REMOVAL OF FERRUM(II) FROM INDUSTRIAL WASTEWATER USING WATER HYACINTH (*Eichhornia Crassipes)*

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ABSTRACT

Heavy metals have been excessively released into the environment due to rapid industrialization and have created a major global concern. Cadmium, zinc, copper, nickel, lead, mercury and chromium are often detected in industrial wastewaters, which originate from metal plating, mining activities, smelting, battery manufacture, tanneries, petroleum refining, paint manufacture, pesticides, pigment manufacture, printing and photographic industries. The main purpose of this research is to study the capability of water hyacinth in removing Ferum (II) from industrial wastewater. The scope of this study is divided to two which is the effect of contact time and the effect of amount water hyacinth on Ferrum (II) removal. For the methodology, firstly, the biosorbent which is water hyacinth was collected from ponds, lake and swamp nearby UMP. Then, the biosorbents were washed from any dirt particles and impurities. The sample of wastewater was collected from Hunstman Tioxide Industries. The capacity of Ferrum absorbed by water hyacinth was measured in this experiment by determining the concentration of Ferrum before and after the addition of biosorbent by using UV-Vis spectrophotometer. From the obtained result, the percentage removal of Ferrum (II) from the industrial wastewater is increases with increasing the contact time and the amount of aquatic plants. Water hyacinth exhibits high potential for wastewater treatment because of its ubiquity, rapid growth rate, ease of harvest and extended growing and harvesting periods. Therefore the water hyacinth could be harvested in water systems to remove heavy metals effectively, and hence indicates the potential of these plants for pollution monitoring of these metals.

ABSTRAK

Logam berat telah berlebihan tanggal ke persekitaran kerana industrialisasi yang pesat dan telah membuat keprihatinan global utama. Kadmium, zink, tembaga, nikel, plumbum, merkuri dan kromium sering dikesan di dalam air sisa industri, yang berasal dari logam plating, kegiatan perlombongan, peleburan, pembuatan bateri, penyamakan kulit, penyulingan minyak bumi, pembuatan cat, racun perosak, pembuatan pigmen, percetakan dan fotografi industri. Tujuan utama dari penelitian ini adalah untuk mengetahui kemampuan keladi bunting dalam menghilangkan ion besi (II) dari air sisa industri. Skop kajian ini dibahagi kepada dua iaitu pengaruh masa dan pengaruh jumlah keladi bunting dalam penghapusan besi (II) . Untuk kaedah, pertama, bio-penyerapan yang mana keladi bunting diperolehi dari kolam, tasik dan paya berdekatan UMP. Kemudian, bio-penyerapan dicuci dari partikel debu dan kotoran. Sampel air sisa diambil dari Hunstman Industri Tioxide. Kapasiti Ferrum diserap oleh keladi bunting diukur dalam percubaan ini dengan menentukan kepekatan besi sebelum dan selepas penambahan bio-penyerapan dengan menggunakan UV-Vis spectrophotometer. Dari hasil yang diperoleh, peratusan penghapusan besi (II) dari air sisa industri meningkat dengan peningkatan masa dan jumlah tanaman. Keladi bunting menunjukkan potensi tinggi untuk memproses air kumbahan kerana sentiasa ada, pertumbuhan yang cepat, senang untuk tuai dan dilanjutkan pertumbuhan dan tempoh tuai. Oleh kerana itu, keladi bunting boleh dituai dalam sistem air untuk menghilangkan logam berat secara efektif, sehingga menunjukkan potensi tanaman ini untuk memantau pencemaran logam ini.

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WH-	Water Hyacinth
Fe(II)-	Ferrum ions 2
Cu^{2+}-	ions 2
Zn^{2+}-	Zinc ion 2
H_2O_2-	Hydrogen peroxide

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CHAPTER 1

INTRODUCTION

1.1 Background

Wastewater may be defined as combination of the liquid or water-carried wastes removed from residences, institutions, commercial and industrial establishments, after they have been used for various cultural, physiological and technological purposes. Industrial wastewater may contain acids or alkalis, heavy metal, flammable and radioactive materials. Rapid industrialization and urbanization have resulted in elevated emission of toxic heavy metals entering the biosphere (Gazso *et al.*, 2001). Activities such as mining and agriculture have polluted extensive areas throughout the world (Shallari *et al.*, 1998). The release of heavy metals in biologically available forms by human activity may damage or alter both natural and man-made ecosystems (Tyler *et al.*, 1989). Heavy metal ions such as $Cu^{2+,}$ $Zn^{2+,}$ $Fe^{2+,}$ are essential micronutrients for plant metabolism but when present in excess, can become extremely toxic (Xiaomei Lu et al., 2004).

