Loading Modell 100-800;Memmert)

Figure 3.4 ICP (Optima 7000DV;Perkin Elmer, Uberlingen, Germany).

3.7 Metal uptake capacity

Volesky (2004) states that the quality of the biosorbent is judged according to the amount of metal ions it can attract and retain in an “immobilized” form. Therefore, the uptake of metal ions must be determined as the amount of metal ions bound by the unit of biosorbent and calculated from a metal mass balance yielding:

$$q = \frac{v(c_i - c_f)}{m} \tag{3.1}$$

where,

q = mg metal ions per g dry biosorbent

V = reaction volume

C_i = initial concentration (mg/L)

C_f = residual concentration (mg/L)

m = amount of dry biosorbent (g)

The efficiency of heavy metal removal was calculated from the amount of metal ions adsorbed on the biosorbent and the amount of metal ions available in the synthetic lead solutions. The percentage removal of Pb(II) ions from the synthetic lead solution can be calculated by using the equation shown below:

$$Percentage\ removal = \frac{mg\ heavy\ metal\ ion\ removal}{mg\ heavy\ metal\ ion\ available} \times 100 \qquad (3.2)$$

However, the percentage removal is an approximation that it could lead to outright misleading conclusions on the relative sorption performance. The equation for the "percentage removal" can only serve the purpose of crude orientation, perhaps adequate only for quick and very approximate screening biosorbent materials (Kratochvil & Volesky, 1998).

3.8 Biosorption isotherms

The sorption performance of the biomass can be assessed by using several isotherm models. Table 3.1 exhibits the advantages and disadvantages of the adsorption isotherms typically used in the biosorption equilibrium studies.

Table 3.1 Adsorption Isotherms. Source: (Kapoor & Viraraghavan, 1995).

Isotherm	Equation	Advantages	Disadvantages
Langmuir	$Q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e}$	Interpretable parameters	Not structured; monolayer sorption
Freundlich	$Q_e = K_F C^{1/n}$	Simple expression	Not structured; multilayered sorption
Redlich-Peterson	$q = \frac{K_{RP} C_e}{1 + a_{RP} C_e^{\beta}}$	Approaches Freundlich at higher concentrations	-
BET	$q_e = \frac{B Q^{o} C_e}{(C_s - C_e)[1 + (B - 1) C_e / C_s]}$	Multilayer sorption	-

The two widely accepted and easily linearized equilibrium adsorption isotherm models are Langmuir and Freundlich model which describe monolayer adsorption and the latter, multilayer adsorption.

3.8.1 Langmuir isotherm model

Researches on biosorption of heavy metals have mainly focus on the adsorption efficiencies and adsorption equilibrium for different biosorbent materials, where the analysis of experimental data is important to serve as a basis for the development of continuous or large scale biosorption process (Sag & Kutsal, 2001). The Langmuir adsorption isotherm has traditionally been used to quantify and contrast the performance of different biosorbents (Davis, Volesky & Alfonso, 2003).

Biosorption equilibrium is more frequently done with Langmuir than Freundlich equation, because a clear *plateau* can be distinguished on the sorption isotherms (Chojnacka, 2010). In the searching of new biosorbents, the equilibrium sorption curve is useful in identifying the better sorption performance of different biosorbents based on the fitting of data into Langmuir isotherm model.

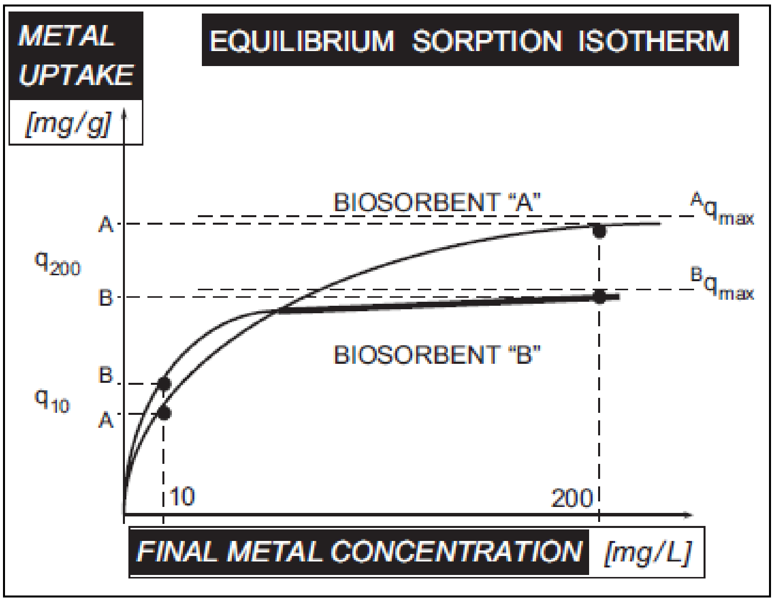


Figure 3.5 Comparative example of sorption isotherm curve.

(Source: Volesky, 2007)

A sorbent is considered as a “good” sorbent if it has a high Q_{max} and a steep initial sorption isotherm slope as indicated by low values of Langmuir parameter b (Volesky, 2004). Figure 2.3 shows that “B” performed better than “A” at lower equilibrium concentrations. The following assumptions are valid for Langmuir model:

i. Fixed number of adsorption sites; at equilibrium, at any temperature and gas pressure a fraction of the surface sites, θ is occupied by adsorbed molecules, and the fraction $1-\theta$ is free.
ii. All sorption sites are uniform (i.e. constant heat of adsorption).
iii. Only one sorbate.
iv. One sorbate molecules reacts with one active sites.
v. No interaction between sorbed species.

