THE EFFECT OF pH AND BIOMASS CONCENTRATION ON LEAD (Pb) ADSORPTION BY *Aspergillus niger* FROM SIMULATED WASTE WATER

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I declare that this thesis entitled "*The effect of pH and Biomass Concentration on Lead Adsorption By Aspergillus Niger From Simulated Wastewater*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ……………………….........

Name of Candidate : …………………...........

Date : ………………...............

Special dedicated of my grateful feelings to my parents, Mr. Joseph a/l Solomon & Nallammal Darmmaknoo for their love and encouragement. I also dedicated this to my brother Simon Prakash and to all my friends who understand and help me in everything I do.......

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ABSTRACT

Heavy metals released by a number of industrial processes are major pollutants in marine, ground, industrial and even treated wastewaters. Lead is widely used in many industrial applications such as storage battery manufacturing, printing, pigments, fuels, photographic materials and explosive manufacturing. Lead is highly toxic as its presence in drinking water above the permissible limit (5 ng/mL) causes adverse health effects such as anemia, encephalopathy, hepatitis and nephritic syndrome. Conventionally, the following methods are employed for the removal of heavy metals from effluents such as oxidation and reduction, precipitation, filtration, electrochemical treatment, evaporation. The alternative method is discovered which is biosorption, refers to a physic-chemical binding of metal ions to biomass. It can be considered as an alternative technology for industrial wastewater treatment, The biosorption rate largely depends on parameters such as pH, initial biomass concentration and temperature. In this study, the biosorption of lead (Pb^{2+}) from simulated waste water using *Aspergillus niger* was investigated. The biosorption process was carried out in a shake flask at different initial pH and biomass concentration. Results from experiment shows, among the different initial pH used, percentage of biosorption was highest is at pH 4.0 (77.99%) and for the experiment conducted at different initial biomass concentration, the highest percent of biosorption was obtained by using 20mg/l (40.58%) biomass. From the results also, it was observed that, the percentage of biosorption is directly proportional to the biomass concentration.

ABSTRAK

Pelepasan logam dari kebanyakan industri adalah pencemaran utama dalam marin, tanah, dan juga pencemaran air yang telah dirawat. Plumbum digunakan secara luas di kebanyakan industri seperti industri bateri, percetakan, minyak, fotografi dan lain-lain. Plumbum adalah logam yang sangat toksik, oleh itu kehadiran logam ini dalam air minuman yang melebihi paras keterlarutan (5ng/mL) menyebabkan kesan kepada kesihatan dan ia menyebabkan penyakit seperti Anemia, hepatitis dan juga kanser. Oleh yang demikian, kaedah berikut adalah digunakan untuk penyingkiran bahan tercemar iaitu pengoksidaan dan penurunan, penapisan, elektrokimia dan penjerapan. Kaedah alternatif iaitu penjerapan bio akan digunakan dalam merawat air sisa yang mengandungi logam toksik. Kaedah penjerapan bio adalah merujuk kepada pelekatan ion logam dengan dinding sel mikroorganisma secara fizikal-kimia. Ini menunjukan kadar penjerapann bio adalah sangat bergantung kepada beberapa penentuan seperti nilai pH, kepekatan awal biojisim dan suhu. Penjerapan logam plumbunm daripada simulasi air sisa akan dijalankan dengan mengunakan fungi (*aspergillus niger*). Dalam kajian ini, proses penjerapan bio akan dijalankan dalam kelalang goncang pada pH dan kepekatan awal biojisim yang berlainan. Daripada keputusan eksperimen yang dijalankan, pada pH yang berbeza, peratus penjerapan bio adalah paling tinggi pada pH 5.5 iaitu sebanyak 55.36%. Manakala bagi eksperimen yang dijalankan pada kepekatan biojisim yang berbeza menunjukan bahawa peratus penjerapan bio adalah paling tinggi dengan mengunakan biojisim berkepekatan 2mg/l iaitu 45.08%. Daripada keputusan yang diperolehi juga, dapat dilihat bahawa peratus penjerapan bio adalah berkadar langsung dengan kepekatan awal biojisim dalam larutan.