Ferum is one of the vital elements for human and for other forms of life. Nevertheless, high doses of ferum are known to causes hemorrhagic necrosis, sloughing of mucosa areas in the stomach, tissues damage to a variety of organs by catalyzing the conversion of H_2O_2 to free radical ions that attack cell membranes, proteins and break the DNA double strands and cause oncogene activation (Mahesh W. Jayaweera et al., 2008).

The removal of heavy metals from industries effluent can be achieved by using ion exchange, chemical oxidation, and chemical precipitation (Aksu et al., 1992). For advanced purification, different physic-chemical methods such as 2ctive carbon adsorption, ion exchange and reverse osmosis can be used. As an alternative to these methods, recently, the method of the removal heavy metal contaminants by mean bacteria has been focused on. Biological removal of heavy metal contaminants from aquatic effluents offers great potential when metals are present in trace amounts (Vinita and Radhanath, 1992).

Many microbial species such as bacteria, fungi, yeast and algae are known to be capable of adsorbing heavy metal on their surface and accumulating within their structure (Vinita and Radhanath, 1992). It is possible that microorganism can be used in removal of toxic ions from the water. Physical adsorption or ion exchange at the living cell surface is very rapid and occur in a short time after microorganisms come into contact with heavy metal ions. Accumulation occurs in living cells and is slow, related to metabolic activity (Nourbakhsh et al., 2002).

Biosorption, biopreciptation, and uptake by purified biopolymers derived from microbial cells provide alternative and/or additive processes for conventional physical and chemical methods. Intact microbial cells live or dead and their products can be highly efficient bioaccumulators of both soluble and particulate forms of metals. The cell surfaces of all microorganisms are negatively charged owing to the presence of various anionic structures. This gives the bacteria the ability to bind metal cations. Various microbial species, mainly *Pseudomonas*, have been shown to be relatively efficient in metal uptake from polluted effluents (Hussien *et al.*, 2004).

Biosorption can be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material (Gadd, 1993).The article on the use of aquatic plants for wastewater treatment induced an interest in the use of aquatic plants for removal of iron from industrial wastewaters (Win *et al.*,2002).

Aquatic plants are known to accumulate metals from their environment and affect metals fluxes through those ecosystems. Water hyacinth (*Eichhornia crassipes*) is highly effective in removing excess nutrients, heavy metals toxic metals minerals and organic chemical, and herbicides from polluted water. Water hyacinth has variable chemicals contents which have been related to differences in study site, season, plant parts and the chemical nature of the habitat (M.E Soltan and M.N Rashed, 2001).

Eichhornia crassipes is a weed growing in shallow waters, etc, especially in tropical and subtropical waters. It can absorbs heavy metals, mineral nutrients and organic chemicals due to its rapid growth. And it has been widely used for the treatment of a variety of wastewaters. Water hyacinth is a floating macrophyte whose appetite for nutrients and explosive growth rate has been put to use in cleaning up municipal and agriculture wastewater. It has been discovered that water hyacinth's quest for nutrients can be turned in a more useful direction (Xiaomei Lu et al,. 2004)

1.2 Objective

The main objective of this research is to study the capability of water hyacinth (*Eichhornia crassipes*) in removing ferum (II) from industrial wastewater.

1.3 Scope of Study

i. To study the effect of amount water hyacinth in removing Ferum (II) from wastewater.
ii. To investigate the effect of contact time towards the removal of Ferum (II) from wastewater.

1.4 Problem Statement

Ferrum is considered as a pollutant and hazardous to human and ecosystems. Ferrum also leads to diabetes mellitus, atherosclerosis and related cardiovascular diseases, hormonal abnormalities, and a dysfunctional immune system. The expose of ferrum into environment by various industries has been aesthetically undesirable and too much of it will eventually cause serious environmental effect, aquatic or non-aquatic. This is due to its properties which are mostly toxic, mutagenic, and carcinogenic. Ferrum are causing pollution to the environment, for example, ferrum adsorb and reflect sunlight from entering water and thus interfere the aquatic ecosystem. It is evidently, therefore, investigating the removal of ferrum is significant environmental, technical, and commercially important.

Water hyacinth is a free-floating aquatic weed originating from tropical areas in so many countries. It is naturally a rapid and uncontrollable growth plant, thus it has become a major cause of water irrigation especially during raining season, where it can be found blocking the drains and water sources. It has caused high costs and labour requirements to control the plant, leaving only temporary removal of the water hyacinths and abundantly growing. This resulted in a major massive growth of mosquito's pest which will lead to serious health problems to the society. Therefore, in making this plant a better use, it is proposed as a biosorbent to remove wastewater.