(Langmuir, 1918)

The equation was developed by Irving Langmuir in 1916. It is expressed in a hyperbolic form:

$$Q_e = \frac{Q_{max} K_L C_e}{1+K_L C_e} \tag{3.3}$$

The Langmuir equation can be transformed to the linear form:

$$\frac{1}{Q_e} = \frac{1}{Q_{max}} + \frac{1}{(Q_{max} K_L) C_e} \tag{3.4}$$

where,

Q_e = metal ion sorbed, mg/g

C_e = equilibrium concentration of metal ion solutions, mg/L

Q_{max} = maximum amount of metal ion which can be taken up by biosorbent, mg/g

K_L = Langmuir constant, L/mg (affinity)

(Langmuir, 1918)

3.8.2 Freundlich isotherm model

The Freundlich equation is an empirical relationship describing the adsorption of solutes from a liquid to a solid surface. In other words, it does not indicate a finite uptake capacity of the biosorbent and thus can only be applied in the low to intermediate concentration ranges. Freundlich (1907) stated that Freundlich isotherm relationship is exponential with the following general form of:

$$Q_e = K_F C_e^{1/n} \tag{3.5}$$

The Freundlich equation can be linearized by taking logarithm of both sides of the equation to give:

$$\log Q_e = \log K_F + \frac{1}{n} \log C_e \tag{3.6}$$

where,
K_F and $1/n$ = Freundlich constant
(Source: Freundlich, 1907)

3.9 Biosorption kinetic studies

Batch kinetic studies were carried out to determine the equilibrium time which is defined as the time needed to reach equilibrium (point beyond which there is very limited removal of metal).

The initial lead concentration is adjusted to 50 mg/L. Then 0.5 mg of dry fungal biomass was added to the plumbum solution in each of the 34 conical flasks of 250mL volume. The samples were placed on the rotary and orbital shaker at 225rpm. The samples were collected tentatively in a duplicate in duration of 48 hours, in the intervals of 20mins for the first hour, and then on an hourly basis. Upon collection, samples are immediately filtered through filter papers of 0.45 µm pore sized into centrifugal tubes. The final pH values of each sample were noted and the lead concentrations in the filtrates are then determined by using ICP. The kinetics studies of biosorption were usually described and analyzed with pseudo-first or pseudo-second order model (Chojnacka, 2010).

3.9.1 Pseudo-first order kinetics model

The reaction order is related with the mechanism of biosorption, which is most frequently ion-exchange or surface precipitation (metal hydroxide, sulphide or carbonate). Literatures have reported that the rate limiting step is chemisorption which involves valent forces by sharing or exchange of electrons between sorbent and sorbate. In kinetic modelling, the pseudo-first order kinetic equation which also known as Lagergren (1898) equation has the following equation:

$$\frac{dQ_t}{dt} = k_1'(Q_e - Q_t) \tag{3.7}$$

Taking the logarithm of both sides:

$$log(Q_e - Q_t) = log\, Q_e - \frac{k}{2.303} \tag{3.8}$$

where,

Q_t = amount of adsorbed ions on the biosorbent at time t,

k = rate constant of Lagergren first-order biosorption

The equation above assumes that metal cation binds only to one sorption site on the sorbent surface (Lagergren, 1898).

3.9.2 Pseudo-second order kinetics model

Ho and McKay (1999) used a pseudo-second order rate equation for a comparison of chemisorption kinetic models applied to pollutant removal on various sorbents. The pseudo-second order equation has the following equation:

$$\frac{dQ_t}{dt} = k_2'(Q_e - Q_t)^2 \tag{3.9}$$

The equation can be linearized to:

$$\frac{1}{t} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e} t \tag{3.10}$$

where,

k_2 = rate constant of second-order biosorption

The equation assumes that metal cations are bound to two binding sites on the sorbent surface (Ho & McKay, 1999).

CHAPTER 4

RESULTS AND DISCUSSIONS

This chapter presents the results of the experiments conducted using different initial lead concentrations and pH of synthetic solutions. The performance of the biosorbent is evaluated using two adsorption isotherm models, Langmuir and Freundlich. The kinetic studies were carried out based on the pseudo first-order and pseudo second-order reaction models. The effect of the two parameters: the initial lead ions concentration and pH of the metal solutions is also discussed in this chapter.

4.1 Batch isotherm studies

The equilibrium of biosorption of heavy metal was modelled using adsorption-type isotherms, Langmuir and Freundlich models, to describe the biosorption equilibrium.

The studies of isotherm of lead adsorption on the dry fungal biomass, *A. niger* were carried out by varying the initial lead ions concentration and pH of solutions with the methods stated in section 3.5 (page 35). Adsorption isotherms were plotted as a function of the adsorbed quantity per unit weight of adsorbent, $Q_{e.}$ at equilibrium and the final equilibrium concentration of residual sorbate remaining in the solution, C_e.

Figure 4.1 shows the adsorption isotherm for *A.niger* biosorption of lead ions at pH 1 - 6. The sorption isotherms depict the experimentally determined performance of biosorbent. The biosorbents’ performance in lead uptake can be assessed using the Langmuir and Freundlich isotherm to determine mechanism of monolayer or multilayer adsorption, respectively.

