REMOVAL OF FERUM FROM AQUEOUS SOLUTION BY USING WATER HYACINTH (*Eichhornia crassipes)*

MOHD IZZAT BIN SISWO

UNIVERSITY MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS

JUDUL: **REMOVAL OF FERRUM FROM AQUEOUS SOLUTION BY USING DRIED WATER HAYCINTH (*Eichhornia crassipes*)**

SESI PENGAJIAN: **2007/2008**

Saya **MOHD IZZAT BIN SISWO**

(HURUF BESAR)

mengaku membenarkan kertas projek ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut :

1. Hakmilik kertas projek adalah di bawah nama penulis melainkan penulisan sebagai projek bersama dan dibiayai oleh UMP, hakmiliknya adalah kepunyaan UMP.
2. Naskah salinan di dalam bentuk kertas atau mikro hanya boleh dibuat dengan kebenaran bertulis daripada penulis.
3. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian mereka.
4. Kertas projek hanya boleh diterbitkan dengan kebenaran penulis. Bayaran royalti adalah mengikut kadar yang dipersetujui kelak.
5. *Saya membenarkan/tidak membenarkan Perpustakaan membuat salinan kertas projek ini sebagai bahan pertukaran di antara institusi pengajian tinggi.
6. ** Sila tandakan (ü)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan).
☐	TIDAK TERHAD	

Disahkan oleh

(**MOHD IZZAT BIN SISWO**) (**PN. JUN HASLINDA BINTI HJ. SHARIFFUDDIN**)

Alamat Tetap**: 22/81 Rumah Kakitangan,**
.FELCRA Sg. Ara, 81900
Kota Tinggi, Johor.

Tarikh: 14 Mei 2008

Tarikh: 14th Mei 2008

CATATAN: * Potong yang tidak berkenaan.
** Jika Kertas Projek ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali tempoh tesis ini perlu dikelaskan sebagai SULIT atau TERHAD.

♦ Tesis ini dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Laporan Projek Sarjana Muda (PSM)

"Saya/Kami* akui bahawa saya telah membaca karya ini dan pada pandangan saya/kami* karya ini adalah memadai dari segi skop dan kualiti untuk tujuan penganugerahan ijazah Sarjana Muda/~~Sarjana/Doktor Falsafah~~

....Kejuruteraan Kimia.....".

Tandatangan : .....

Nama Penyelia : Puan Jun Haslinda Binti Hj. Shariffuddin

Tarikh : 14th May 2008

BIOSORPTION OF FERUM FROM AQUEOUS SOLUTION BY WATER HYACINTH (*Eichhornia crassipes)*

MOHD IZZAT BIN SISWO

A thesis submitted in fulfillment of the
Requirement for the award of the degree of
Bachelor of Chemical Enginering

Faculty of Chemical and Natural Resources Engineering Technology
University Malaysia Pahang

MAY 2008

"I declare that this thesis is the result of my own research except as cited references. The thesis has not been accepted for any degree and is currently submitted in candidate of any degree."

Signature :……..

Name of Candidate : Mohd Izzat Bin Siswo

Date : 14th May 2008

Dedicated especially to
my father and mother, Along, Fahmi, Pudin and my friends
who give me inspiration and support that made this work possible.

ACKNOWLEDGEMENT

I would like to take this opportunity to extend my deepest gratitude to the following persons who have helped me a lot in this project, which enable me to complete the research project in time a partial fulfillment of the requirement of the degree of Bachelor Engineering (Chemical Engineering).

Firstly and foremost, a special thank to my supervisor Mrs. Jun Haslinda Binti Hj. Shariffuddin, who helped me a lot during the progress of the research project, for all support, continuous patience and supervision given throughout the project. Without her time in sparing their precious time to guide me and answer my doubts, this project would not accomplish successfully.

Apart from that, I would like to thank our lecturer Mr. Mior Ahmad Khusyairi Bin Mohd Zahari and Miss Siti Kholijah Binti Abdul Mudalip for his guidance and coordination in this final year project.

Last but not least, my special thanks, I would like to direct to my family members for their continuous support and advice from the early stage of my studies.

ABSTRACT

The used of nonliving aquatic plants for the removal of toxic heavy metals as biosorbent has been study recently. The objective of this study is to investigate the capability of *Eichhornia crassipes* as the biosorbent in removal of ferrum from aqueous solution in laboratory scale. Biosorbent dosage, contact time, temperature and pH were chose as the parameters for this study. The experiment was carried out in conical flask with initial concentration of ferrum solution was 10 mg/L and then agitated in orbital shaker at 175 rpm. HACH spectrophotometer was used to measure the final concentration of the ferrum solution. Increasing the biosorbent dosage result more surface area for ferrum ions to bind with functional groups exist in biosorbent. The removal of ferrum increased in early stage and the optimum contact time was 60 minutes. Prolonged the contact time in this study, do not give better result for removal of ferrum. Optimum temperature was at 25°C and removal of ferrum decreased by increasing the temperature because it can damage the binding site in biosorbent. Competition with H^+ will occur at acidic condition and at alkaline condition cause the precipitation of ferrum. Optimum removal of ferrum was achieved at pH 6. As the conclusion, water hyacinth (*Eichhornia crassipes*) is suitable for development of efficient biosorbent for the removal of ferrum from wastewater stream.

ABSTRAK

Kajian penggunaan tumbuhan akuatik sebagai agen penyerap dalam pengambilan logam berat bertoksid dari kawasan industri telah dilakukan. Oblektif kajian ini adalah untuk mengkaji kebolehan atau keupayaan *Eichhornia crassipes* atau keladi bunting sebagai agen penyerap dalam merawat ferum, Fe(II) atau besi dari larutan dalam skala makmal. Pengaruh atau kesan jumlah dos, masa, pH dan suhu dalam eksperimen ini telah dikaji. Eksperimen ini dijalankan di dalam kelalang kon yang digoncang. Kepekatan awal larutan ferum adalah 10 mg/L dan digoncang pada 175 rpm. HACH spectrophotometer digunakan untuk menentukan kepekatan akhir larutan ferum. Penambahan kuantiti serbuk keladi bunting akan memberikan lebih luas permukaan untuk proses penyerapan ion ferum oleh kumpulan berfungsi yang wujud pada keladi bunting. Pengurangan ferum berlaku pada peringkat awal eksperimen dan mencapai optimum setelah 60 minit eksperimen dijalankan. Melanjutkan masa tidak memberikan keputusan kajian yang bagus. Suhu optimum adalah pada suhu 25°C dan penambahan suhu akan mengurangkan penyerapan ferum. Ini kerana, suhu yang tinggi akan merosakkan bahagian pengikat pada keladi bunting. Kepekatan ion H+ pada keadaan asid dan pemendakan ferum pada keadaan beralkali mengurangkan berkesanan penyerapan ion ferum dari larutan ferum. pH optimum adalah pada pH 6. Kesimpulannya, keladi bunting sesuai dengan pembangunan agen penyerap yang efektif dalam penyerapan ferum daripada sisa-sisa yang dikeluarkan oleh industri.

TABLE OF CONTENTS

LIST OF SYMBOLS

Fe	-	Ferrum/Iron
Cd	-	Cadmium
Cr	-	Chromium
Pb	-	Plumbum/Lead
Mn	-	Manganese
Hg	-	Mercury
Ni	-	Nickel
Zn	-	Zinc

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background

Water contamination with heavy metal discharged from industrial activities and urbanization is a very important problem in the current world which can affect the ecosystem of environment and human health. Attention has been paid to find the methods to remove heavy metals from wastewater from dispose because they will cause the serious problems to environment and human health. Several studies have shown that the biological materials have the ability to accumulate heavy metal from wastewater. The process has gained importance due to its advantages over conventional separation techniques such as chemical precipitation, ion exchange, reverse osmosis, membrane filtration, and activated carbon adsorption, which are used to remove toxic metals from wastewater.

Biosorption is an alternative way to treat the heavy metal waste. It uses the biological materials. Biological materials have been divided to two types which are living biomass and non-living biomass. For living biomass there are like aquatic plants, fungi, algae and moss. Non-living biomass is like bark, lignin and peanut hull. Those biological materials have been use as biosorbent. Most of them are easily to find and live wildly in a large group. The most versatile and commonly used as a sorbent is activated carbon but it is too expensive, so there have been considered other sorbent materials there are biosorbents. Heavy metal also can be removed by inexpensive biological materials. The

performance of any biosorbent depends on biomass characteristics, physico-chemical characteristics of the target metals and the microenvironment of contact solution such as solution pH, temperature, and interaction with other ions (Tsezos *et al*, 1997).

The researchers have study the capability of the living submerged aquatic plants in removing the heavy metals by making the wetland with the artificial wastewater with heavy metals inside it and gets the good result (Xiaomei Lu *et al*, 2003; K. S. Low *et al*, 1994). Some other researchers have been develop a new way in removing heavy metals, there is by change the living aquatic plants into dose form (I. A. H. Schneider and J. Rubio, 1999; Mentaky *et al*, 2004). It might be through some methods to produce the dose from aquatic plants but it still low in cost and no chemicals are needed. Heavy metals produce phytotoxic effects on plant such as inhibition of chlorophyll synthesis and it also lead to death of the plants.

Heavy metals ions such as Cu^{2+}, Fe^{2+} and Zn^{2+} are needed in plant metabolism but when they are present in excess they can become extremely toxic (Sungai Buloh Nature Park, 2001). Iron (Fe) is one of the toxic heavy metal. In plant, Fe is involved in the production of chlorophyl. Iron (Fe) also a component of many enzymes and associated with sulfur to produce other compound that can be catalyze other reaction. Plant also have can accumulate nonessential metal like Cd and Pb. Iron (Fe) applications go from food containers to family cars, from screwdrivers to washing machines, from cargo ships to paper staples. Steel is the best known alloy of iron, and some of the forms that iron takes include: pig iron, cast iron, carbon steel, and wrought iron, alloy steels, iron oxides. It also has been used in textile industry and ink manufacturing.

Water hyacinth (*Eichhornia crassipes*) is a floating macrophyte, can grow and spread rapidly in freshwater and slow-moving water. They also can live with extremes of pH level, temperature and even grow in toxic water. The useful of non-living water hyacinth in removing metal ions have been investigated and it shown that it have the potential of being a new resources of biosorbent in removing the metal ions. The aim of this research is to study the removal of iron (Fe) from aqueous solution by using dried

water hyacinth as biosorbent at different biosorbent dosage, contact time, pH and temperature.

1.2 Problem Statement

The discharge of heavy metals into aquatic ecosystems had become a matter of concern over the last few decades. These pollutants are introduced into the aquatic system significantly as a result of various industrial operations. The pollutants of concern include lead, chromium, mercury, cadmium, arsenic, uranium, cooper and nickel. The toxic materials may be discharge from mining operations, sludge disposal, fly ash from incinerators, the processing of radioactive materials, metal plating, paints, alloys, pesticides or preservative.

Iron have been applied worldwide for commercial purposes and produced in large amount. It is applicable mostly in manufacturing industry such as automobile parts, laundry machines and containers. It also uses as essential components in producing alloy and steel. It also has been used in ink manufacture industry. Industries that involve ferrous alloys and those that use iron salts, such as ink manufacture and tanneries, discharge iron-contaminated waters. These can pollute both surface and ground waters (David Tin Win *et al*, 2002)

Aquatic plants have been considered as an alternative way in removing metals. There are many of aquatic plants that have been tested their ability in removing metals such as *Potagometon lucens, Salvinia hergozii, Eichhornia crassipes, Myriophyllum brasiliensis, Cabomba sp. and Ceratophyllum demersum* (Schneider and Rubio, 1999). It has been considered as one of a cheapest biosorbent resource in removing heavy metals from waste stream. Water hyacinth is easy to find, grow rapidly in pools, lakes, swamps and drainage systems as long as there is freshwater and slow-moving flow. The community of water hyacinth obstructs the flow of water and that can cause flood. It also

degrade water quality by reduce oxygen level in water will eliminate underwater animals. It also block sun light, it will cause elimination of underwater plants. Water hyacinth has also capable to live at water with high level pH, water with high level of toxic.

1.3 Objective

The objective of this research is to study the capability of dried water hyacinth in removing ferrum from aqueous solution.

1.4 Scope of Study

The scope of study of this research is to study the effects of four parameters; biosorbent dosage, contact time, pH and temperature toward the biosorption process of the removal of Ferum. For biosorbent dosage, it is concentrated in how the amount of sorbents can affect the rate of metal uptake. Obviously the amount of metal that have been uptake by sorbent will increase with increasing the amount of sorbent. pH also might be effect the rate of metal uptake. The effect of Ph towards the removal of Fe will also be studied. For the contact time, we will investigate the capacity of the dried water hyacinth. We also study about the effect of temperature to biosorbent in removing ferum from the aqueous solution.

CHAPTER 2

LITERATURE REVIEW

2.1 Wastewater

2.1.1 Definition

Wastewater can be defined as water that which is affected by anthropogenic influence. It consists of liquid waste discharged by domestic residences, commercial properties, industry and agriculture. It can also cause the existences a wide range of potential contaminants and concentration.

Wastewater usually consists organic and inorganic compound which is organic compound was biodegradable while the inorganic compounds such as toxic heavy metal and ammonia cannot be degraded naturally. So it needs some processes or materials that can be use to recover or remove the inorganic compound from wastewater before it dispose to environment. Biodegradable means that the compounds able to decompose by the action of microorganisms or other biological materials into innocuous product which not harm the environment.

To regulate the hazardous waste handling, it is important to identify the hazardous wastes. Both qualitative and quantitative, have been used by regulatory agencies of different countries for this purpose. Generally, hazardous wastes can be identified based on their characteristics and the lists of specific hazardous wastes provided in the

legislation. A waste can be considered hazardous if it exhibits one or more of the following characteristics show in Table 2.1 (Chongrak Polprasert and Liyanage, 1996);

Table 2.1: Type of hazardous and their characteristics (Chongrak Polprasert and Liyanage, 1996)

Hazard Identification	**Characteristics**
Ignitability	Ignitable wastes can create fires under certain conditions. Examples include liquids that readily catch fire, substances which are friction-sensitive or cause fire through absorption of moisture and ignitable compressed gases.
Corrosivity	Corrosive wastes include those that are strongly acidic or basic and those that are capable of corroding metal (such as containers, drums and barrels).
Reactivity	Reactive wastes are unstable under normal conditions. They can create explosions, toxic fumes, gases and vapors when mixed with water or heated in confinement.
Toxicity	Toxic wastes are harmful or fatal when ingested or absorbed. The toxicity can be chronic or acute. Toxic wastes can cause carcinogenic, mutagenic and teratogenic effects on human or other life forms.