In this research, a method of biosorption using aquatic plant is used because of some advantages. The major advantages of biosorption over conventional treatment methods include:

- No additional nutrient requirement;
- High efficiency in removing heavy metals.
- Renewable biosorbent (lower cost)
- Minimization of chemical used
- Possibility of metal recovery

1.5 Rationale and Significance

The purpose of this study is to remove Ferrum (II) which can cause environmental pollution because it can cause excessive levels of iron in the blood. High blood levels of free ferrous iron react with peroxides to produces free radicals, which are highly reactive and can damage DNA, proteins, lipids and other cellular components. Unlike organic wastes, heavy metals are non-biodegradable and they can be accumulated in living tissues, causing various diseases and disorders; therefore they must be removed before discharge.

Besides that, some advantages using water hyacinth for wastewater treatment includes simple technique, requires little processing, free availability and easy regeneration. Water hyacinth can settle that problem because water hyacinth can cause blockage of canals and rivers. It’s also low cost absorbent and high efficiency of metal removal from wastewater.

In this study biosorption will be used because biosorption treatment technology has received much attention as it offered low cost biosorbent and non-hazardous biomaterial.

CHAPTER 2

LITERATURE REVIEW

2.1 Water Hyacinth (*Eichhornia crassipe)*

Water Hyacinth or *Eichhornia crassipes* is a weed growing in shallow waters, especially in tropical and subtropical waters (Tan *et al.*, 2007). It can absorb heavy metals, mineral nutrients and organic chemicals due to its rapid growth (Zhou et al., 2007). This plant also has been widely used for the treatment of a variety of wastewaters (Reeta and Sooknah, 2004). Excessive growth cause serious environmental problems, such as the growth of other plants will be limited and even killed by *E. crassipes* when the surface of water is full of *E. crassipes*.After its death, decomposition of its residual exhaust oxygen from the water column, and cause suffocation to aquatic animal life. Now, *E. crassipes* has been proved to be a persistent and expensive aquatic problem weed costing millions of dollars to control, together with an uncountable millions of dollars more due to damage to the environment, irrigation systems and crops (Mathur and Singh, 2004). Over the years, various control methods have been used, including chemical, biological and mechanical means, but with no lasting success (Ahluwalia and Goyal, 2007). Conventional treatment technologies for removal of heavy metals from aqueous solution are not economical and generate huge quantity of toxic chemical sludge, so there is little economic return for these control methods. If bio-material of *E. crassipes* body could be made into some useful products, it will be very good both for the society and environment.

Water hyacinth is fast growing perennial aquatic macrophyte. It is a member of pickerelweed family (*Pontederiaceae*) and its name *Eichhornia* was derived from well known 19^{th} century Prussian politician J.A.F. Eichhorn (Aquatics, 2010). This tropical plant spread throughout the world in late 19^{th} and early 20^{th} century (Wilson *et al*., 2005). Today it is well known for its reproduction potential (De Casabianca and Laugier, 1995) and as a plant that can double its population in only twelve days (Apiris, 2010). Water hyacinth is also known for its ability to grow in severe polluted waters (So *et al*., 2003). *E. crassipens* is well studied as an aquatic plant that can improve effluent quality from oxidation ponds and as a main component of one integrated advanced system for treatment of municipal, agricultural and industrial wastewaters (Maine *et al*., 2001). To regret water hyacinth is often described in literature as serious invasive weed (Wilson *et al*., 2005) and it is ranked on eight places in the list of world's ten most serious weeds.

During the past decade, there has been increasing interest in the use of aquatic vascular plants for the removal of pollutants from domestic and industrial sewage effluents. Of the several plants studied, water hyacinth is the most commonly cited and appear to have the greatest potential for use in water pollution control. Water hyacinths have rapid growth rates and are known to accumulate nutrients and potentially toxic water pollutants (Kay et al., 1983).

The majority of the studies on the use of water hyacinth in water pollution control have consisted of short-term accumulation experiments in the laboratory, whereas data on biomass production have come largely from field studies. In such studies, estimations of plant's ability to remove pollutants are extrapolations from laboratory studies to field situations, without consideration for the effect that these pollutants can have upon plant growth and primary production. Very little information is available concerning the effects of heavy metals on the growth of water hyacinth, but available information indicates growth will be reduced (Kay et al., 1983)

2.2 Wastewater

Wastewater is water that has come into contact with any of a variety of contaminants and is not fit for human consumption most often; wastewater has its source in domestic settings, commercial operations, industry and agriculture.