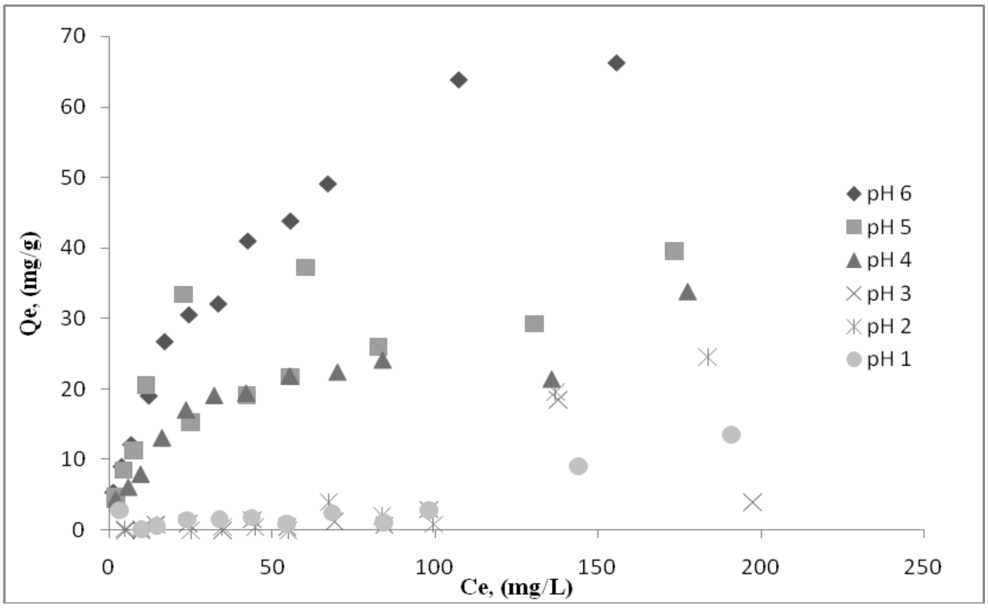


Figure 4.1 The adsorption isotherms for lead at different pHs.

There are several research studies on biosorption of metals using *A.niger* fungal biomass from year 2006 to 2008. Table 4.1 shows the adsorption equilibrium models applied for the biosorption processes in these studies. Dursun (2006) had demonstrated that the adsorption isotherms of *A. niger* to remove Pb(II) was of a saturated type kinetic model where the experimental data fitted to three different adsorption isotherms namely, Langmuir, Freundlich and Redlich-Peterson. The latter, Redlich-Peterson isotherm model is normally proposed as an isotherm compromising the features of the Langmuir and Freundlich isotherms (Yu & Ya-Juan, 2008).

Table 4.1 Equilibrium and kinetic studies of heavy metal adsorption onto *Aspergillus niger*. (Source: Lesmana et. al., 2009)

Biosorbent	Heavy metal	Adsorption Equilibria	Adsorption kinetic	Reference
A. niger	Cu(II) Pb (II)	Langmuir, Freundlich & Redlich-Peterson	Saturation type kinetic model	Dursun et. al., (2006)
	Cr(VI)	Langmuir & Freundlich	Pseudo-first & pseudo-second order	Mungasavalli et. al., (2007)
	Cr(VI)	Langmuir & Freundlich	-	Kumar et. al., (2008)

Isotherm models provide information on the biosorbent uptake capabilities and also reflect the equilibrium behaviour. Langmuir isotherm includes parameter K_L which relates to the energy of adsorption. K_L value is obtained from the reciprocal of *b* value, which represents the affinity of the biosorbent towards the heavy metal ions.

Meanwhile, Freundlich isotherm is an indication of a finite uptake capacity of the sorbent (Volesky & Holan, 1995). The magnitude of K_F and *n* shows the ease of uptake of heavy metal ions from an aqueous solution and high adsorption capacity (Akar & Tunali, 2006). In a comparative study by Dursun (2006), K_F was used as a relative measure of adsorption capacity while *n* was related to intensity of adsorption.

The Langmuir constant, K_L and maximum adsorption capacity, Q_m were obtained from the intercept and slope of Figure 4.2 and 4.3. The results showed that the maximum adsorption happened at pH 6, and it decreased as the pH of lead ion solutions decreased. The K_L values obtained from the Langmuir isotherm models indicated that the affinity of biosorbent towards lead ions increased when pH increased from 1 to 6.