Several types of the industrials which have been known as the wastewater generators are timber product processing, leather tanning and finishing, iron and steel manufacturing, petroleum refining, inorganic chemicals manufacturing, textile mills, organic chemical manufacturing, non-ferrous metal manufacturing, paving and roofing materials, paint and, ink formulation and printing, soap and detergent manufacturing,

laundries, plastic, synthetic materials, pulp, paperboard mills, paper products, rubber processing, miscellaneous, chemicals plastics processing, porcelain enamel, mechanical products, electrical and electronic components, electroplating and extractive industries.

Every contaminant from wastewater has used the different treatments depend on the physical properties and chemical properties obtained by each contaminants. Several types of common contaminants and treatment system that have been used are shown in Table 2.2.

Table 2.2: Common contaminants and treatment system used in industry (Howard *et al.* 1985)

Contaminant	**Treatment system**
Suspended solids	Sedimentation, screening & comminuting, filtration, floatation, chemical-polymer add, coagulation/ sedimentation, land treatment
Biodegradable organics	Activated-sludge, fixed film, lagoon & oxidation pond, sand filtration, land treatment, physical-chemical system
Pathogens	Chlorination, hypo-chlorination, ozonation, land treatment systems
Nitrogen	Suspended-growth nitrification & denitrification, fixed-film, ammonia stripping, ion exchange, breakpoint chlorination, land treatment system
Phosphorus	Metal-salt addition, lime coagulation/ sedimentation, bio-chemical phosphorus removal, land treatment
Refractory organics	Carbon adsorption, tertiary ozonation, land treatment system
Heavy metals	Chemical precipitation, ion exchange, land treatment system
Dissolved inorganic solid	Ion exchange, reverse osmosis, electro dialysis

2.1.2 Heavy metal

The metals of most immediate concern are Cr, Mn, Fe, Cu, Zn, Hg, Pb and Cd. These metals are widely distributed in materials which make up the earth's surface (Namasivayam and Senthilkumar, 1995**).** Heavy metal is chemical elements with a specific gravity that is at least 5 times the specific gravity of water. The specific gravity of water is 1 at 4ºC (39ºF). Simply stated that specific gravity is a measure of density of a solid substance when it is compared to an equal amount of water. Some well-known toxic metallic elements with a specific gravity that is 5 or more times that of water are arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 (Life Extension^{TM}, 2003). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture, manufacturing, pharmaceutical, industrial, or residential settings.

2.1.2.1 Iron (Fe)

Figure 2.1: Iron (Fe)

Iron is a shiny, bright white metal that is soft, malleable, ductile and strong. It is known to exist in four distinct crystalline forms. Iron rusts in moist air, but not in dry air. It dissolves readily in dilute acids. Iron is chemically active and occurs as the cations Fe^{2+}, ferrous iron, and Fe^{3+}, ferric iron. Fe^{2+} will gladly donate an electron in an acid

solution or in the presence of oxygen, so it is a reducing agent, oxidizing itself to Fe^{3+}. In alkaline solution, this ion will gladly accept an electron, so it is an oxidizing agent, reducing itself to the ferrous state.

Heavy metal ions used in various industries due to their technological importance (Kaustubha Mohanty *et al*, 2005). Iron is one of the heavy metal. Iron is the most frequently encountered metal in daily life, always in the form of manufactured objects, and usually covered with a protective coating or buried deep within the object. Concrete structures contain essential reinforcing iron such as electrical machines, including transformers, depend on iron. It also has been applied worldwide for commercial purpose and has been produced in large amount. Its applications go from food containers to family cars, from screwdrivers to washing machines, from cargo ships to paper staples. Steel is the best known alloy of iron, and some of the forms that iron takes include: pig iron, cast iron, carbon steel, wrought iron, alloy steels and iron oxides.

Industries that involve ferrous alloys and those that use iron salts such as ink manufacture and tanneries, discharge iron-contaminated water. These can pollute both surface and ground waters (David *et al*, 2002). Mining activities can expose minerals to air and water, causing the formation of extremely acidic metal-rich water. Mining areas has extremely high concentrations of iron, copper, arsenic, cadmium and zinc in solution (Edward, 2000). It will cause change color of the river, stream and groundwater to orange-red by bacterial action and can cause high erosion rate or industrial pollution (Bureau of Land and Water Quality, 2002)

Heavy metal ions such as Cu^{2+}, Zn^{2+}, Fe^{2+} are essential micronutrient for plant metabolism but when it present in excess it can become extremely toxic (Williams LE *et al*, 2000). Iron has been used in metabolism which is in production of chlorophyll. It can be a catalyst when associate with sulfur. In human body, iron (Fe) is an essential part of hemoglobin; the red colouring agent of the blood that transports oxygen through our bodies. Iron may cause conjunctivitis, choroiditis, and retinitis if it contacts and remains

in the tissues. A more common problem for humans is iron deficiency, which leads to anaemia.

2.2 Biosorption

2.2.1 Introduction

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be define as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). Algae, bacteria, fungi and yeast have proved to be potential metal biosorbents (Ahalya et al, 2001). The ability of aquatic plants, both living and dead to remove heavy metals has been studied extensively (Brierley, 1990). Aquatic plants generally live wildly at stream, lake, drainage system and wetland. The major advantages of biosorption using biological materials over conventional treatment methods include:

- Use of renewable biomaterials, which can reduce production costs
- High efficiency of metal removal from dilute solution
- Easy desorption of metals by pH changes, which can generate the sorbent
- High selectivity of biosorbents (possible to recover valuable metal)
- Low affinity with competing cations (calcium and magnesium)
- No nutrients requirement
- No production of secondary compounds which might be toxic (Ahalya *et al*, 2003)

The biosorption process has gained importance due to its advantages over conventional separation techniques such as chemical precipitation, ion exchange, reverse osmosis, membrane filtration, and activated carbon adsorption, which are used to remove toxic metals from waste streams. Biosorption process involves a solid phase (sorbent or biosorbent; biological materials) and liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution.

2.2.2 Water hyacinth (*Eichhornia crassipes*)

Figure 2.2: Water hyacinth (*Eichhornia crassipes*)

Water Hyacinths in scientific was named *Eichhornia crassipes* and in malay there were named as `keladi bunting'. Water hyacinths are generally seen in aquatic environments like streams, lakes, drainage system and wetlands. Water hyacinth is in Pontederiaceae or Pickerelweed Family.

The Water Hyacinth has special properties that allow it to grow and spread rapidly in freshwater. They grow best in still or slow-moving water. The main method of reproduction is vegetative, through stolons. A single plant under ideal conditions can produce 3,000 others in 50 days, and cover an area of 600 square meters in a year. Water hyacinth is not winter-hardy; its minimum growth temperature is 12ºC (54ºF), its optimum growth was in range 25º-30ºC (77ºF-86ºF); while its maximum growth temperature is 33ºC-35ºC (92ºF-95ºF) (Kasselmann, 1995).

Water hyacinth is one of the worst weeds in the world even weeds in aquatic or terrestrial (Holm et al, 1977). The fast-growing water hyacinth can become a noxious weed outside its native habitat. Plants interlock in such a dense mass that a person could walk on a floating mat of them from one bank of a river to the other. The presence of water hyacinth disrupts all life in the water. They can block the waterways and preventing river travel, block irrigation canals, destroy rice fields, and ruin fishing grounds. By shading the water, these plants deprived native aquatic plants of sunlight and animals of oxygenated water. There is a sharp increase in nutrient levels in the water, which spark off algal growths that further reduces oxygen levels.

Water Hyacinths are difficult to destroy. In US, arsenic was used on a large scale which only partially cleared the weeds but poisoned the ecosystem. Fire and explosives were also attempted, but the plants reproduce rapidly even from the tiniest fragment and simply grew back. The most effective measures are biological controls, hundreds of which have been studied for this purpose. Two weevils, a moth and two types of fungi have been introduced to successfully control the plant. Other creatures that keep the plant in check include fish (Chinese grass carp (*Ctenopharyngo idella*) and *Tilapia melanopleura* and *T. mossambica*) and manatees (Sungai Buloh Nature Park, 2001).

The ability of aquatic plants, both living and dead to remove heavy metals has been studied extensively (Brierley, 1990). Some fresh water macrophytes like *Myriophyllum spicatum*, *Potamogeton lucens*, *Salvinia herzogoi*, *Cabomba* sp., *Ceratophyllum demersum* have been investigated for the removal of heavy metals (Schneider and Rubio, 1999). The economic success of a biomass depends to a large extent on the growth rate of the plants. Among all freshwater macrophytes, the floating water hyacinth has the highest growth rate with potential yield of about 500 kg ha^{-1} day^{-1} (Reddy *et al*; 1997)

2.2.3 Biosorbent

Biosorbent is an important material in biosorption process. Biosorption is a metabolism-independent process and thus can be performed by both living and dead cells (Volesky, 1990).A wide range of non-living biomass like bark, lignin, and peanut hulls as well as living biomass like fungi, and, bacteria and, yeast and, moss, aquatic plants and algae and has been used as biosorbents.

Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metal. Some laboratories have used easily available biomass whereas others have isolated specific strains of microorganisms. For example, Machado *et al*, in 2007 have done the research removal of heavy metals using brewer's yeast strain of *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is a species of budding yeast. Brewer's yeast strain was used to remove heavy metals from a synthetic effluent. They also study the flocculation ability of the strain in solid-liquid separation process. The yeast strain was able to sediment in the present of Cu^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+} and Cr^{3+}, which proved that flocculation can be use as the cheap and natural separation process for an enlarge industrial effluents. For biomass concentration higher than 0.5 g/L, more than 95% of the cells have been settle in 5 minutes and this prove that the auto-aggregation of yeast

biomass is a rapid and efficient separation process. But the process have been interrupt with high temperature which the cell will inactivated at 45ºC and it will lose partially flocculation at 80ºC.

Some of the existing raw biomass has been processed to certain degree to improve the biosorption properties. Physical pretreatment methods such as heating, autoclaving, freeze-drying, boiling and chemical pretreatment such as using acids, alkali and organic chemical showed enhancement or reduction in metal biosorption depending upon the biomass and treatment procedures used (Huang *et al*, 1988; Kuyucak & Volesky, 1989; Kapoor & Viraraghvan, 1998).

Bishnoi *et al* in 2006 have done a research upon algal biomass *spirogyra* spp in removal of Cr(III) from aqueous solution. The algal biomass was treated with 0.1 M NaOH, 0.2 M $CaCl_2$ and 5% HCHO. The biosorption efficiency was compared with untreated biomass. The effects of physico-chemical parameters were studied, such as the effect of pH 3.0–6.0, initial metal ions concentration 20–150 mg L^{-1}, algal dose 1.0–3.0 g L^{-1}, and contact time 15–180 min. Biosorption of Cr(III) is highly pH dependent. The maximum biosorption of Cr(III) was obtained at pH 5.0 with untreated and treated biosorbents. Because at lower pH, functional groups like hydroxyl, carboxyl, sulfonate, amine, and phosphate, etc. are present on algal surface spp. *spirogyra* become positively charged due to increase in hydrogen ion concentration responsible for reduction in biosorption capacity (Gupta *et al*, 2006 and Bishnoi *et al*, 2004). The sorption mechanism mainly precedes by ion exchange between cations and existences functional groups within the suitable pH. The maximum metal uptake was observed as 0.2 M $CaCl_2$ treated algal biomass indicate good biosorbents than other treated and untreated biomass.

Not all biosorbents have the ability to remove all the heavy metals. Some of them only remove certain metals only. Schneider and Jorge Rubio in 1999 have run experiments which involved studies of physical and biochemical properties of different dead or dried biomass of macrophyte. The batch sorption was carried out in agitation flasks at laboratory scale. The sorption mechanism by the biomaterials was found to

proceed by ion exchange reaction between metal ions and cationic weak exchanger groups present on the plant surface. In this experiment, the freshwater aquatic plants such as *Potagometon lucens, Salvinia hergozii, Eichhornia crassipes, Myriophyllum brasiliensis, Cabomba sp. and Ceratophyllum demersum* have been used in removing Cr(III), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II). Ivo Andre H. Schneider and Jorge Rubio have proceeded with removal copper from aqueous solution. The biosorption results of copper by the biomass of different macrophytes are shown in Table 2.3.

Table 2.3: Sorption of copper by selected freshwater macrophytes
(Ivo Andre H. Schneider & Jorge Rubio, 1999)

Aquatic macrophyte	**Initial concentration (mg/L)**	**Final concentration (mg/L)**	**Removal (%)**
Potamogeton lucens	6.3	0.3	95
Salvinia herzogii	6.3	0.4	94
Eichhornia crassipes	6.3	1.3	79
Myriophyllum brasiliensis	6.3	1.4	78
Cabomba sp.	6.3	3.4	46
Ceratophyllum demersum	6.3	3.9	38

(2 g of biosorbent/ L, igitial pH 5.5 ± 0.2, 30 min with agitation)

Schneider and Jorge Rubio (1999) want to mention that ion exchange is the main mechanism in biosorption by biomass of different dead macrophytes. It shows that functional groups such as of carboxyl and hydroxyl groups at dead biomass of macrophyte have been considered responsible to remove the metals especially copper from aqueous solution at suitable pH. The carboxyl groups appear to be related to the protein content in the tissue of plants and from the experiment show that *P. lucens* have the highest protein content. The metals binding probably occur with carboxyl groups that present in glutamic and aspartic amino acids of the protein chains. The present of anionic

functional groups inside the biomass such as phosphoryl, carboxyl, carbonyl, sulfydryl and hydroxyl groups are able to immobilize metal ions (Volesky 1987). This mechanism has also been proposed for metal binding properties of *Sphagnum* mosses (Breuer and Melzer, 1990) and marine algae (Leusch *et al*, 1995).

Figure 2.3 shows the effect of pH on sorption of copper onto *P.lucens, S. herzogii* and *E. crassipes* biomass. Maximum removal of Cu^{2+} was attained between pH 5.5 and pH 6.6. The Cu^{2+} residual species after the sorption, 0.3 mg/L *for P. lucens*, 0.4 mg/L for *S. herzogii* and 1.3 mg/L for *E. crassipes*, represents 99.8% of the total species present in aqueous solution at pH 5.5. No sorption occurs at very low pH values because for lower pH values (2-3) can be explained because H^+ ions were present in high concentration and compete with Cu^{2+} ions for the binding sites.