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	=	Atomic Absorption Spectrometry
T	=	Temperature
t	=	time
cm	=	centimeter
m	=	meter
g	=	gram
°C	=	Degree Celcius
%	=	Percentage
µL	=	Micro liter
Pb	=	Plumbum (Lead)
Cu	=	Copper
Zn	=	Zinc
g/mL	=	gram per mililiter
mg/L	=	miligrm per liter
K	=	Rate constant
C□ and C (mg/l)	=	The initial and equilibrium (residual) concentrations of metal in the solutions
rpm	=	rotation per minute

CHAPTER 1

INTRODUCTION

1.1 Background

Heavy metals in wastewater generated from various industries are hazardous to human and other living organisms when discharged into the environment. This concern has led to the development of various methods for the removal of metals from wastewater such as biological treatment, ion exchange, chelating, reverse osmosis, coagulation–precipitation, electrochemical operation, filtration. The major shortcoming of conventional treatment includes low efficiency at low concentration of heavy metals and expensive handling and safe disposal of toxic sludge.

Biosorption has been recognized as a promising technology that shows potential as an alternative to conventional processes for the treatment of water with trace levels of metal contaminant. Microorganisms have rich contents of polysaccharides and several important functional groups, which are mainly responsible for higher metal biosorption. Ion exchange, surface complex formation and micro precipitation are used to explain the biosorption mechanisms. Microorganisms including bacteria, fungi and algae have been investigated in metal adsorption studies. Among the microorganisms used for biosorption, *Aspergillus niger* is an inexpensive, readily available source of biomass for heavy metal removal from wastewater than other microorganisms where poor mechanical strength and low adsorption capacity.

Besides that, this investigations is conducted by several researchers demonstrated that *aspergillus niger* is capable of accumulating heavy metals such as Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^{2+} and Ni^{2+} (Y.Goksungur, 2004).

1.2 Problem Statement

The increase in industrial activity during recent years is greatly contributing to the increase of heavy metals in the environment, mainly in the aquatic systems. Water pollution due to heavy metals is an issue of great environmental concern. Heavy metal ions such as cobalt, copper, nickel, chromium, lead and zinc are detected in the waste streams from mining operations, tanneries, electronics, electroplating, batteries and petrochemical industries as well as textile mill products.

Major lead pollution is through automobiles and battery manufacturers. Heavy metals have a harmful effect on human physiology and other biological systems when they exceed the tolerance levels. Exposure to lead can cause anemia, diseases of the liver and kidneys, brain damage and ultimately death. Besides, chronic exposure to these contaminants present even at low concentrations in the environment can prove to be harmful to the human health. For these reasons, heavy metals must be removed as much as possible from industrial effluents. In Malaysia, domestic wastewater is treated separately from industrial wastewater, which in turn generates safer water in the sense of environmental and human health aspects.

Under the 9th Malaysia Plan, the government were promoted agro-based industries so that there be a steady rise on the demand of water treatment.

1.3 Objectives

The objective of this study is to determine the effect of pH and biomass concentration on **Lead (Pb)** adsorption by ***Aspergillus niger*** from simulated waste water.

1.4 Research Scope

In order to achieve the objective of this study, there are two scopes that have been identified in this study:

i Study on the effect of pH on lead adsorption by *Aspergillus niger* from simulated waste water.

ii Study on effect of initial biomass concentration on lead adsorption by *Aspergillus niger* from simulated waste water.

CHAPTER 2

LITERATURE REVIEW

2.1 Biosorption

2.1.1 Introduction

Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metals from industrial waste streams. Most studies of biosorption for metal removal have involved the use of either laboratory-grown microorganism or biomass generated by the pharmacology and food processing industries or wastewater treatment units. Many aquatic microorganisms, such as bacteria, yeast and algae can take up dissolved metals from their surroundings onto their bodies and can be used for removing heavy metal ions successfully. Here the term of biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Tsezos and Volesky, 1981). Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents. The major advantages of biosorption over conventional treatment methods include :

- Low cost
- High efficiency
- Minimization of chemical and low biological sludge
- No additional nutrient requirement
- Regeneration of biosorbent
- Possibility of metal recovery.

The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be absorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the absorbed species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound absorbed species and its portion remaining in the solution. The degree of sorbent affinity for the absorbed determines its distribution between the solid and liquid phases (Volesky, 1986).