Wastewater is water that has come into contact with any of a variety of contaminants and is not fit for human consumption. Most often, wastewater has its source in gasoline storage tanks, leaking septic tanks, accidental spills and industrial waste disposal domestic settings, commercial operations, industry and agriculture. Groundwater becomes contaminated from many sources.

Wastewater is the flow of used water from a community. The characteristics of the wastewater discharges will vary from location to location depending upon the population and industrial sector served, land uses, groundwater levels, and degree of separation between storm water and sanitary wastes. Domestic wastewater includes typical wastes from the kitchen, bathroom, and laundry, as well as any other wastes that people may accidentally or intentionally pour down the drain. Sanitary wastewater consists of domestic wastewater as well as those discharged from commercial, institutional, and similar facilities. Industrial wastes will be as varied as the industries that generate the wastes. The quantities of storm water that combines with the domestic wastewater will vary with the degree of separation that exists between the storm sewers and the sanitary sewers.

Rapid economic changes have resulted in elevated level of toxic heavy metals and radionuclides entering the biosphere (Gazso, 2001). The heavy metals such as lead, cadmium, copper, nickel and zinc are among the most common pollutants found in industrial effluents. Solid and/or liquid wastes containing toxic heavy metals may be generated in various industrial processes such as chemical manufacturing, electric power generating, coal and ore mining, smelling and metal refining, metal plating, and others (Yahaya, 2008).

Water pollution is one of the most serious problems of today’s civilization. The consumption of water has been doubling on every twenty years but the reduction of this period is expected if today’s trends in water use continue (Nevena Nesic and Ljubinko Jovanovic, 2005). These two statements justify people’s fear that whole areas of the world will remain without biochemical safe water suitable for drinking and other needs. One can say situation is already alarming if it is known that because of fresh water disposition on Earth only one third of its territory is well provide with water, and if drastic efforts in water protection are not made by year 2025 2.3 billion people will live in areas with chronic water shortage (WHO, 2010). There are many technologies for wastewater treatment that can help in re-establishing and preserving physical, chemical and biological integrity of water. All of these technologies can be classified in two basic groups:

1. Conventional methods for purification of wastewater (wastewater treatment is carried out by physical, chemical and biological processes) and

2. Alternative methods for purification of wastewater (wastewater treatment is carried out by imitating self-purification process present in natural wetlands).

Today these conventional wastewater treatment facilities fail in satisfying all demands of ecologically aware societies. This is because they: do not harmonize with basic principles of water conservation, do not enable reclamation and reuse of water and nutrients, generate toxic sludge as by product and use chemicals, harmful to environment and people, in the treatment process (Davis for EPA, 2004). So scientist sought for other solutions that will go beyond all problems mentioned above. All of the answers were found in natural wetlands which then served as model for construction of systems for wastewater purification by aquatic plants.

There are many different types of these alternative systems (constructed wetlands, aquatic systems, etc.) but all of them have the same major characteristic, microorganisms, algae, substrates and water they have the ability to remove organic and inorganic matter, nutrients, pathogens, heavy metals and other pollutants from wastewater in a completely natural way. In last few years a great interest has been shown for research of aquatic macrophytes as good candidates for pollutant removal or even as bioindicators for heavy metals in aquatic ecosystems. Water hyacinth (*Eichhornia crassipes*), just one of the great number of aquatic plant species successfully used for wastewater treatment in decades, was of particular importance. It is important to emphasize that *E. crassipes* has a huge potential for removal of the vast range of pollutants from wastewater and that a great number of aquatic systems with water hyacinth as basic component were construct, but this macrophyte is also one of the most dangerous and the most invasive aquatic weed in the world (Nevena Nesic and Ljubinko Jovanovic, 2005).

2.3 Biosorption

Both living and dead biomass as well as cellular products such as polysaccharides can be used for metal removal. Heavy metal pollution is one of the most important environmental problems today. Various industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metalliferous, surface finishing industry, energy and fuel production, fertilizer and pesticide industry and application, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation etc. Thus, metal as a kind of resource is becoming shortage and also brings about serious environmental pollution, threatening human health and ecosystem. Three kinds of heavy metals are of concern, including toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.) (Wang and Chen, 2006).