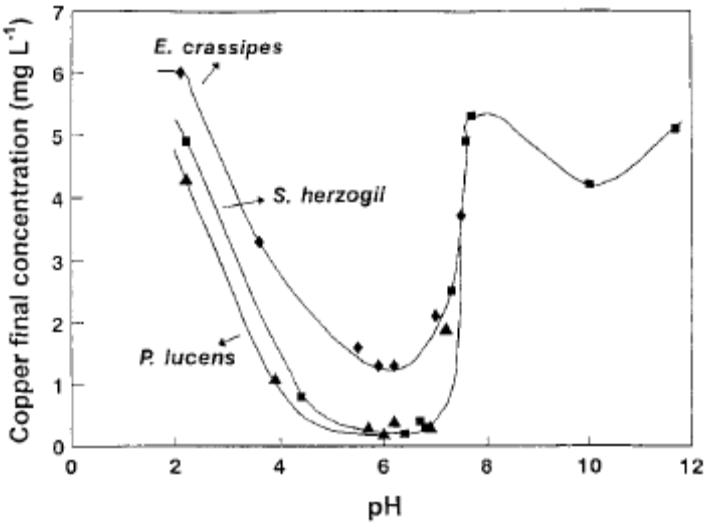


Figure 2.3: Effect of pH on copper sorption by *P. lucens, S. herzogii* and *E. crassipes* biomass. (Schneider & Jorge Rubio, 1999)

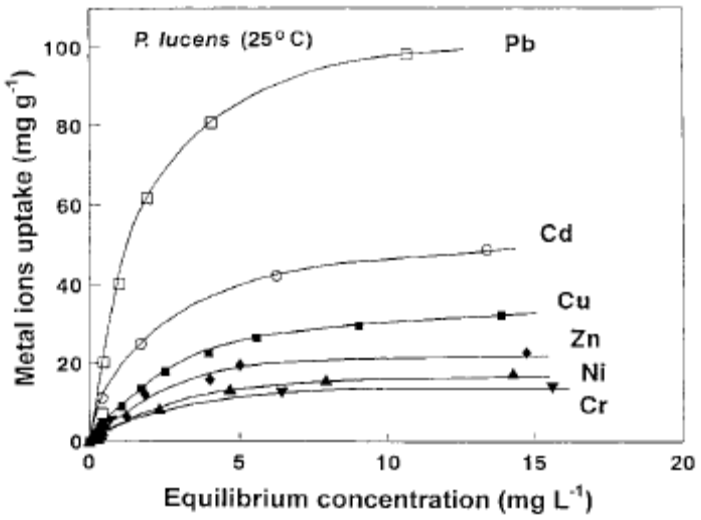


Figure 2.4: Sorption isotherm of Cr(III), Ni, Cu, Zn, Cd and Pb on *P. lucens*biomass (initial pH 5.5 ±0.2). (Ivo Andre H. Schneider & Jorge Rubio, 1999)

Figure 2.4 shows that the highest metal ions removed by *P. lucens* is Pb. *P.lucens* remove mostly 100 mg/g ions of Pb. Cr(III) is the lowest metal ions removed by *P. lucens*. Dried biomass of *P. lucens*, *S. herzogii* and *E. crassipes* were tested with each of metal solutions. Sorption isotherms were measured at 25ºC by varying initial metal ion concentration and keeping the biosorbent mass constant. Ivo Andre H. Schneider and Jorge Rubio (1999) found that the *P. lucens* are greater than *S. herzogii* and *E. crassipes* as biosorbent.

Some of the physical and biochemical properties of the dried biomass of *P. lucens, S. herzogii* and *E. crassipes* are summarized in Table 2.4.

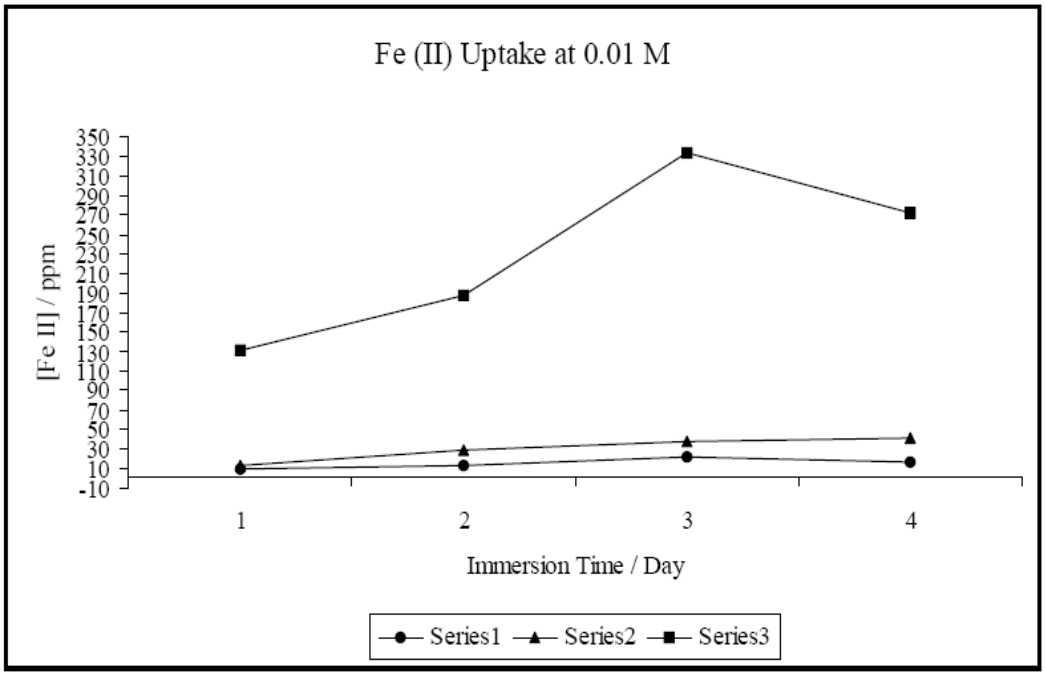
Table 2.4: Physical and biochemical properties of the biosorbents (Schneider & Jorge Rubio, 1999)

Properties	**E. crassipes**	**S. herzogii**	**P. lucens**
Partical size	< 0.59 mm	<0.59	<0.59
Bulk ensity	0.13 g/cm^3	0.13 g/cm^3	0.15g/m^3
Density	1.1 g/m^3	1.1 g/m^3	1.2 g/m^3
Water retention	3.2 g/g	4.2 g/g	3.1 g/g
Specific surface area	250 m^2/g	270 m^2/g	415 m^2/g
Proteins	10.0 %	11.5%	21.7%
Carbohydrates	69.0 %	77.2%	66.0%
Lipids	0.7 %	1.1%	0.9%
Ash	20.3 %	10.2%	11.4%
Ion exchanger behavior	Cationic weak	Cationic weak	Cationic weak
Carboxyl groups	0.7 mequiv/g	0.9 mequiv/g	1.5 mequiv/g
Phenolic groups	0.9 mequiv/g	2.2 mequiv/g	1.3 mequiv/g

.

David *et al.* (2002) have done a research for iron removal from industrial waters by water hyacinth (*Eichhornia crassipes*). In studying the iron uptake process, the experiment was carried with hydrophonic water hyacinth. A preliminary study of iron uptake by water hyacinth similar to that done with copper uptake (Aung and Win, 1994). The water hyacinth has been cut into three segments (roots, petiole and leaves).From the experiment, David *et al* (2002) have indicated that there were three main types of graph pattern in iron uptake;

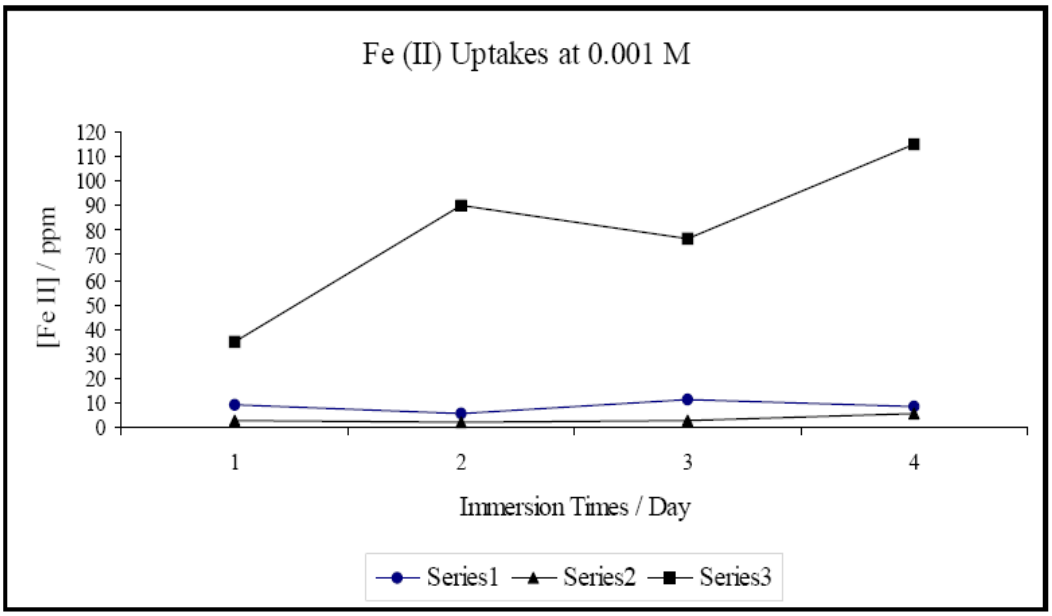
- A fast rising show in initial uptake which means that iron was essential for plant metabolism.
- Declining traces due to saturation of cells with iron.
- Oscillate traces means that the traces with alternate rising and falling (absorp and desorp process will alternate continuously).



series 1=leaf, series 2=petiole, series 3=roots

Figure 2.5: Iron (II) uptake at 0.01 M test solution concentration

(David *et al*, 2002)



series 1=leaf, series 2=petiole, series 3=roots

Figure 2.6: Iron (II) uptake at 0.001 M test solution concentration

(David *et al*. 2002)

From Figure 2.5 iron content of the roots rapidly at concentration 0.01 M to the third day and dropped in fourth day. A same type of curve was seen for the leaves and for petiole it just starting to become saturated towards day 3 and day 4. In Figure 2.6, the oscillating type curve was observed for roots and leaves. It shows that the petiole iron content was much lower than iron content in leaves when concentration of the solution was 0.001 M and it was higher than leaf when the concentration of solution was 0.01 M. This showed that iron was tending to transport to leaves initially. Iron accumulate in petiole occurred only when the leaves become saturated. Obviously, it was found that absorption of iron was predominantly in the roots because iron content in roots was highest than in leaves and petiole in both concentration (0.01 M and 0.001 M).

Kaustubha Mohanty *et al* (2003) in their research have use the biomass (both roots and stems) nonliving Eichhornia crassipes as biosorbent to remove Cr(VI) from solution. The effect of physiso-chemical parameters like pH, sorbent dose, contact time and initial concentration were investigated. The Freundllich isotherm was found to

represent the measured sorption data very well. To confirm the type of functional groups, infrared spectra of the native and chromium-loaded were obtained using a Fourier transform infrared spectrometer. The FT-IR showed that the hydroxyl group was the chromium-binding site within pH range (pH 1-5) where chromium does not participate. Peak observed at 3419 cm^{-1} is indicative of the existence of bonded hydroxyl group and peak observed at 1646 cm^{-1} have assigned to the C=C group. There were significant shifts of absorption peaks can be seen when comparing the FT-IR spectra of native and chromium-loaded biomass in Figure 2.7 and Figure 2.8.

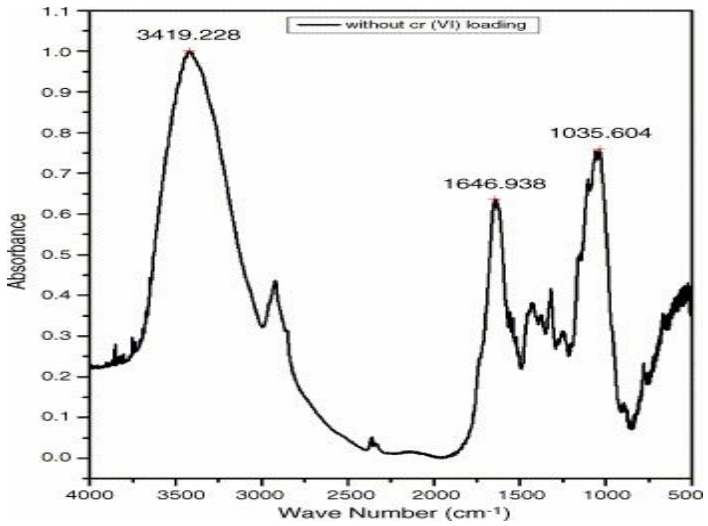


Figure 2.7: FT-IR spectra of native biomass

(Kaustubha Mohanty *et al*, 2003)

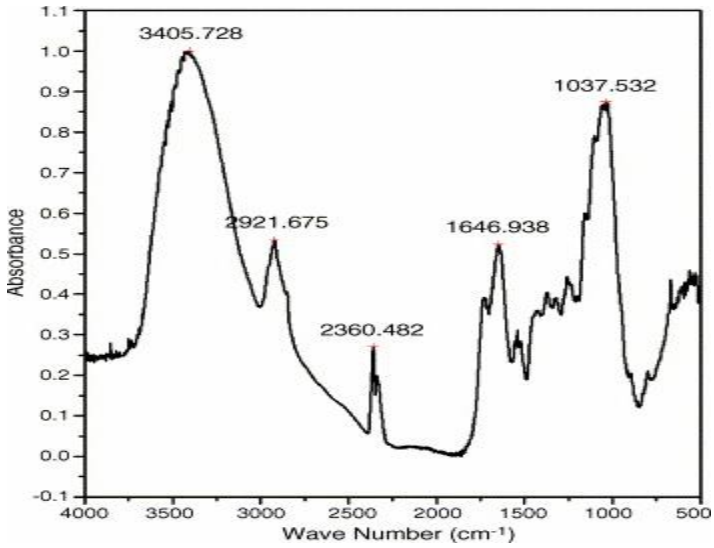
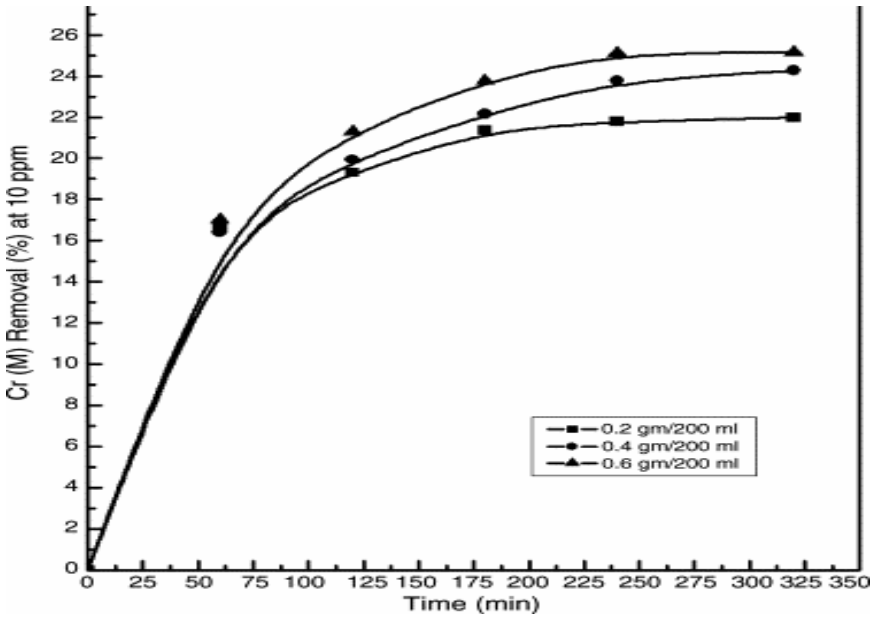


Figure 2.8: FT-IR spectra of chromium loaded biomass

(Kaustubha Mohanty *et al*, 2003)

For kinetic studies, batch sorption studies were conducted in conical flasks at natural pH 5.85. Then dry biomass with different weight (0.2, 0.4, 0.6g) was mixed individually with 200 mL of chromium solution with concentration 10mg/L and it were shaken in incubator-shaker at room temperature(25ºC). Samples were drawn from the conical flask at required time and filtered through Whattman No. 1 filter paper. Then the filtrates were analyzed. Results of percent chromium removal as a function of time at three different initial sorbent doses have been shown on Figure 2.9.