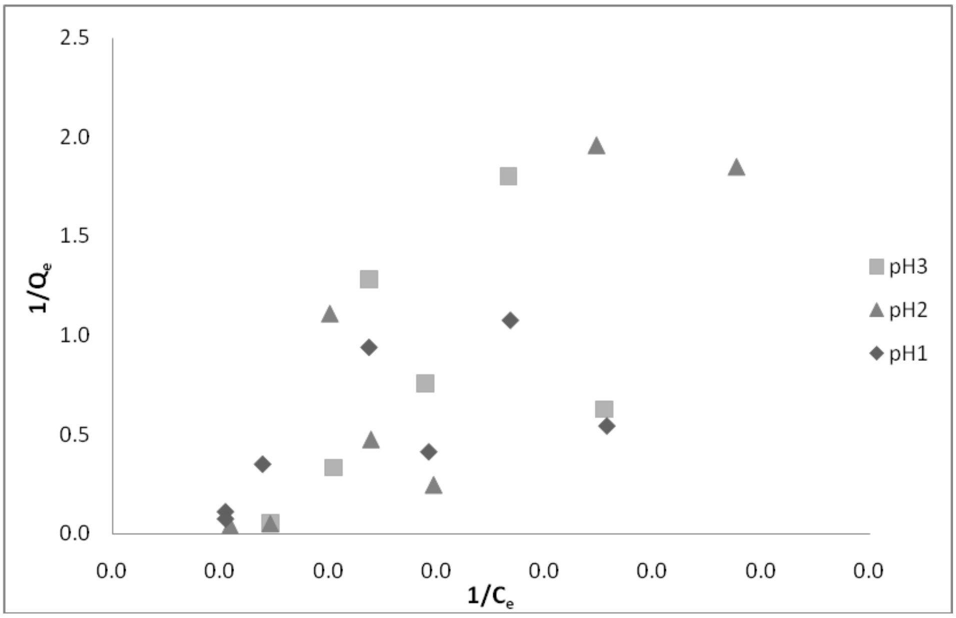


Figure 4.2 Langmuir isotherms for lead removal at pH 1 – 3.

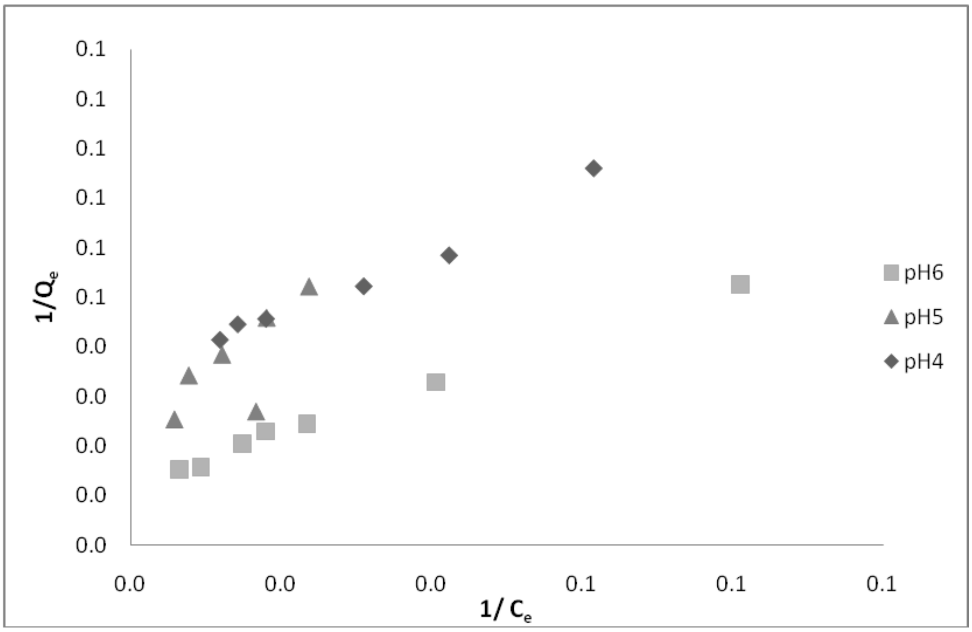


Figure 4.3 Langmuir isotherms for lead removal at pH 4 – 6.

The Freundlich constants K_f and n were calculated from the intercept and slope of plot $ln\ Q_e$ versus $ln\ C_e$ respectively, as shown in Figure 4.4 and 4.5. These values were listed in Table 4.2 with their correlation coefficients, R^2. The constant n values (pH 3 – 6) obtained for Freundlich isotherm were above 1.0, which indicated that Pb(II) ions are favourably adsorbed by the biomass at these pH values (Dursun, 2006).

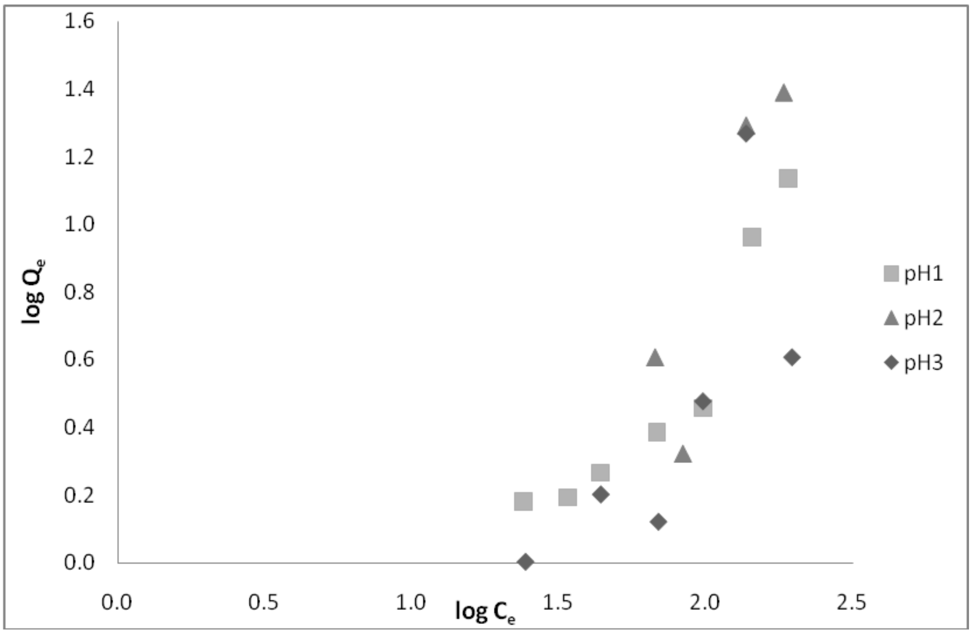


Figure 4.4 Freundlich isotherms for lead removal at pH 1 – 3.