2.1.2 Biosorbent material

Accumulation of metals by microorganisms has been known for a few decades but has received more attention in recent years, because of its potential application in environmental protection or recovery of precious or strategic metals. The general term `biosorption` has been used to describe a property of microbial biomass to retain ions of mainly heavy metals and radionuclide's. Increased interest in sequestering these elements by nonliving microbial biomass has been based on potential technological applications of biosorption in metal recovery and industrial wastewater treatment.

Recent biosorption experiments have focused attention on waste materials, which are by-products or the waste materials from large-scale industrial operations. For e.g. the waste mycelia available from fermentation processes, olive mill solid residues, activated sludge from sewage treatment plants, biosolids, aquatic macrophytes, etc.(Tae-Young Kim, *et al.* 2005).

The mechanism of biosorption is complex, mainly ion exchange, chelations, adsorption by physical forces, entrapment in inter and intrafibrilliar capillaries and spaces of the structural polysaccharide network as a result of the concentration gradient and diffusion through cell walls and membranes (N. Ahalya, *et al*, 2001).

2.1.3 Choice of metal for biosorption process

The appropriate selection of metals for biosorption studies is dependent on the angle of interest and the impact of different metals, on the basis of which they would be divided into four major categories:

- Toxic heavy metals
- strategic metals
- precious metals and
- Radio nuclides.

In terms of environmental threats, it is mainly categories (i) and (iv) that are of interest for removal from the environment and/or from point source effluent discharges. A whole new family of suitably "formulated" biosorbents can be used in the process of metal removal and detoxification of industrial metal-bearing effluents.

The sorption packed-column configuration is the most effective mode of application for the purpose. Recovery of the deposited metals from saturated biosorbent can be accomplished because they can often be easily released from the biosorbent in a concentrated wash solution which also regenerates the biosorbent for subsequent multiple reuse (N. Ahalya, *et al*. 2001). This and extremely low cost of biosorbents makes the process highly economical and competitive particularly for environmental applications in detoxifying effluents of e.g:

- metal-plating and metal-finishing operations,
- mining and ore processing operations,
- metal processing, battery and accumulator manufacturing operations,
- thermal power generation (coal-fired plants in particular),
- nuclear power generation

Apart from toxicological criteria, the interest in specific metals may also be based on how representative their behavior may be in terms of eventual generalization of results of studying their biosorbent uptake. The toxicity and interesting solution chemistry of elements such as chromium, arsenic and selenium make them interesting to study. Strategic and precious metals though not environmentally threatening are important from their recovery point of view (N. Ahalya, *et al.* 2001).

2.2 Atomic Absorption Spectrometry (AAS)

2.2.1 Introduction

Atomic absorption spectrometry (AAS) is an analytical technique that measured the concentration of elements. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (dm^{-3}) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another higher energy level. Atomic absorption spectrometry has many uses in different areas of chemistry.

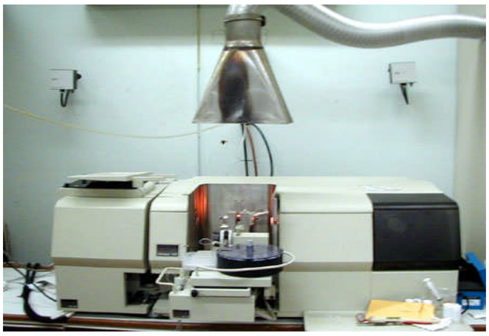

Figure 2.1 *Picture of atomic-absorption spectrometer*

2.2.2 Operation of Atomic Absorption Spectrophotometer (AAS)

The process of atomic absorption spectroscopy (AAS) involves two steps:

1. Atomization of the sample
2. The absorption of radiation from a light source by the free atoms

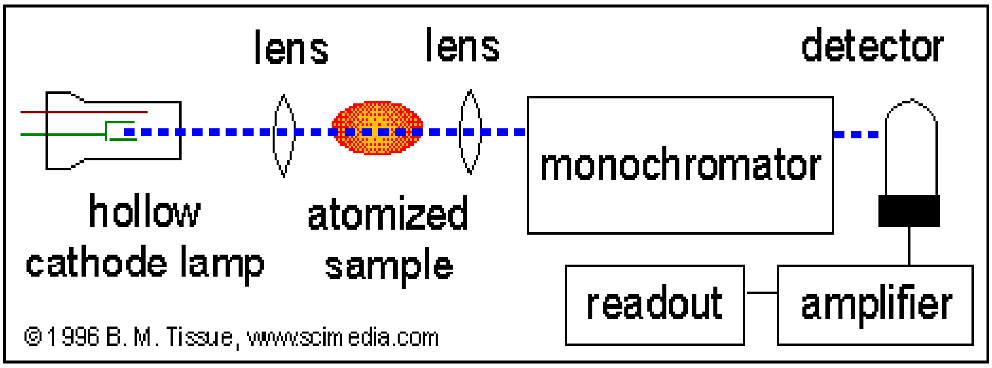

Figure 2.2 Schematic Diagram of Atomic Absorption Spectrometer (AAS)

Atom of different elements absorb characteristic wavelength of light. Analyzing a sample is to see if it contains a particular element means using light from that element. For example with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample. In AAS, the sample is atomized converted into ground state free atoms in the vapor sate- and a beam of electromagnetic radiation emitted from excited lead atom is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample.

The greater the number of atoms there is the vapor, the more radiation is absorb. The amount of the light absorbed is proportional to the number of lead atoms. A calibration curve is constructed by running several samples of known lead concentration under the same condition as the unknown. The amount the standard absorbs is compared with the calibration curve and this enable the calculation of the lead concentration in the unknown sample. Consequently an atomic absorption spectrometer needs the following three components: a light source; a sample cell to produce gaseous atoms; and of measuring the specific light adsorbed. A typical atomic absorption instrument holds several lamps each for a different element. The lamps are hosed in a rotating turret so that the correct lamp can be quickly selected.
(R. Levinson *et al* 1998)

2.3 Aspergillus niger

2.3.1 Introduction

Aspergillus niger is a fungal that capable of removing heavy metals such as lead, cadmium and copper from aqueous solutions. The role played by various functional groups in the cell wall of *A. niger* in biosorption of lead was investigated. Currently the research on *Aspergillus niger* tell that the size of A. niger is estimated to be between 35.5 and 38.5 mega bases (Mb) divided among eight chromosomes or linkage group that vary in size from 3.5-6.6Mb.

Aspergillus niger is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual,although perfect forms (forms that reproduce sexually) have been found. *Aspergillus* are ubiquitous in nature. They are geographically widely distributed, and have been observed in abroad range of habitats because they can colonize a wide variety of substrates. *A. niger* is commonly found as a saprophyte growing on dead leafs, stored grain, compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil.

The primary uses of *A. niger* are for the production of enzymes and organic acids by fermentation. While the foods, for which some of the enzymes may be used in preparation, are not subject to TSCA, these enzymes may have multiple uses, many of which are not regulated except under TSCA. Fermentations to produce these enzymes may be carried out in vessels as large as 100,000 liters (Finkelstein, *et al*. 1989).

A. niger is also used to produce organic acids such as citric acid and gluconic acid.The history of safe use for *A. niger* comes primarily from its use in the food industry for the production of many enzymes such as a-amylase, amyloglucosidase, cellulases, lactase,invertase, pectinases, and acid proteases (Bennett, 1985a; Ward,1989).

In addition, the annual production of citric acid by fermentation is now approximately 350,000 tons, using either A.niger or Candida yeast as the producing organisms. Citric acid fermentation using *A. niger* is carried out commercially in both surface culture and in submerged processes (Berry *et al.*, 1977;Kubicek and Rohr, 1986; Ward, 1989).

Aspergillus niger has some uses as the organism itself, in addition to its products of fermentation. For example, due to its ease of visualization and resistance to several anti-fungal agents, Aspergillus niger is used to test the efficacy of preservative treatments (Jong and Gantt, 1987). In addition, *A. niger* has been shown to be exquisitely sensitive to micronutrient deficiencies prompting the use of A. niger strains for soil testing (Raper and Fennell,1965). There is also interest in using this fungus to perform certain enzymatic reactions that are very difficult to accomplish by strictly chemical means, such as specific additions to steroids and other complex rings (Jong and Gantt, 1987).