(Initial Cr(VI) concentration = 10 ppm)

Figure 2.9: Percentage removal of chromium with effect of time

(Kaustubha Mohanty *et al*, 2003)

The figure show that the sorption of Cr(VI) increases with time (0-240 min) and after that it become constant to the end of the experiment. From the figure it shows that the rate of Cr(VI) binding with the biomass is more at initial stages then gradually decreases and remains almost constant after an optimum period of 240 min for 10 mg/L initial concentration. Kaustubha Mohanty and members were told that the pattern of curve for initial concentration of 20 mg/L and 30 mg/L as a function of time.

The effect of sorbent dosages on the percentage removal of chromium has been shown in Figure 2.10. Percentage sorption increase when sorbent dosages were increase and reach a saturation level at high doses. The removal of chromium increased from 73% to 89% for initial concentration of 10 mg/L, 70% to 88% for initial concentration of 20 mg/L and 67%- 87% for initial concentration of 30 mg/L with changes of dosage from 0.05 g to 0.2 g. The increase in removal efficiency is obvious, as the doses of adsorbent increases, the surface area available is more to adsorb the Cr(VI).

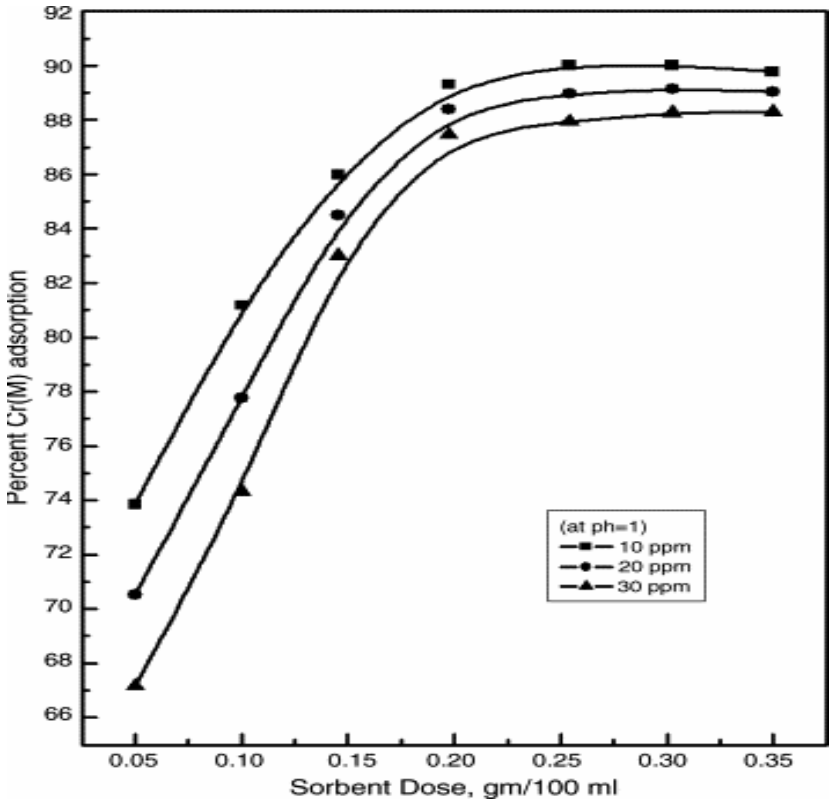


Figure 2.10: Effect of sorbent dosages on the percentage removal of chromium.

(Kaustubha Mohanty *et al*, 2003)

Solution pH is an important parameter affecting biosorption of heavy metals. Figure 2.11 shows the removal of Cr(VI) as function of pH at different sorbent doses;

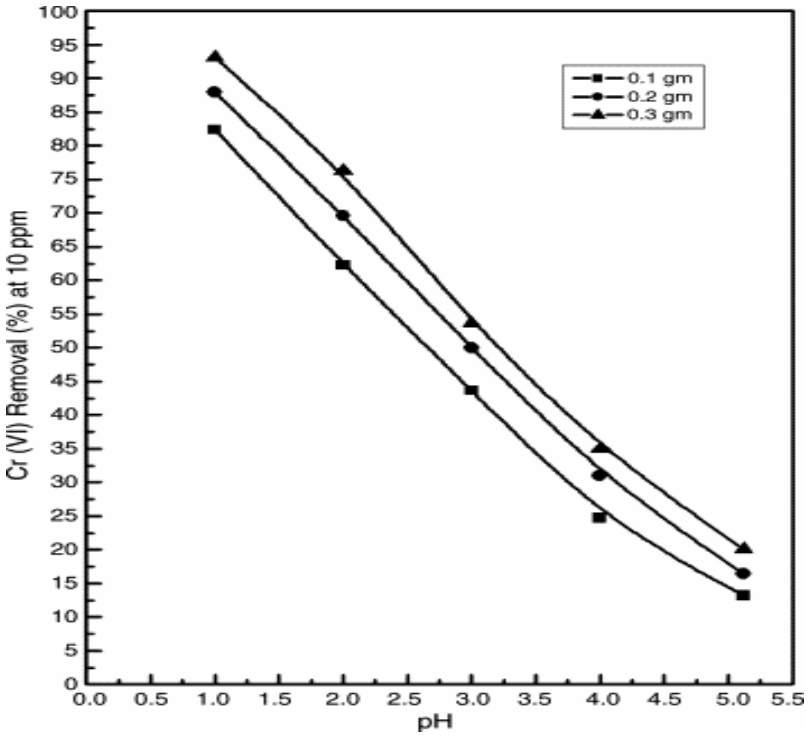


Figure 2.11: Effect of pH for initial Cr(VI) concentration = 10 ppm

(Kaustubha Mohanty *et al*, 2003)

From the figure the percent removal of Cr(VI) is maximum for all the concentration at pH 1.0 and decrease with increasing of pH. Kaustubha and members mention that this behavior can be attributed to the presence of a large number of surface functional groups on cell wall of the biomass. The pH dependence of metal adsorption can largely be related to type and ionic state of these functional groups and also on the metal chemistry in solution (Matheickel *et al*, 1999). They have concluded that biomass of water hyacinth is suitable for development of efficient biosorbent for remove chromium and physico-chemical characteristic of the developed biosorbent were characterized and show that the parameters are affecting the biosorption process.

Low *et al.* (1994) have investigated the potential of the biomass of nonliving, dried and roots of water hyacinth to remove basic dye, methylene blue and Victoria blue. Parameters studied included pH, sorbent dosage, contact time and initial concentrations. They also make a comparative sorption of dye by different sorbents. They used activated

carbon, moss, water hyacinth roots and banana pith to remove metylene blue. Table 2.5 shows the percent of removal of methylene blue by different sorbents;

Table 2.5: Sorption of methylene blue by different sorbents (Low *et al*, 1994)

Sorbent	**Uptake (%)**
Activated carbon	99.9
Moss	97.7
Water hyacinth roots	94.9
Banana pith	92.2

It shows that all sorbents have the great sorption capacity for metyhlene blue. Water hyacinth roots and the other biosorbents could be cheaply and readily available; they could make a significant contribution to the removal of related dyes in full-scale aqueous systems. The uptake was unaffected in the pH range of 5-12. At low pH sorption was unfavorable because of the excess H^+ ions competing for sorption sites on the tissue. A similar observation was also reported by Perineau et al (1983) in their study on the adsorption of ionic dyes on charred plant materials. The effect of pH on sorption of methylene blue on water hyacinth roots are shown in Table 2.6.

Table 2.6: Effect of pH on the uptake of methylene blue by water hyacinth roots. (Low *et al*, 1994)

pH	**Uptake (%)**
1.99	46.46
3.49	91.72
6.34	96.44
9.62	97.09
11.96	96.99

For the effect of biosorbent dosage, it followed the predicted pattern of increasing percentage sorption as the biosorbent dosage increased. The sorbent dosage was varied from 0.1 g to 1.0 g using a fixed volume of 50 mL of 500 mg/L of dye solution. The effect of sorbent dosage on methylene blue sorption is shown in Figure 2.12.

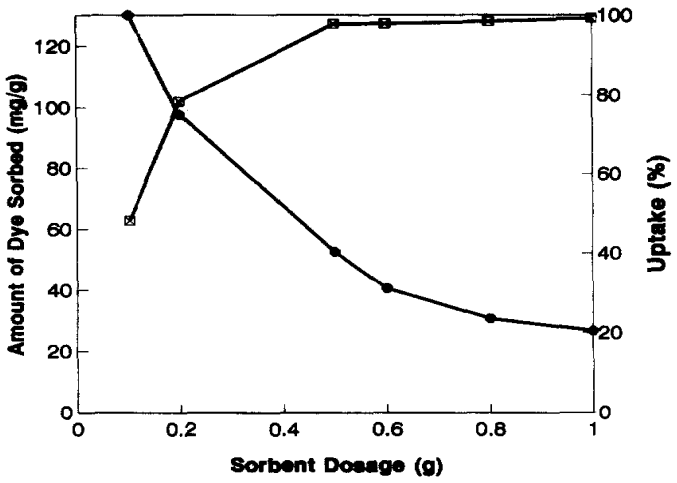


Figure 2.12: Sorption of methylene blue by dried biomass of water hyacinth as a function of sorbent dosage. (Low *et al*, 1994)

Low *et al.* (1994) and members have studied the effect of contact time and initial concentration to sorption of methylene blue by dried biomass of water hyacinth. To study contact time, they have used methylene blue, Victoria blue, basic blue, acid blue and reactive yellow. The rate of removal of those type of dyes are high at the initial sorption process then become constant until the end of experiments because the sorbents have been saturated. For their research the Langrnuir isotherm was found to represent the measured sorption data well. They concluded that water hyacinth roots could represent a cheap source of biosorbent for basic dyes as they are readily available in great abundance.

Kumar *et al* (2002) have study the accumulation of lead (Pb), zinc (Zn) and copper (Cu) in the edible aquatic plants *Trapa bispinosa* Roxb. and *Nelumbo nucifera* Geartn. Water chestnut (*Trapa bispinosa* Roxb, Trapaceac) and Indian lotus (*Nelumbo*

nucifera Geartn, Nympheaceae) is cultivated in wetlands of India. The region have known for galvanizing and electroplating industry, steel factories, mining and metal processing, cement factories and agriculture activities. The plant part of *N. nucifera* and *T. bispinosa* were oven dried, cut into small pieces, and digested in clean Kjeldahl digestes tubes in sulphuric acid peroxide solution through heating at different temperature and contact time in drying oven. Metal analysis for Cu, Zn, Pb and Cd was carried out by using atomic absorption spectrophotometry. As the result, the concentration of Cd was high at pericarp. The concentration of Pb was found high at cotelydon. The accumulation of Cu and Zn was high at rhizomes, fruiting torus and carpels. Accumulation of heavy metals has been reported in vegetables such as spinach, leek, cabbage lettuce and many in industrial areas (Fyatianos et al, 2001). For the same propose *Ipomoea aquatica* (water spinach) also have been use in accumulation of metals (Fe, Cu, Mn, Cr and Pb) in wetland of the India by Upendra N. Rai and Sarita Sinha in 1999.

Many of the freshwater aquatic plants have showed the potential in removing different metals from the aquatic environment or from the aqueous solutions. However removing process has demonstrated that the incorporation of heavy metals produces phytotoxic effects on plants resulting in inhibition of chlorophyll synthesis, it will decrease in biomass production and cause plant necrosis and also lead to death (Satyakala and Jamil, 1992; Delgado *et al*, 1993). Conversely, the metal sorption capacity of the dried or dead biomass of aquatic plants has been recently recognized. The main advantages in using the dead biomass appear to be following:

- Prevent the problem of metal toxicity on plant metabolism, plant
. deterioration, odor liberation and insect proliferation
- Dried biomass present advantages for conservation, transport and handling
- Possible too recover the sorbed heavy metals by elution techniques using sorption or desorption cycles (Schneider and Rubio, 1999).

All aquatic plants are not equally effective for removal of heavy metals. Plants such as *Phragmites cornmunis*, *Scirpus lacustris*, *Eichhornia crassipes*, *Spirodela polyrrhiza*, *Elodea canadensis*, *Egeria densa*, *HydriUa* sp., *Ceratophyllum demersum*, *Bacopa monnieri*, *Limnanthernum cristatum* and the algal macrophyte, *Hydrodictyon reticulatum*, have been found suitable for the removal of different metals (Wolverton et al., 1975; Rai and Chandra, 1989, Rai and Chandra, 1992; Garg and Chandra, 1990; Sinha and Chandra, 1990; Tripathi and Chandra, 1991).