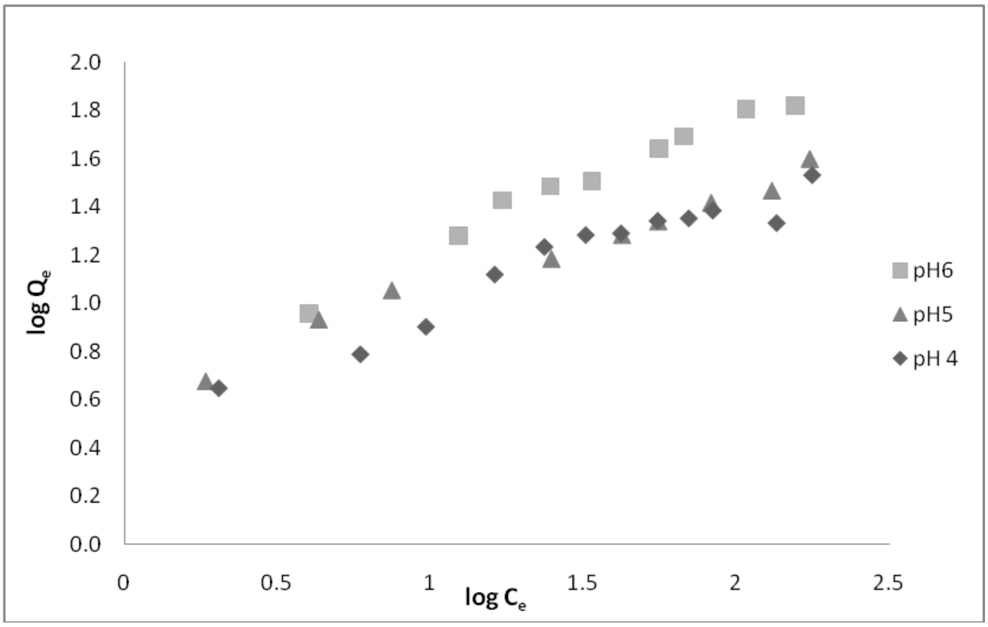


Figure 4.5 Freundlich isotherms for lead removal at pH 4 – 6.

The experimental data obtained in this study were well fitted into both Langmuir and Freundlich isotherm models, except at pH 2 and pH 3. This study's analysis agreed with the study by Dursun et. al., (2006) describing the biosorption process involving both monolayer and multilayer mechanisms.

Table 4.2 Langmuir and Freundlich isotherm model constants.

pH	Langmuir isotherm			Freundlich isotherm		
	Q_{max}, (mg/g)	K_L	R^2	K_F	n	R^2
1.0	-	-	0.97	-	0.757	0.88
2.0	0.10	0.11	0.69	-	0.831	0.83
3.0	18.59	20.41	0.84	-	1.118	0.38
4.0	23.98	26.34	0.99	0.871	1.780	0.93
5.0	31.65	34.75	0.96	0.469	2.410	0.97
6.0	66.23	72.73	0.99	0.450	1.833	0.97

4.2 Batch kinetic studies

The kinetics of heavy metal adsorption was modelled using pseudo first-order and pseudo second-order equations described in details in section 3.9 (page 41). According to Yu & Ya-Juan (2008), pseudo first-order and second-order kinetic equations have been widely used to describe time evolution of biosorption under non-equilibrium conditions. The criterion in the determination of the adequacy of kinetic model is based on the value of the correlation coefficient, R^2 of the plot (Ahmed & Mohammed, 2008).

The reaction rate can be obtained by plotting a linear plot of $ln(Qe - Qt)$ against *t*. The slope obtained is the rate constant of Lagergren first-order biosorption, *k* from equation 3.8 (page 42).

Meanwhile, the rate constant of second-order biosorption, k_2 can be obtained from the slope of curve plotted with t/Q_e versus t.

The parameters of the kinetics models (pseudo-first, pseudo-second) with their correspondent coefficients of determination are calculated from the slopes and intercepts of the linear plot of these models as shown in Figure 4.6 and 4.7, respectively.