Biosorption is a promising method for removal of toxic ions from wastewater. Its advantage is especially in the treatment of large volumes of effluents with low concentration of pollutants. Biosorption of heavy metals by inactive non-living biomass of microbial or plant origin is an innovative and alternative technology for removal of these pollutants from aqueous solution (Ahluwalia and Goyal, 2007). Biosorption of metal ions using biological materials such as algae, bacteria, fungal and yeast have received greater attention due to its advantages over conventional method (Yahaya, 2008). It has been defined as the property of biomass such as algae, bacteria, fungal and yeast to bind with metal ions from aqueous solutions (Dursun, 2006). Biosorption process could involve several mechanisms such as ion-exchange, physical adsorption, complexation and precipitation (Mahvi, 2008). According to Ahalya *et al* (2003), biosorption mechanisms can be divided into metabolism dependent and nonmetabolism dependent. Metabolism dependent is a slow process include of transport across cell membrane and precipitation. While non-metabolism dependent is a rapid process include of precipitation, physical adsorption, ion exchange and complexation (Yahaya,2008).

The process is classified as (Ahalya *et al*., 2003):

i. extracellular accumulation/ precipitation
ii. cell surface sorption/precipitation and
iii. Intracellular accumulation

Biosorption or bioremediations consists of a group of applications which involve the detoxification of hazardous substances instead of transferring them from one medium to another by means of microbes and plants. This process is characterized as less disruptive and can be often carried out on site, eliminating the need to transport the toxic, materials to treatment sites (Gavrilescu, 2004). Biosorbents are prepared from naturally abundant and/or waste biomass. Due to the high uptake capacity and very cost-effective source of the raw material, biosorption is a progression towards a perspective method. Various biomaterials have been examined for their biosorptive properties and different types of biomass have shown levels of metal uptake high enough to warrant further research (Volesky and Holan, 1995). Biosorbents of plant origin are mainly agricultural by-products such as, maize cob and husk, sunflower stalk, medicago sativa (Alfalfa), cassava waste, wild cocoyam, sphagnum peat moss, sawdust, chitosan, Sago waste, peanut skins, shea butter seed husks, banana pith, coconut fiber, sugar-beet pulp, wheat bran, sugarcane bagasse and so on (Igwe and Abia, 2006) .

2.3.1 Mechanism of biosorption process

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

(i) Physical adsorption

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

(ii) Complexation

Metal ions removals from aqueous solution also take place by complex formation on the cell surface after the interaction between metal ions and active groups. Metal ions can be biosorbed or complexed by carboxyl groups found in the microbial polysaccharides or other polymers. Aksu *et al.* (1992) reported that copper biosorption onto *Zoogloea ramigera* and *Chlorella vulgaris* involve both adsorption and formation of coordination bonds between metals and carboxyl and amino groups of the cell wall.

(iii) Ion exchange

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

(iv) Precipitation

This mechanism is dependent or independent on cellular metabolism. Metal ions removal from aqueous solution often associates with active defense system of microorganisms. This active system is a system that produces compounds favoring the precipitation process (Yahaya, 2008).

2.4 Heavy Metal

The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment (Faisal and Hasnain, 2004). The rate at which effluents are discharged into the environment especially water bodies have been on the increase as a result of urbanization. Most of these effluents contain toxic substances especially heavy metals. The presence of heavy metals in the environment is a major concern because of their toxicity, bio-accumulating tendency, threat to human life and the environment (Horsfall and Spiff, 2005). Lead, cadmium and mercury are examples of heavy metals that have been classified as priority pollutants by the U.S Environmental protection Agency (U.S EPA).

Heavy metals are among the conservative pollutants that are not subject to bacterial attack or other break down or degradation process and are permanent additions to the marine environment. As a result of this, their concentrations often exceed the permissible levels normally found in soil, water ways and sediments. Hence, they find their way up the food pyramid. When they accumulate in the environment and in food chains, they can profoundly disrupt biological processes. The primary sources of heavy metals pollution in coastal lagoons are input from rivers, sediments and atmosphere, which can affect aquaculture profitability in certain areas (Krishnani et al., 2004). The anthropogenic sources of heavy metals include wastes from the 1168 Afr. J. Biotechnol electroplating and metal finishing industries, metallurgical industries, tannery operations, chemical manufacturing, mine drainage, battery manufacturing, leather tanning industries, fertilizer industries, pigment manufacturing industries, leachates from landfills and contaminated ground water from hazardous waste sites (Faisal and Hasnain, 2004). Heavy metals are also emitted from resource recovery plants in relatively high levels on fly ash particles.

Heavy metals, industrial pollutants, in contrast with organic materials cannot be degraded and therefore accumulate in water, soil, bottom sediments and living organisms. Water contamination with heavy metals is a very important problem in the current world. Occurrence of toxic metals in pond, ditch and river water affect the lives of local people that depend upon these water sources for their daily requirements. Consumption of such aquatic food stuff enriched with toxic metals may cause serious health hazards through food-chain magnification (Miretzky et al., 2004).

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

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