2.2.4 Biosorption mechanisms

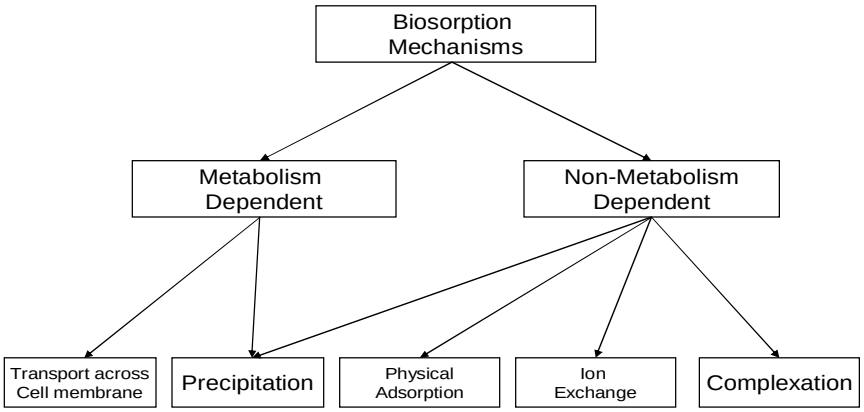


Figure 2.14: Biosorption mechanisms classified according to the dependence on the cell's metabolism (F. Veglio *et al*. 1997)

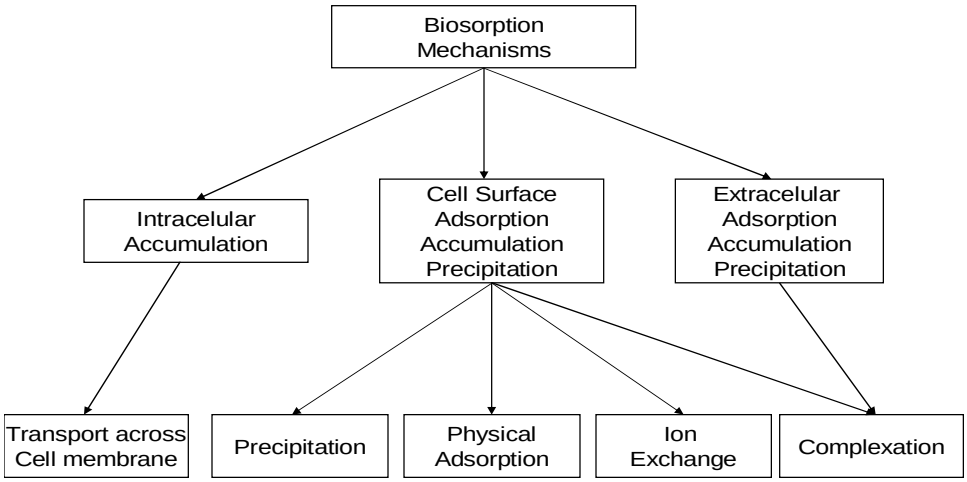


Figure 2.15: Biosorption mechanisms classified according to the location where the metal removed is found (F. Veglio *et al*. 1997)

The complexity of the microorganism's structure implies that there are many ways for the metal to be captured by the cell. Biosorption mechanisms are therefore various and in some cases they are still not very well understood. They may be classified by the following different criteria. According to the dependence on the cells' metabolism, biosorption mechanisms can be divided into:

- Metabolism dependent;
- Non-metabolism dependent.

According to the location where the metal removed from the solution is found, biosorption may be classified as:

- Extracellular accumulation/precipitation;
- Cell surface sorption/precipitation;
- Intracellular accumulation.

Figure 2.14 and Figure 2.15 show schematically the various biosorption mechanisms and location of removed metal found. Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cells metabolism. This implies that this kind of biosorption may take place only with viable cells. It is often associated with an active defense system of microorganisms, which react in the presence of a toxic metal. Obviously, in this case biosorption is not immediate, since it requires the time for the reaction of the microorganism.

In the case of physicochemical interaction between the metal and functional groups of the cell surface, based on physical adsorption, ion exchange and complexation, we have cell surface sorption, which is not dependent on the metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids, offer particularly abundant metal-binding functional groups, such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. This physicochemical phenomenon of metal biosorption, non-metabolism dependent, is relatively rapid and can be reversible (Kuyucak *et al*, 1988). In the presence of such a mechanism, which fortunately is the most common, biomass has all the chemical characteristics of an ion exchange resin or of an activated carbon, implying many advantages in the industrial application of biosorption.

In the case of precipitation, the classification is not unique. In fact the precipitation of the metal may take place both in solution and on the cell surface (Ercole *et al*, 1994). Furthermore, it may be dependent on the cells' metabolism if, in the presence of toxic metals, the microorganism produces compounds which favor the precipitation process. On the other hand, precipitation may not be dependent on the cells' metabolism, occurring after a chemical interaction between the metal and the cell surface. Below, examples found in literature for each mechanism are reported.

2.2.4.1 Transport across the cell membrane

As mention in Figure 2.14, this mechanism is associated with cell metabolism. Unfortunately, it is the toxicity of some elements which does not allow investigation of biosorption in the presence of high metal concentrations. In fact, little information is available about this kind of mechanism. Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically essential ions, such as potassium, magnesium and sodium. The metal transport system may become confused by the presence of heavy metal ions of the same charge and ionic radius (Brierley *et al*, 1990).

This kind of mechanism often takes place contemporary to biosorption phenomena not linked to metabolic activity. There are many examples in the literature where biosorption by living microorganisms comprises two basic steps. Firstly, an independent metabolism binding to cell walls and secondly, metabolism-dependent intracellular uptake, whereby metal ions are transported across the cell membrane into the cell (Costa *et al*, 1990; Gadd *et al*, 1988; Gourdon *et al*, 1988; Huang *et al*, 1990).

2.2.4.2 Physical adsorption

This mechanism associated with the presence of van der Waals' forces are included (Crowell *et al*, 1966). Tsezos and Volesky in 1982 have been verified that thorium and uranium biosorption by fungal biomass of *Rhizopus arrhizus* is also based on physical adsorption in the cell-wall chitin structure. The hypothesis that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomass of algae, fungi and yeasts takes place through electrostatic interactions between ions in solution and cells walls (Kuyucak *et al*, 1988). Electrostatic interactions have been demonstrated to be responsible for copper biosorption by bacterium *Zoogloea ramigera* and alga *Chlorella vulgaris* (Aksu *et al*, 1992), for chromium biosorption by fungi *Ganoderma lucidum* and

Aspergillus niger as well as for cadmium biosorption by marine algae (Venkobachar *et al*, 1990; Holan *et al*, 1993). Physical adsorption is furthermore responsible for copper, nickel, zinc, cadmium and lead biosorption by *Rhizopus arrhizus* (Zhou *et al*, 1991; Fourest *et al*, 1992).

2.2.4.3 Ion exchange

Cell walls of microorganisms contain polysaccharides as basic building blocks. The ion exchange properties of natural polysaccharides have been studied in detail and it is a well established fact that bivalent metal ions exchange with counter ions of the polysaccharides (Tsezos *et al*, 1982). Alginates of marine algae usually occur as natural salts of K^+, Na^+, Ca^{2+} and Mg^{2+}. These metallic ions can exchange with the counter ions such as Co^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+}, resulting in the biosorption uptake of the metals (Kuyucak *et al*, 1988). The uptake of uranium and lead by *Streptomyces longwoodensis* takes place through ion exchange between metal ions and phosphodiester residue counterions, both present in the cell wall and cytoplasmic fractions (Friis *et al*, 1986). Ion exchange was also found to be responsible for copper biosorption by fungi *Ganoderma lucidum* and *Aspergillus niger* (Muraleedharan *et al*, 1990; Venkobachar *et al*, 1990).

2.2.4.4 Complexation

The metal removal from solution may also take place through complex formation on the cell surface after interaction between the metal and active groups. Metal ions can bind to ligands or through chelation (Cabral *et al*, 1992). Thorium and uranium biosorption by *Rhizopus arrhizus* has a mechanism not only based on physical adsorption, as above mentioned, but also on complex formations, metals coordinate with the nitrogen of the chitin cell wall network (Tsezos *et al*, 1982). Aksu *et al* in 1992 also hypothesized that biosorption of copper by *C. vulgar* and *Z. ramigera* takes place through

both adsorption and coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides. Complexation was found to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by *Pseudomonas syringae* (Cabral *et al*, 1992).

2.2.4.5 Precipitation

Precipitation may be either dependent on the cellular metabolism or independent of it. In the former case, the metal removal from solution is often associated with an active defense system of microorganisms. They react in the presence of a toxic metal, producing compounds which favor the precipitation process. Scott and Palmer in 1992 have found that cadmium elimination from solution by some *Arthrobacter* and *Pseudomonas* species was determined by detoxification systems that precipitate cadmium on the cell surface. In the case of precipitation not dependent on the cellular metabolism, it may be a consequence of the chemical interaction between the metal and the cell surface. This phenomenon is the terminal step of uranium biosorption by *Rhizopus arrhizus* (Tsezos *et al*, 1982); the formation of the complex uranium-chitin was followed by the complex hydrolysis and the precipitation of the hydrolysis product (uranyl hydroxide) in the cell wall. Holan *et al.* (1993) proposed that an additional mechanism, such as entrapment of metals in the form of insoluble micro deposits, can greatly contribute to cadmium biosorption by the biomass of marine algae.

It is evident from the literature mentioned that biosorption mechanisms are not only various, but they can also take place simultaneously. A global analysis of the results found in literature suggests the following about the influence of operating conditions on the equilibrium of the biosorption process:

- Temperature seems not to influence biosorption performances in the range 20°C - 35°C (Aksu *et al*, 1992).

- pH seems to be the most important factor in the biosorption process: it affects the solution chemistry of the metals, the activity of functional groups in the biomass, and the competition of metallic ions (Galun *et al*, 1987; Ramelow *et al*, 1992).

- Biomass concentration in solution seems to influence the specific uptake: for lower values of biomass concentrations there is an increase in the specific uptake (Fourest *et al*, 1992; Gadd *et al*, 1988). Gadd *et al* have suggested that an increase in biomass concentration leads to interference between binding sites. Fourest and Roux invalidated this hypothesis, attributing the responsibility of the specific uptake decrease to metal concentration shortage in solution. This factor would, however, need to be taken into account in any application of biomass as adsorbent;

- Biosorption is in some cases selective. This aspect has to be investigated in detail. Biosorption is mainly applied to treat waste water containing metal ions and the removal of one metal may be influenced by the presence of other metals. Thorium uptake by *Rhizopus arrhizus* was not influenced by the presence of other ions, such as Fe^{2+} and Zn^{2+} in solution (Tsezos *et al*, 1982). Uranium uptake by biomass of some bacteria, fungi and yeasts was not affected by the presence of manganese, cobalt, copper, cadmium, mercury and lead in solution (Sakaguchi *et al*, 1991). In contrast, the presence of Fe^{2+} and Zn^{2+} was found to influence uranium uptake by *Rhizopus arrhizus* and cobalt uptake by different microorganisms seemed to be completely inhibited by the presence of uranium, lead, mercury and copper (Sakaguchi *et al*, 1991; Tsezos *et al*, 1982).

CHAPTER 3

METHODOLOGY

3.1 Introduction

Collected water hyacinths have been washed by tap water, and then dry it in the oven. Dried water hyacinth was blended into powder form by using blender. Prepare the 1000 mg/L of ferrum stock solution by mixing ferrous sulphate powder with distilled water and then dilute the stock solution to make 10 mg/L working solution.

There are four parameters that have been studied in removal of ferrum by dried water hyacinth from aqueous solution which are effect of biosorbent dosage, contact time, temperature and pH. Experiments were carried out in 250 mL conical flasks with varying the amount of biosorbent dosage from 0.0 g to 1.0 g. The biosorbent were mixed individually with 100 mL of ferrum solution with initial concentration is 10 mg/L. In incubator shaker, the samples were shaking with mixing speed at 175 rpm and temperature was ranged from 25°C to 80°C. Vary the contact time from 0 to 150 minutes with 30 minutes interval time. pH of ferrum solution was adjusted from 2 to 10 using hydrochloric acid and sodium hydroxide. HACH Spectrophotometer (Model DR/2400) was used to measure the concentration of ferrum solution before and after treatment for each experiment.

(a)

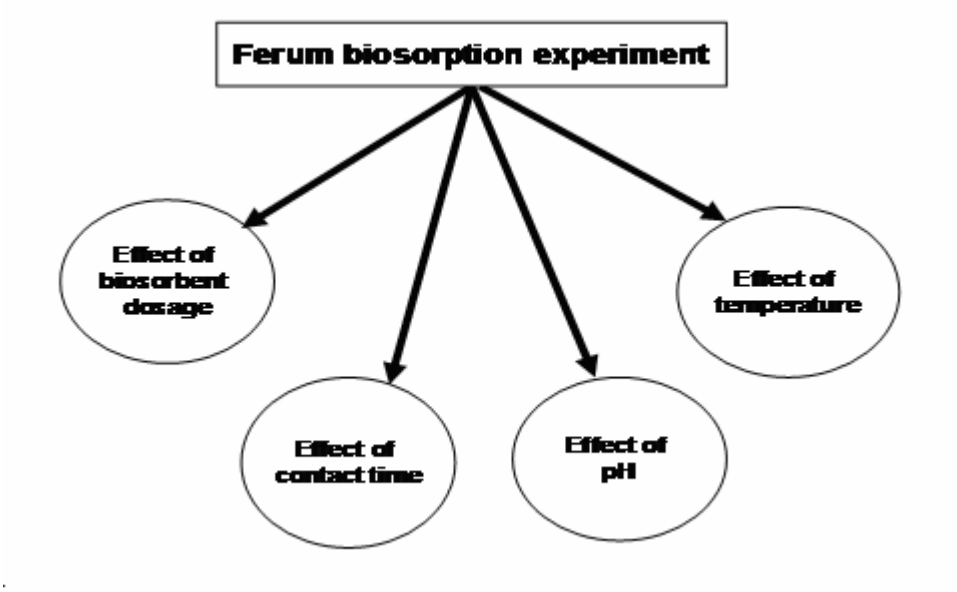


Figure 3.1: Parameters for ferrum biosorption experiment

(b)

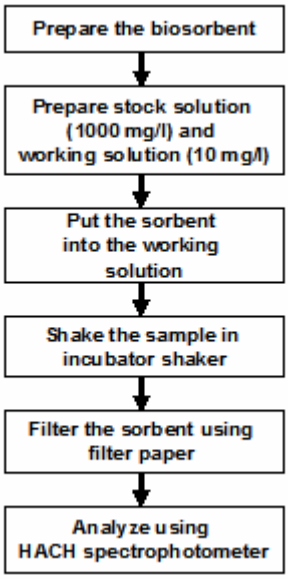


Figure 3.2: Main steps of methodology

(c)

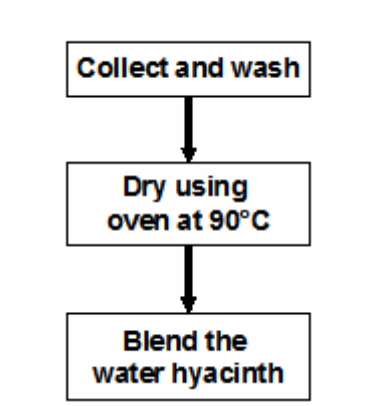


Figure 3.3: Preparation of dried water hyacinth

3.2 Equipments/apparatus

(a) HACH Spectrophotometer (Model DR/2400)
(b) Incubator shaker
(c) Oven
(d) pH meter
(e) 500 mL volumetric flask
(f) 250 mL conical flask
(g) Sample cell (I-inch square, 10 mL)
(h) Filter paper

3.3 Reagents

(a) FerroVer® Method Reagent Powder Pillows
(b) Ferrous sulphate powder
(c) Dried water hyacinth

3.4 Preparation of biosorbent

Water hyacinths were collected from the Pahang River at Pekan, Pahang and washed with tap water and dried under the sunlight and then dried at 60ºC for 24 hours. The dry water hyacinths were blended using blender to make them into powder form. This powder of water hyacinth was the biosorbent for the removal of ferrum ions from aqueous solution.