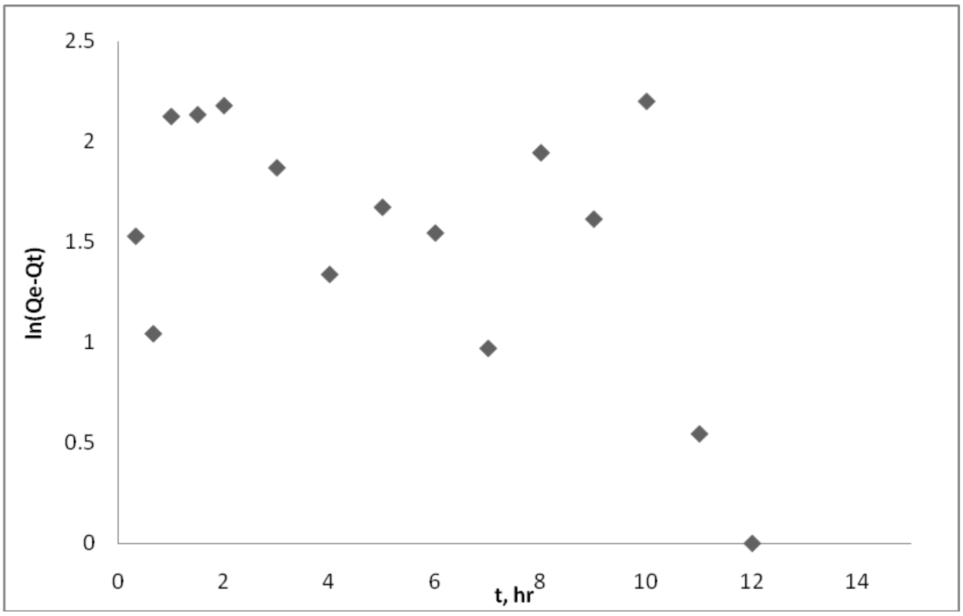


Figure 4.6 The pseudo first-order kinetics data for lead adsorption on *A.niger*.

The results obtained for the linearized pseudo second-order showed correlation coefficient of 0.99. Thus, the adsorption of Pb(II) ions onto the *A.niger* biomass is regarded as pseudo-second order rather than pseudo-first order.

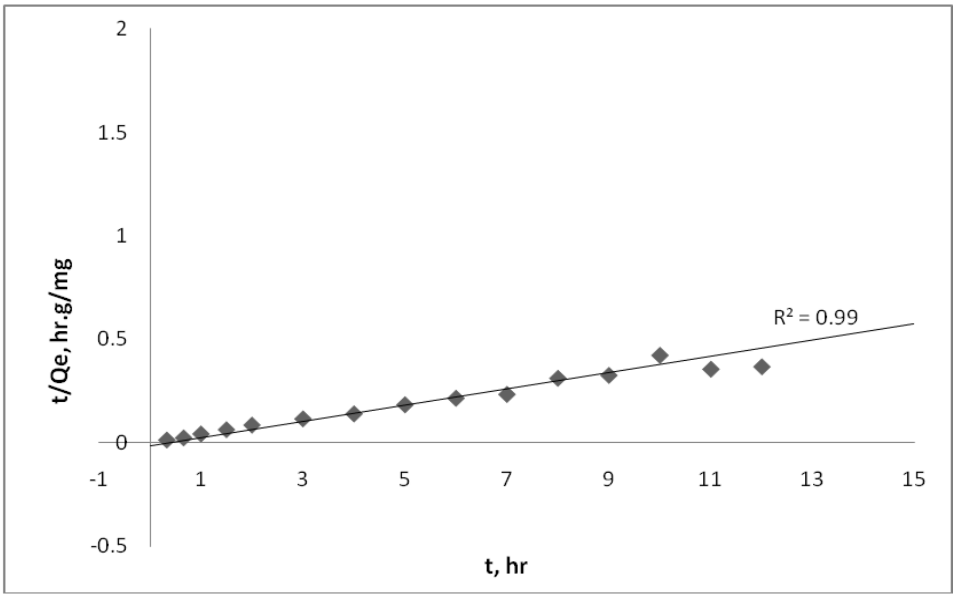


Figure 4.7 The pseudo second-order kinetics for lead adsorption on *A.niger*.

The rate constants were 8.52×10^{-4} and 9.2×10^{-3} g/mg min for the pseudo first order and second-order reaction, respectively. In the kinetic studies of biosorbents, the biosorption is similar to the conventional sorption processes, involves inherently very fast sorption reaction mechanisms based predominantly on chemisorptions.

4.3 Effect of pH on the metal uptake

Since earlier studies on biosorption phenomena, it has been known that the uptakes of heavy metal cations by most biomass types decrease dramatically as pH of the metal solutions decreases from pH 6 to 2.5 (Sag & Kutsal, 2001). The environmental factor, which is the pH of metal ion solutions, influenced the surface metal binding sites of the biosorbents and the chemistry of the cells walls, thus the physicochemistry and hydrolysis of the metals are influenced as well (Tsekova et. al., 2010).

In this study, the effect of pH was investigated by varying the pH of metal ion solutions from 1.0 to 6.0. The results obtained were analysed by Langmuir isotherm equation to compare the maximum adsorption values for each pH value as tabulated in Table 4.2.

Figure 4.8 depicts the maximum adsorption capacity for lead at pH 1 to pH 6. It shows the influence of pH in terms of adsorption uptake. It shows that the adsorption happens in pH range of 4 – 6. The biosorption capacity is reduced as the pH of lead ion solutions is changed towards acidic range since there was little or no biosorption of lead determined for pH range less than 2. The heavy metal removal capacity increased greatly from 31.65 to 66.23 mg/g with an increase in pH from 5.0 to 6.0 at, respectively.