3.5 Preparation of ferrum solution

.

All chemicals used were of analytical-reagent grade. 1000 mg/L ferrum stock solution was prepared by dissolving 1000 mg ferrous sulfate in 1 L of distilled water in volumetric flask. 10 mg/L working solution of was prepared by diluting 1 mL of stock solution with ratio 1:100.

3.6 Ferrum biosorption experiment

3.6.1 Effect of biosorbent dosage

Amount of biosorbent (water hyacinth) was varying from 0.2 g to 1.0 g into 100 mL of ferrum solution in conical flask (250 mL). Initial concentration of the ferrum working solution is 10 mg/L and pH of the solution was recorded at 5.83. Shake the samples in incubator shaker for 30 minutes at 25ºC. Mixing speed of the shaker was 175 rpm.

3.6.2 Effect of contact time

This experiment was done with each sample has different contact time varying from 0 to 150 minutes with 30 minutes interval time. 1.0 g of biosorbent was put into 10 mg/L of the ferrum working solution (pH = 5.83). Shake the samples in incubator shaker with temperature 25ºC at 175 rpm.

3.6.3 Effect of pH

This experiment was done at pH 2.0, 4.0, 6.0, 8.0 and 10 in 100 mL of ferrum working solutions. The initial concentration of the solutions was 10 mg/L. The pH value of the solution was adjusted using hydrochloric acid (HCl) and potassium hydroxide (NaOH). Amount of biosorbent used for each samples was 1.0 g. Set the temperature in the incubator shaker at 25ºC and shake the samples for 30 minutes at 175 rpm

3.6.4 Effect of temperature

This experiment was done at temperature 25ºC, 30ºC, 40ºC, 60ºC and 80ºC. Put 1.0 g of biosorbent into 100 mL of ferrum working solution in 250 mL conical flask. Initial concentration of the solutions was 10 mg/L and pH value of the solutions was 5.83. Shake the samples with 30 minutes contact time at 175 rpm.

Table 3.1: Summary of the experiment parameter

Parameters	**Variables**
1. Biosorbent dosage	- biosorbent dosage: 0.0 mg/L - 10 mg/L - contact time: 30 min - pH: 5.83 - temperature: 25ºC - concentration: 10 mg/L - mixing speed: 175 rpm
2. Contact time	- biosorbent dosage: 10 mg/L - contact time: 0-150 min (interval period = 30 min) - pH: 5.83 - temperature: 25ºC - concentration: 10 mg/L - mixing speed: 175 rpm
3. pH	- biosorbent dosage: 10 mg/L - contact time: 30 min - pH: 2.0-10.0 - temperature: 25ºC - concentration: 10 mg/L - mixing speed: 175 rpm
4. Temperature	- biosorbent dosage: 10 mg/L - contact time: 30 min - pH: 5.83 - temperature: 25ºC-80ºC - concentration: 10 mg/L - mixing speed: 175 rpm

3.7 Analysis

Concentration of ferrum (Fe) is determined using absorbance value measured before and after the treatment at 510 nm with HACH Spectrophotometer (Model DR/2400)

CHAPTER 4

RESULT AND DISCUSSION

4.1 Effect of biosorbent dosage

Ferrum ions uptake by nonliving water hyacinth (*Eichhornia crassipes*) was studied using different biosorbent dosage range from 0.0 g to 1.0 g. The effect of biosorbent dosages on the percentage removal of ferrum has been shown in Figure 4.1. It follows the predicted pattern of increasing percentage removal as the increasing biosorbent dosages (Kausthuba Mohanty *et al*, 2005). Percentage of removal increased from 0% to 95.7% for 10 mg/L initial concentration of the solution, when the dosages change from 0 g to 1.0 g. First sample act as control for this experiment. From the result, it shows that the percentage removal of ferrum has been increase instantly from 0% to 89.6% because existence of metal binding site in biosorbent, then the percentage removal was keep increase to 95.7% (1.0 g). The increase of the removal efficiency is obvious, when the dose of biosorbent is increased it will increase the available surface area for bind or adsorb the ferrum ions. The main functional groups that available for metal binding in the biosorbent was identified such carboxyl and hydroxyl (I. A. H. Schneider *et al*, 1999; N. Ahalya *et al*, 2003).

Table 4.1: The effect of biosorbent dosage on Fe removal

(contact time = 30 minutes, temperature = 25ºC, pH = 5.83, mixing speed = 175 rpm)

Sample	Initial Concentration (mg/L)	Amount of biosorbent (mg/L)	Final concentration (mg/L)	% of metal removal
1	10.00	0.00	10.00	0.00
2	10.00	2.00	1.04	89.60
3	10.00	4.00	0.94	90.60
4	10.00	6.00	0.64	93.60
5	10.00	8.00	0.55	94.50
6	10.00	10.00	0.43	95.70

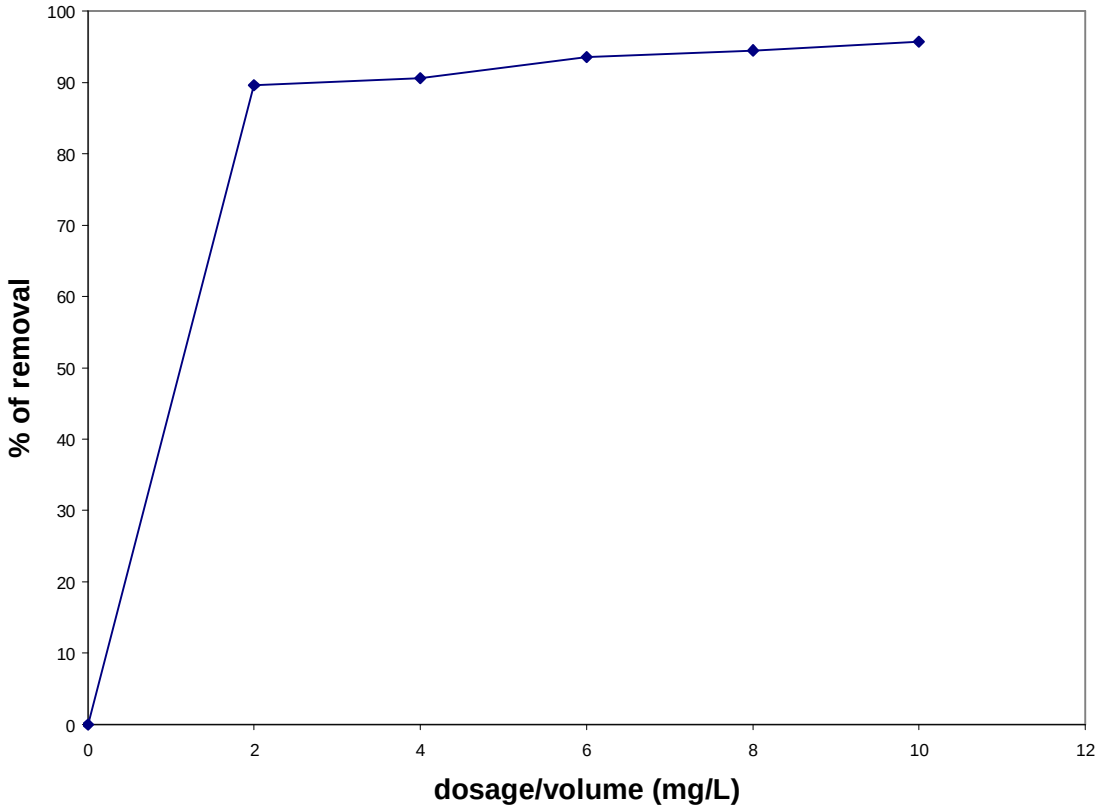


Figure 4.1: Effect of with dosage on Fe removal

(initial Fe concentration = 10 mg/L, temperature = 25°C, contact time = 30 minutes, pH = 5.83)

4.2 Effect of contact time

As illustrated in Figure 4.2, rapid removal of ferrum ion from 0% to 95.4% was observed during the initial 30 minutes of biosorbent contact. Ferrum ions uptake by nonliving water hyacinth was studied using different contact time range from 0 minutes to 150 minutes with 30 minutes interval time. It has increased slowly to 96.9% at 60 minutes contact time. After 60 minutes contact time, the removal process become constant with percentage removal of ferrum ions was 97% (90 minutes), 96.9% (120 minutes) and 97.2% (150 minutes). Optimum contact time was achieved at 60 minutes with 96.9% percentage removal of ferrum ions. This is because the kinetic of metal adsorption which depend on physical adsorption on the cell surface is usually rapid during the early period of contact between the sorbent and sorbate (Sudha Bai R *et al*, 2000; Gourdon *et al*, 1997). The metal binding site of the biosorbent also known as active adsorption site such as carboxyl and hydroxyl was involved ferrum complexation as soon as the biosorbent enter the solution. Therefore, prolonged contact between biosorbent and the ferrum solution will not yield better percentage removal of ferrum ions.

Table 4.2: The effect of contact time on Fe removal
(biosorbent dosage = 10 mg/L, pH = 5.83, temperature = 25°C, mixing speed = 175 rpm)

Sample	Initial Concentration (mg/L)	t (minute)	Final concentration (mg/L)	% of metal removal
1	10.00	0	10.00	0.00
2	10.00	30	0.46	95.40
3	10.00	60	0.31	96.90
4	10.00	90	0.30	97.00
5	10.00	120	0.31	96.90
6	10.00	150	0.28	97.20

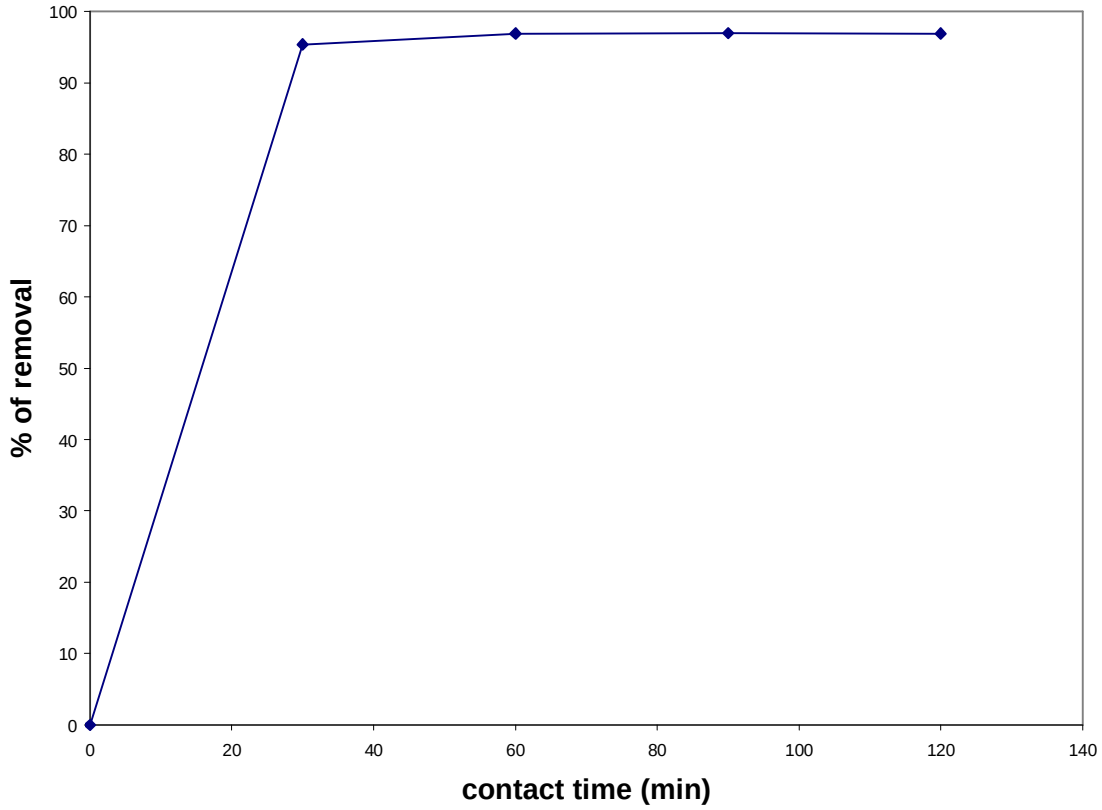


Figure 4.2: Effect of contact time on Fe removal
(initial Fe concentration = 10 mg/L, water hyacinth dosage = 10 mg/L,
temperature = 25°C, pH = 5.83)

4.3 Effect of temperature

Optimum temperature for ferrum removal was observed at 25ºC as show in Figure 4.3. At 25ºC the percentage removal of ferrum ions was 97.2% and at 40ºC percentage of removal was 97.1%. Then the percentage removal of ferrum ions was decreases to 96% at 60ºC and rapidly decreases to 60% at 80ºC. From the result, at temperature of 25ºC to 40ºC are acceptable for biosorption of ferrum ions because the percentage removal of ferrum at both temperatures was higher than the percentage removal at others temperature. From previous research by Sudha Bai R *et al* in 2000 and Eneida Sala Cossich et al in 2002 discussed that increasing temperature from 30ºC to 45ºC have

enhanced the removal of chromium ions by *Rhizopus nigricans and Sargassum* sp. biomass. However, the percentage removal of ferrum ions was decrease when the temperature increases to 60ºC due to the damage of active binding site in biosorbent.

Table 4.3: The effect of temperature on Fe removal
(water hyacinth dosage = 10 mg/L, pH = 5.83, contact time = 30 minutes, mixing speed = 175 rpm)

Sample	Initial Concentration (mg/L)	Temperature (ºC)	Final concentration (mg/L)	% of metal removal
1	10	25	0.28	97.20
2	10	40	0.29	97.10
3	10	60	0.40	96.00
4	10	80	4.00	60.00

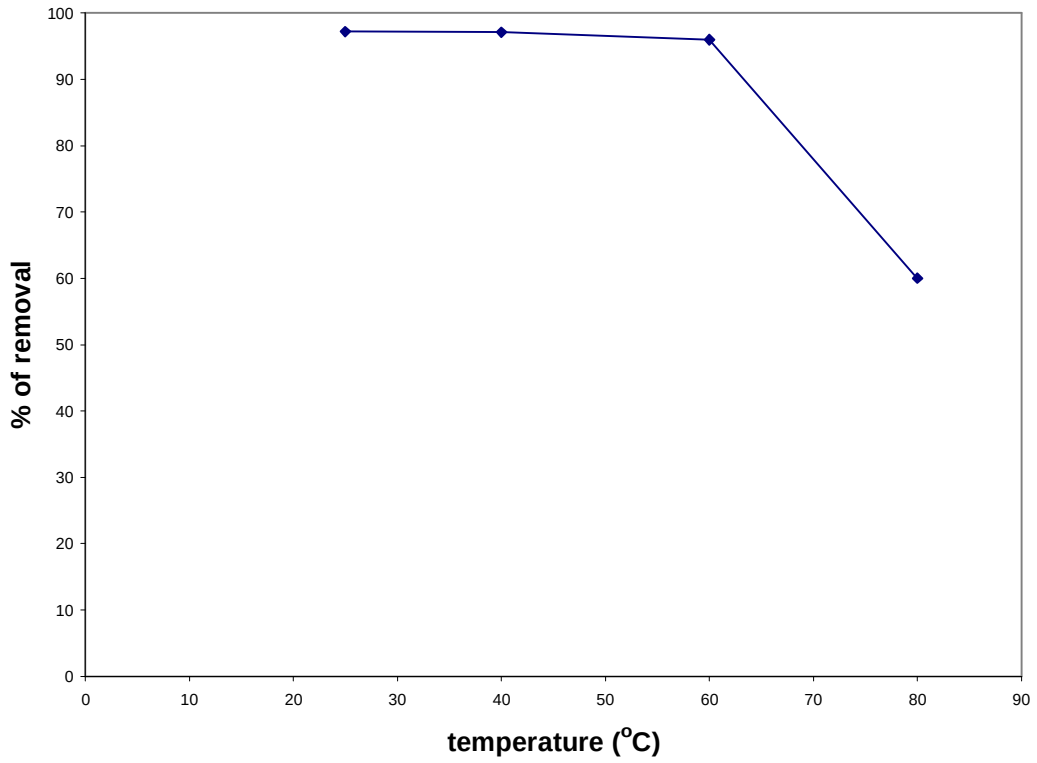


Figure 4.3: Effect of temperature on Fe removal
(initial Fe concentration = 10 mg/L, water hyacinth dosage = 10 mg/L,
contact time = 30 minutes, pH = 5.83)

4.4 Effect of pH

From the study of pH effect, it shows that optimum pH for biosorption of ferrum ions on to nonliving water hyacinth was observed at 6.0. As illustrated in Figure 4.4, 99.3% of ferrum ions from a solution of 10 mg/L had been adsorbed at pH 6.0. This experiment had been done from pH 2.0 to 10.0. At pH 2.0, the percentage removal of ferrum ions from ferrum working solution was 70%. At acidic condition (pH 2.0), concentration of hydrogen ions was too high. There will be the competition between hydrogen ions (H^+) and ferrum ions, so it makes the biosorbent difficult to adsorb the ferrum ions (K. S. Low *et al*, 1995; Perineau *et al*, 1983). From the figure, percentage removal of ferrum ions was increase from pH 2.0 to pH 6.0 near to neutral pH 7.0 and

decrease at pH 8.0 to pH 10.0. At pH 8.0 and pH 10.0 which is alkaline condition, ferrum ions will be precipitate due to high concentration of hydroxide ions (OH^-) in the solution. The reduction metal removal with increasing pH beyond its optimum pH values has attributed to reduced solubility and precipitation (Harris *et al*, 1990; Zhou *et al*, 1991). The adsorption of metal ions depends on solution pH, which influences electrostatic to ions corresponding metal groups (N. Ahalya *et al*, 2005).

Table 4.4: The effect of pH on Fe removal
(water hyacinth dosage = 10 mg/L, contact time = 30 minutes, temperature = 25°C, mixing speed = 175 rpm)

Sample	Initial Concentration (mg/L)	pH	Final concentration (mg/L)	% of metal removal
1	10	2	3.00	70.00
2	10	4	0.59	94.10
3	10	6	0.07	99.30
4	10	8	0.16	98.60
5	10	10	0.40	96.00

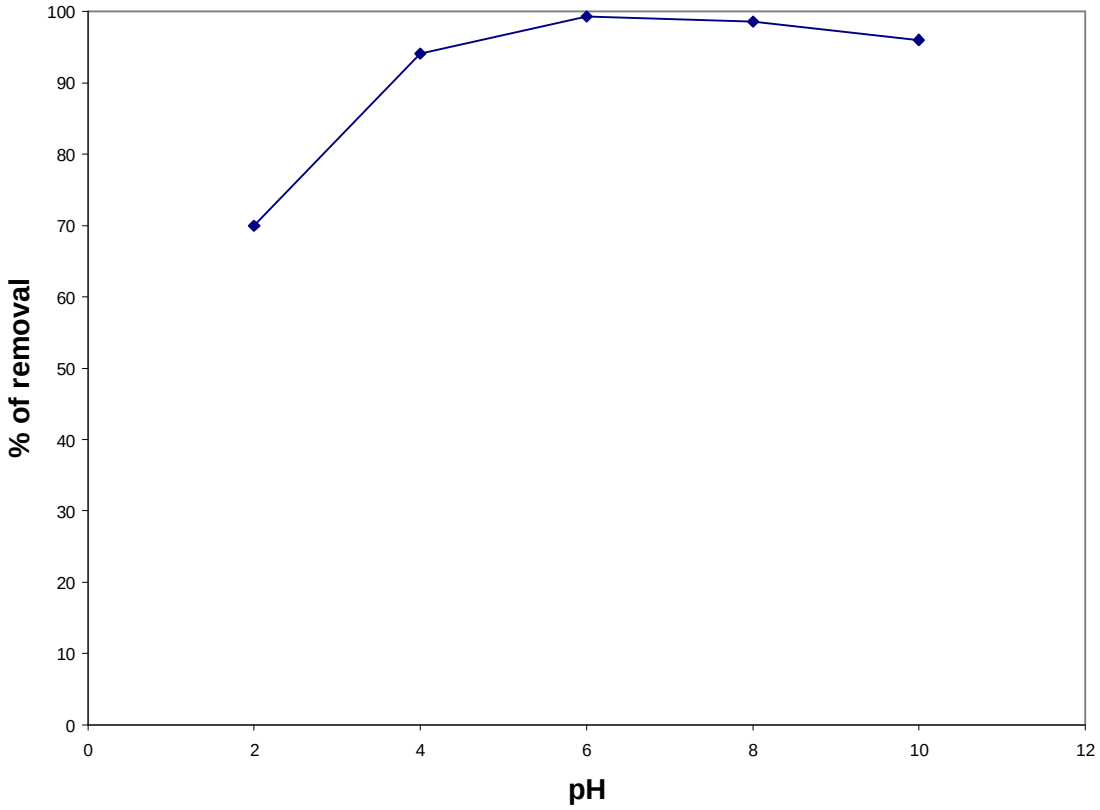


Figure 4.4: Effect of pH on Fe removal

(initial Fe concentration = 10 mg/L, water hyacinth dosage = 10 mg/L, contact time = 25°C, temperature = 25°C)

CHAPTER 5

CONCLUSION & RECOMMENDATION

5.1 Conclusion

As the conclusion, from the study of nonliving water hyacinths we found that water hyacinth were capable to be used as adsorbent for removal of iron (Fe) from aqueous solution. From the study of biosorbent dosage, it shows that percentage of removal can be enhancing by increasing the amount of biosorbent dosage. There will be more metal binding site such as carboxyl and hydroxyl groups that used to adsorb the ferrum ions. Optimum removal of ferrum ions was achieved within 60 minutes. Prolonged contact time not yield better percentage of removal of ferrum ions. It shows the advantage of time effectiveness of nonliving water hyacinth as the biosorbent. For effect of temperature, from the study it shows that the highest removal of ferrum ion was achieved at $25^{o}C$. Increase the temperature will damage the active binding site in the biosorbent. For effect of pH, highest removal of ferrum ions was achieved at 6.0. At acidic and alkaline condition, not yield better percentage of removal because of the high concentration of H^{+} and precipitation of ferrum ions.

5.2 Recommendation

Metal adsorption to the biomass can be manipulated by pretreating the biomass with alkalines, acids or organic solvents which may increase the amount of the metal adsorbed. These types of pretreatment modify the cell surface which is essential for biosorbent either by removing or exposing more metal binding site (Gupta *et al*, 2000) In the case of alkaline pre-treatment, in a study by Galun *et al* (1987) with NaOH pretreatment to the *Pencillium digitatum* have showed enhancement of cadmium, nickel and zinc biosorption. Removal of surface impurities, rupture of cell membrane and exposure of available binding sites for metal biosorption after pretreatment may be the reason for the increase in metal biosorption (Galun *et al*, 1987). Biosorbent can be treating with organic solvents like methanol, dimethyl sulfoxide and formaldehyde (Charumathi *et al*, 2007; Kapoor and Viragavhan, 1998). Huang and Huang (1996) reported that the pretreated biomass with formaldehyde cause methylation of amino groups which reduce the biosorption capacity. From the previous researches, pretreatment of nonliving water hyacinth can be consider because it can enhance the performance of nonliving water hyacinth in removing the ferrum.

The study of nonliving water hyacinth in removing ferrum can be continuing by using another part of the plant such as leaf and root to compare the performance for each part of the plant in removing ferrum from aqueous solution. From previous research by David *et al* in 2002, the removal of iron from industrial water have been done by three separated part; leaf, root and petiole. From the research resulted that iron accumulated mainly in roots of water hyacinth.

REFERENCES

Aksu Z., Sag Y. and Kutsal T. (1992), The biosorption of copper(II) by *C. vulgaris* and *Z. Ramigera*. Environmental Technology. Volume 13: 579-586.

A. Kapoor and T. Viraraghvan (1998), Biosorption of heavy metals on *Aspergillus niger*: Effect of pre-treatment. *Bioresource Technology*. Volume 63: 109-113.

Aung W. W. and Win T. (1994), Aquatic plant treatment of cation polluted water. *Science Resource*. Volume 19: 207-210.

B. Volesky and Z. R. Holan (1995), Biosorption of heavy metals: review. *Biotechnology Programs*. Volume 11: 235-250.

B. Volesky (1987), Biosorbents for metal recovery. *Trends Biotechnology*. Volume 5: 96-` 101.

Bereau of Land and Water Quality (2002). Unidentified Floating Objects. Washington DC, USA.

Breuer and K. Melzer (1990).*Oecologia*. Volume 82: 461-467.

Brierley C. L. (1990), Bioremediation of metal-contamined surfaces and groundwaters. *Geomicrobiology J.* Volume 8: 201-223.

Cabral J. P. S., Selective binding of metal ions to *Pseudomonas syringae* cells. *Microbios*. Volume 71: 47-53

C. Ercole, F. Veglio, L. Tow, G. Ficara and A. Lepidn (1994), Immobilization of microbial cells for metal adsorption and desorption. *Mineral Bioprocessing II. Snowboard*, Utah.

C. P. Huang, D. Westman, K. Quirk and J. P. Huang (1988), The removal of cadmium(II) from dilute aqueous solution by fungal adsorbent. *Water Science Technology*.1988. Volume 20: 369-376.

Chamrumathi D., Das N. And Vimala K., Effect of pretreatment of Cd^{2+} biosorption by mycelial biomass of *Pluerotus florida*. *African Journal of Biotechnology*. Volume 6:2555-2558

Christie John Geankoplis, Liquid- Liquid and Fluid-Solid Separation Processes. *Transport Processes and Separation Process Principles*. International Edition Fourth Edition. 760-771.

Chongrak Polprasert and L. R. J. Liyanage (1996), Hazardous waste generation and processing. *Resources, Conservation and Recycling*. Volume 16: 213-226.

Cronell A. P. (1966), Surface forces and solid-gas interface. In: E. Alison Flood (Editor), The Solid-Gas Interface, Marcel Dekker, New York.

David Tin Win, Myint Myint Than and Sein Tun (2002), Iron removal from industrial waters by water hyacinth. *Department of Chemistry, Yangon University, Myanmar*. Volume 6: 55- 60.

Delgado M., M. Bigeriego and E. Guardiola (1993), Uptake of Zn, Cd and Cr by water hyacinth biomass. *Water Research*. Volume 3: 269-272.

E. Fourest and J. C. Roux (1992), Heavy metal biosorption by fungal mycelial by products: mechanisms and influence of pH. *Application Microbial Biotechnology*. Volume 37: 399-403.

Edward K. J. (2000), New iron-eating microbe major component of mining pollution and iron and sulfur cyling. *Marine Chemistry and Geochemistry Department*, Woods Hole Oceangraphic Institution, Woods Hole, Massachusetts, USA.

Eneida Salai Cossich, Celia Regina Granchen Tavares and Teresa Massako Kabuta Ravagrani (2002), Biosorption of chromium (III) by *Sargassum* sp. biomass. *Process Biotechnology*.

F. Veglio and F. Beolchini (1997), Removal of metals by biosorption: a review. *Hydrometallurgy*. Volume 44: 301-316.

Francesca Pagnanelli, Luigi Toroa and Francesco Veglio (2002), Olive mill solid residues as heavy metal sorbent material: a preliminary study. *Waste Management*. Volume 22: 901-907.

Friis N. and Myers-Keith P. (1986), Biosorption of uranium and lead by *Streptomyces longwoodnesis*. *Biotechnology Bioengineering*. Volume 28: 21-28.

Gadd G. M., White C. and De Rone L. (1988), Heavy metal and radionuclide uptake by fungi and yeast. *Biohydrometallurgy*.

Garg P. and Chandra P. (1990), Toxicity and accumulation of chromium in *Ceratophyllum demersum. L. Bull. Environmental Contaminant Toxicology*. Volume 44: 473-478

Galun M. (1987), Removal of metal ions from aqueous solutions by Penicillium biomass: kinetic and uptake parameters. *Water Air Soil Pollution*. Volume 33: 359-371.

Gourdon R., Bhende S., Rus E. and Sofer S. S. (1990), Comparison of cadmium biosorption by gram-positive and gram-negative bacteria from activated sludge. *Biotechnology Lett*. Volume 11: 839-842.

G. Satyakala and Q. Jamil (1992), Chromium induced biochemical changes in *Eichhornia crassipes* Mort Solms. and *Pistia stratioitess*. *Bull. Environment Contaminant Toxicology.* Volume 48: 921-928.

Holan Z. R., Volesky B. and Prasetyo. L., Biosorption of cadmium by biomass of marine algae. *Biotechnology Bioengineering*. 1993. Volume 41: 819-825.

Holm L. G., Pluckneet D. L., Pancho J. V. and Herberger J. P. (1997), The world's worst weeds: distribution & biology. Honolulu. University Press of Hawaii. pp: 609.