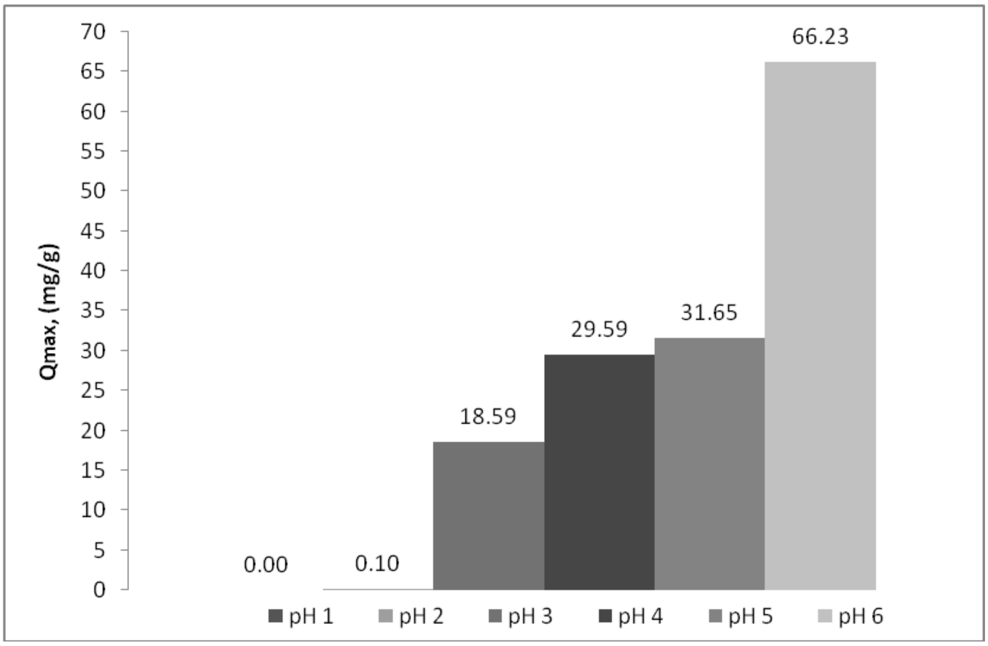


Figure 4.8 The maximum adsorption of Pb on *A.niger* at different pHs.

According Kapoor et. al., (1999) a sudden increase in sorption with a slight increase in pH is often referred to as an “adsorption edge”. The results also showed that the dead fungal biomass, *A. niger* favoured the pH 6 metal ion solution and possessed higher affinity towards the lead metal ions in this environment.

The results of the experiments indicated that pH is an important parameter affecting the biosorption of heavy metals. As depicted in Figure 4.8, at low pH (lower than 3.0) heavy metal removal was inhibited; this phenomenon is possibly a result of a positive charge density on the surface binding sites due to a high concentration of protons in solution.

In other words, at highly acidic pH, the overall surface charge on cells became positive and metal cations and protons compete for binding sites on cell wall, which resulted in lower metal uptake rates. According to Dursun (2006), it has been suggested that at low pH values, cell wall ligands would be closely associated with H_3O^+ that restricts access to ligands by metal ions as a result of growing repulsive force.

Besides of the ionic state of the solutions and adsorbents, highly acidic environment may also serve as desorbing conditions for the purpose of releasing the metal ions from the binding sites (Chojnacka, 2010). This theory has been proved by Jianlong et. al., (2001), where 0.1M of nitric acid was able to effectively elute the biosorbed lead ions from the fungal biomass. The acidic environment was playing a role as to desorb and regenerate the biomass to be reused (Volesky, 2007).

However, with an increase in pH, the negative charge density on the cell surface increases due to deprotonation of the metal binding sites and thus biosorption increased. At pH values above the iso-electric point, there is a net negative charge on the cell surface and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such that so as to promote reaction with metal ions, hence the rapid binding efficiency was obtained.

4.4 Effect of initial concentration on metal uptake

The effect of initial metal ion concentration on the biosorption capacity of *A.niger* was studied under the ambient condition at 25°C and pH 1 – 6 range. The biosorption of Pb(II) ions on *A.niger* increased with increasing initial concentration of metal ions.

Figure 4.9 depicts the metal ions removal percentage ranging from pH 1.0 to 6.0. There is a general trend where the initial metal ions increases, the percentage removal increases as well.