Howard S. Peavy, Donald R. Rowe and George Tchobanoglous (1985), Environmental Engineering.McGraw-Hill Book Company.

Huang C. P. and Morehart A. L. (1990), The removal of Cu(II) from dilute aqueous solution by *Saccharomyces cerevisiae*. *Water Research*. Volume 24: 433-439.

I. A. H. Schneider, R. W. Smith and J. Rubio, Effect of mining chemicals on biosorption of Cu(II) by the non-living biomass of the macrophyte *P. Lucens. Minerals Engineering*. 1999. Volume 12: 255-260.

I. A. H. Scheneider, R. W. Smith and J. Rubio (1999), Effect of mining chemicals on biosorption of Cu(II) by the nonliving biomass of the macrophyte *Patamogen lucens. Minerals Engineering*. Volume 12: 255-260.

J. A. Brierly (1990), Biosorption of heavy metals. CRC Press. USA.

J. T. Matheikal, L. Iyengar and C. Venkobachan (1991), Sorption and desorption of Cu(II) by *Garaderna lucidum*. *Water Pollution Resources J. Canda*. Volume 28: 187-200.

Kasselmann C. (1995), Aquqrienplflazen. Egen Ulner GMBH & CO., Stuggart. pp: 472.

Kaustubha Mohanty, Mousam Jha, B. C. Meikap and M. N. Biswas (2005), Biosorption of Cr(VI) from aqueous solution by *Eichhornia crassipes*. *Chemical Engineering Journal*. Volume 117: 71-77.

K. S. Low, C. K. Lee and K. K. Tan (1994), Biosorption of basic dyes by water hyacinth roots. *Bioresource Technology*. Volume 52: 79-83.

Kuyucak N. and Volesky B. (1988), Biosorbents for recovery of metals from industrial solutions. *Biotechnology Bioengineering*. Volume 2: 137-142.

Leusch A., Holan Z. R., Volesky B. (1995), *J. Chemical Technology Biotechnology*. Volume 62: 279-288.

M. Kumar, S. Chikara, M. K. Chand and A. K. Bhatnagar (2002), Accumulation of lead, cadmium, zinc and copper in the edible aquatic plants *Trapa bispinosa* Roxb. and *Nelumbo nucifera* Gaertn. *Bull. Environmental Contamination and Toxicology*. Volume 69: 649-654.

Manuala D. Machado, Monica S. F. Santos, Claudio Gouveia, Helena M. V. M. Soares and Eduardo V. Soares (2007), Removal of heavy using a bremer's yeast strain of *Saccharomyces cerevisiae*: The flocculation as a separation process. *Bioresources Technology*.

Muraleedharan T. R. and Venkobachar C. (1990), Mechanism of biosorption of copper(II) by *Ganoderma lucidum*. *Biotechnology Bioengineering*. Volume 35: 320-325.

Narsi R. Bishnoi, Rajender Kumar, Sunil Kumar and Suman Rani (2006), Biosorption of Cr(III) from aqueous solution using algal biomass *spirogyra* spp. *Journal of Hazardous Materials*. Volume 145.

N. Ahalya, T. V. Ramachandra and R. D. Kanamadi (2003), Biosorption of heavy metals. *Research Journal of Chemistry and Enviroment*. Volume 7: 71-79.

N. Ahalya, R. D. Kanamadi and T. V. Ramachandra (2005), Biosorption of chromium (VI) from aqueous solutions by the husk of Bangal gram (*Cicer arientinum*). Environmental Biotechnology. Volume 8: No. 3, Issue of December.

N. Kuyucak and B. Volesky (1989), Accumulation of cobalt by marine algae. *Biotechnology Bioengineering*. Volume 33: 809-814.

Namasivayam C. and Senthilkumar S. (1995), Recycling of industrial solid wastes: waste Fe(III)/Cr(III) hydroxide as an adsorbent for the removal of toxic ions and dyes from wastewater. *Adsorption Science Technology*. Volume 12: 293-296.

N. R. Bishnoi, A. Pant and Garima (2004), Biosorption of copper from aqueous solution using algal biomass. *J. Science Industry Resource*.Volume 63: 813-816.

Patricia Miretzky, Andrea Saralegui and Alicia Fernandez Cirelli (2006), Simultaneous heavy metal removal mechanism by dead macrophytes. *Chemosphere*. Volume 62: 247-254.

Perineau F., Molinier J. Gaset A. (1983), Adsorption of ionic dyes on charred plant material. *J. Chem. Tech. Biotechnol*. Volume 32: 749-758.

P. O. Harris and G. J. Ramelow (1990), Binding of metal ions by particulate biomass derived from *Chlorella vulgaris* and *Sceredesmus quadacauda*. *Environmental Science Technology*. Volume 24: 220-228.

Rai U. N. and Chandra P. (1989), Removal of heavy metals from polluted waters by *Hydrodictyon reticulatum* (Linn.) Lagerheim. *Science Total Environmental*. Volume 87/88: 509-515.

Rai U. N. and Chandra P. (1992), Accumulation of copper, lead, manganese and iron by field population of *Hydrodictyon reticulatum* (Linn.) Lagerheim. *Science Total Environmental*. Volume 116: 203-211.

Ramelow G. J., Fralick D. and Zhao Y. (1992), Factors affecting the uptake of aqueous metal ions by dried seaweed biomass. *Microbiology*. Volume 72: 81-93.

Reddy K. R. and Debusk T. A. (1987), *Water Science Technology*. Volume 19: 61-79.

Sakaguchi T., Nakajima A. (1991), Accumulation of heavy metals such as uranium and thorium by microorganism. In: R. W. Smith and M. Misra (Editors). Mineral Bioprocessing. The Minerals, Metals and Materials Society.

Sinha S. and Chandra P. (1990), Removal of Cu and Cd from water by *Bacopa monnieri*. L. Water Air Soil Pollution. Volume 40: 271-276.

S. Sentilkumaar, S. Bharathi, D. Nithyanandhi and V. Subburam (2000), Biosorption of toxic heavy metal from aqueous solutios. *Bioresource Technology*. Volume 75: 163-165.

Scott J. A. and Palmer S. J. (1990), Sites of cadmium uptake in bacteria used for biosorption. *Application Microbiology Biotechnology*. Volume 33: 221-225.

Sudha Bai R. and T. Emilia Abraham (2000), Biosorption of Cr(VI) from aqueous solution by Rhizopus nigricans. Biochemical Processing and Wastewater Technology Division. Volume 79: 73-81.

Triphati R. D. and Chandra P. (1991), Chromium uptake *Spirodela polyrrhiza* (L.) *schneiden* in relation to metal chelators and pH. *Bull. Env. Contam. Toxicology*. Volume 447: 764-769.

Tsezos M. and Volesky B. (1981), Biosorption of uranium and thorium. *Biotechnology Bioengineering*. Volume 23: 583-604.

Tsezos M. and Volesky B. (1982), The mechanism of thorium biosorption by *Rhizopus arrhinus*. *Biotechnology Bioengineering*. Volume 24: 955-969.

Tsezos M. and Volesky B. (1982), The mechanism of uranium biosorption by *Rhizopus arrhizus*. *Biotechnology Bioengineering*. Volume 24: 385-401.

Tsezos M., E. Romendaki and V. Angelaton (1997), A systematic study on equilibrium and kinetics of biosorptive accumulation: The case of Ag and Ni. Entern. Biodeter. Biodegr. Volume 35: 129-153.

Upendra N. Rai and Sarita Sinha (2001), Distribution of metals in aquatic edible plants: *Trapa natans (Roxb.) Makino* and *Ipomoea aquatic Forsk*. *Environmental Monitoring and Assessment*. Volume 70: 241-252.

V. K. Gupta, A. Rastosi, V. K. Saini and N. Jain (2006), Biosorption of copper(II) from aqueous solution by *Spirogyra* species. *J. Colloid Intreference Science*. Volume 296: 59-63.

Venkobachar C. (1990), Metal removal by waste biomass to upgrade wastewater treatment plants. *Water Science Technology*. Volume 22: 319-320.

Williams L. E., Pittman J. K. and Hall J. L. (2000), Emerging mechanisms for heavy metal transport in plants. *Biochemical Biophysical Acta*. Volume 1465: 104-126.

Wolverten B. C., McDonald R. C. and Gordon J. (1975), Water hyacinth and Alligator weeds for final filtration of sewage. NASA Tech. Memo. TM-X-72724.

Xiaomei Lu, Maleeya Kruatrachue, Prayad Pokethitiyook and Kuraporn Homyak (2003), Removal of cadmium and zinc by water hyacinth, *Eichhornia crassipes*. *ScienceAsia*. Volume 30: 93-103.

Zhou J. L. and Kiff R. J. (1991), The uptake of copper from aqueous solution by immobilized fungal biomass. *J. Chemistry Technology, Biotechnology*. Volume 52: 317-330.

http://www.naturia.per.sg/buloh/plants/water_hyacinth.htm
(Accessed on 27th August 2007, 3:10 PM)

http://www.lef.org/protocols/prtd-156.shtml

(Accessed on 26th March 2008. 11.30 PM)

APPENDIX A

Equipments and reagents

A.1 Equipments

(a) Water hyacinth population

Figure A.1: Population of water hyacinth at Pahang River, Pekan.

(b) HACH Spectrophotometer (Model DR/2400)

Figure A.2: HACH Spectrophotometer used to measure final concentration of ferrum solution.

(c) Incubator shaker

Figure A.3: Double stack incubator shaker used to shake samples at 175 rpm and to control the temperature.

(d) Oven

Figure A.4: Oven used to dry the water hyacinth.

(e) pH meter

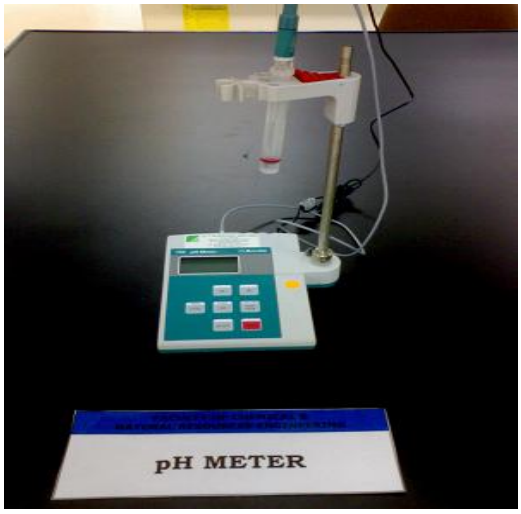


Figure A.5: pH meter used to measure the pH value of the ferrum solution.

(f) Blender

Figure A.6: Blender used to sieve the dried water hyacinth to powder form

(g) Filter paper

Figure A.7: Filter paper was used to separated the loaded biosorbent from the solution before analyze using HACH Spectrophotometer.

(h) 250 mL conical flasks

Figure A.8: Conical flasks used to place the samples during the experiment.

(i) 1000 mL volumetric flask

Figure A.9: Volumetric flask used to prepare the ferrum stock solution

(j) Sample Cells, 1-inch square, 10-mL

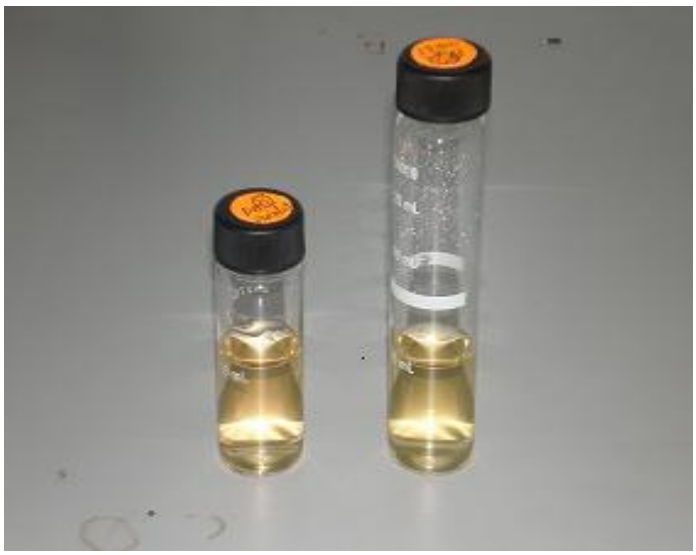


Figure A.10: Sample cells used to replace the samples and blank for analysis using HACH Spectrophotometer.

A.2 Reagents

(a) FerroVer Iron Reagent Powder Pillows

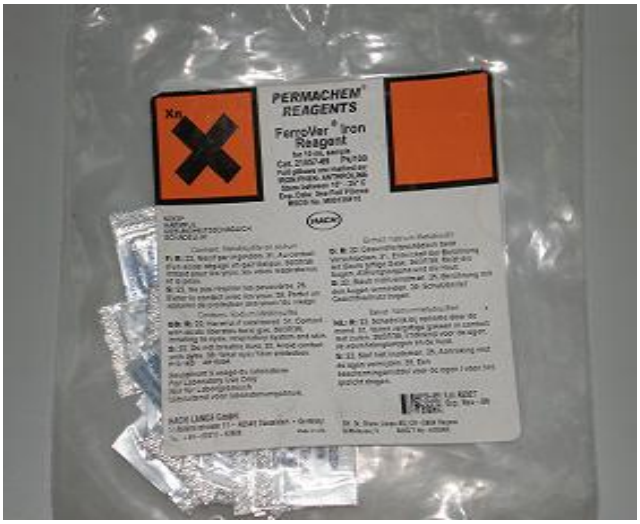


Figure A.11: FerroVer Iron Reagent Powder Pillows as the indicator of ferrum ions in total iron test using HACH Spectrophotometer.

(b) Iron (II) sulfate powder

Figure A.12: Iron (II) sulfate powder used to prepared the ferrum solution by mix with 1 L of distilled water.

(c) Biosorbent (dried water hyacinth)

Figure A.13: Biosorbent was used to remove the Fe from aqueous solution

APPENDIX B

HACH® DR/2400 Method 8008, FerroVer® Method (Iron Total)

Powder Pillows

1. Select '**Hach Programs**', select program '**265 Iron, FerroVer**' and select '**Start**'.
2. Fill a clean, round sample cell with 10 mL of sample.
3. Add the contents of FerroVer Iron Reagent Powder Pillow to the sample cell which was filled with the prepared sample. Then, swirl to mix it.
4. Select the timer icon and touch '**OK**'. A three minutes reaction period will begin.
5. Fill another sample cell with 10 mL as a blank.
6. When the timer beeps, place the blank into the cell holder.
7. Select '**Zero**'. The display will show: 0.00 mg/L.
8. Place the prepared sample into the cell holder. Then touch '**Read**' and the results will appear in mg/L Fe.