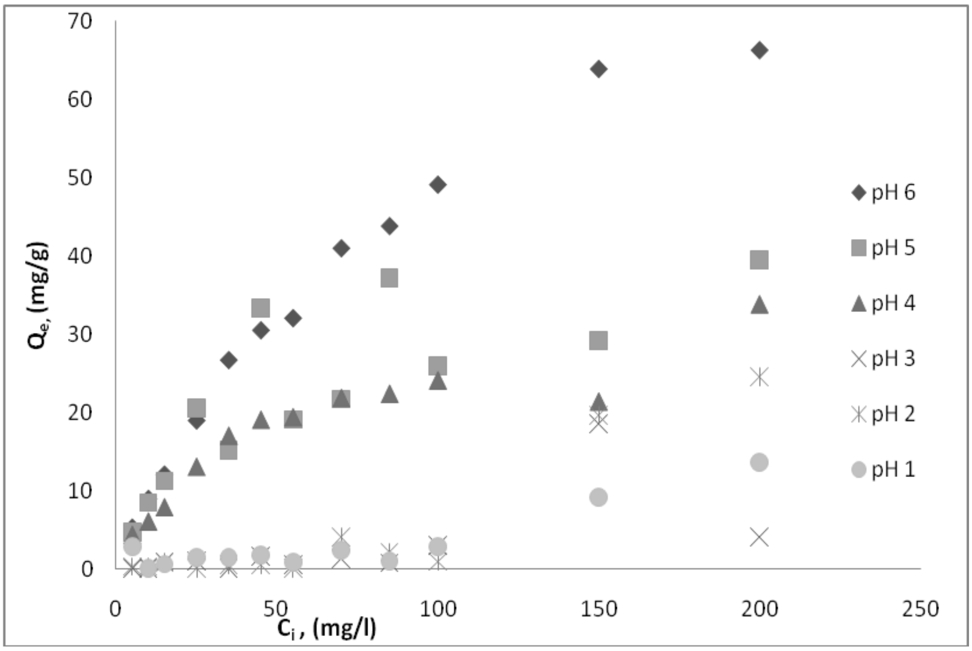


Figure 4.9 Effect of initial lead concentration on biosorption capacity of *A.niger*.

Figure 4.9 shows the effect of initial metal ion concentration on lead biosorption by fungal biomass. There is an obvious trend shown on the graph: the biosorption curves of pH 4 to 6 starts with a slope and reaches equilibrium when the initial metal ion concentrations increase. Despite of the rule stated, these phenomena may be explained by an increase in the number of metal ions competing for the available binding sites on the biomass surface and the lack of binding sites for complexation of Pb(II) ions at higher concentration levels.

Table 4.3 shows data on the increase in the metal ions to biomass ratio which results in decrease of the biosorption efficiencies. For instance, the percentage removal of lead metal ions decreases from 71.20 % to 22.10 % when the metal ions concentration increases from 5 to 200 mg/L. There is a rule stated Dursun (2006): an increase in the initial metal concentration results in an inrease in the biosorption capacity because the initial metal concentration provides a driving force to overcome mass transfer resistance between the biosorbent and biosorption medium.

Table 4.3 Percentage removal of lead by biomass of *A. niger*.

	Lead Percentage Removal, %					
Initial Concentration C_i, mg/L	pH 1	pH 2	pH 3	pH 4	pH 5	pH 6
5	37.94	3.70	0.40	59.40	63.20	71.20
10	0.70	2.00	0.40	41.02	56.90	60.05
15	2.87	3.67	4.20	35.53	50.20	53.87
25	4.04	0.04	2.68	35.08	54.96	50.72
35	2.97	1.03	0.20	32.60	29.09	50.91
45	2.73	0.76	2.36	28.40	49.49	45.24
55	1.13	0.09	0.67	23.62	23.20	38.89
70	2.31	3.86	1.26	20.89	20.74	39.06
85	0.84	1.65	0.61	17.62	29.20	34.39
100	1.91	0.60	2.00	16.12	17.36	32.75
150	4.07	8.73	8.27	9.55	13.00	28.40
200	4.55	8.20	1.35	16.80	13.20	22.10

In other words, at high concentration levels, more Pb(II) ions are left in solution compared to the adsorbed metal ions may have caused the saturation of binding sites on the biomass surface. When the biomass surface are saturated with metal ions, the ion-exchange mechanism could not take place, thus the biosorption is inhibited. On the other hand, at low concentration of metal ions the number of available binding sites on the biomass surface is high and hence biosorption of metals was very effective.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The ability of *A. niger* biomass to adsorb lead, Pb(II) ions was investigated in a batch system. The equilibrium of biosorption of lead, Pb(II) ions on the biosorbent was evaluated using Langmuir and Freundlich models. Both of the adsorption models applied agreed well with the experimental data. However, Langmuir equation was the best isotherm model that represented the biosorption data in this study because the obtained correlation coefficients were higher. The equilibrium data of lead adsorption fitted well to both Langmuir and Freundlich, thus the adsorption is characterized as monolayer and multilayer.

The maximum removal efficiency of Pb(II) ions occurs at pH 6 where the maximum adsorption capacity obtained was 66.23 mg/g using the Langmuir isotherm model.

This study also showed that the dried fungal biomass of *A.niger* is very sensitive to pH changes especially below pH 3. The biosorption favoured the alkaline environment as the maximum adsorption capacity happened at pH 6.

It was also found that the pseudo-second order model was applicable for the whole period of contact time. As a conclusion, the results of this study indicated that A.niger biomass is a suitable biosorbent for the removal of Pb(II) ions from aqueous solution.

5.2 Recommendations

It is obvious that many different endeavours and challenging contributions have to made on the path of developing biosorption from a scientific curiosity to useful applications for the sake of protecting the environment with low cost.

The analysis of experimental data in this study shows that the adsorption of metal ions was best at the pH range of 4 to 6. Thus, it is suggested that the pH range of the study can be narrowed down to this pH range but adding more parameters of interest in this study, for example the biosorbent dosage, agitation rate and temperatures. Meanwhile, the technigue of elution may also be added in the study for the purpose experimenting on the regeneration and reuse of biosorbents.

Volesky, (2007) also stated the importance of the affinity of adsorbent towards the metal ions. This “affinity” is largely contributed by swirl of the electrons, which is thermodynamics. Therefore, more technical and feasibility data are required for the better understanding and effective use of fungal biosorbent. Therefore, it is recommended that the thermodynamics studies can be included as well for the biosorption of Pb(II) ions.

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