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Biosorption of Heavy Metals by the Locally Isolated *Pseudomonas spp*.

A thesis

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By

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Summary

A total of 110 samples were collected from different environments (waste waters, soil, and fresh waters) and locations in Baghdad governorate. Total isolates obtained from these samples were 85, 42 of them were suspected to be *Pseudomonas spp*. depending on their stability to grow on cetrimide agar. Biochemical tests have been done on these suspected isolates, these biochemical tests include catalase test, oxidase test, gelatin hydrolyses test and the ability of these isolate to grow on king A agar and king B agar medium, which give a positive results for catalase and oxidase tests for all isolates, also a positive results for gelatin hydrolyses test, while for isolate growing on king A and king B give a variable results. Ability of these isolates to resist heavy metal (nickel, cobalt, lead and zinc) was examined by using plate diffusion method. *Pseudomonas* isolates exhibited different resistant pattern depending on the various concentration of metals. However, an isolate of *Pseudomonas spp*. namely P36 was the most resistant to nickel and zinc, while another isolate P37 was resistant to cobalt, and for lead isolate no.41.

Optimum conditions for biosorption of the locally isolated *Pseudomonas spp.* P36 were studied. Results showed that the optimum biosorption conditions were; pH 7.0, temperature 25ºC, biomass concentration 0.5gm/L, initial metal concentration 20 mg/L, biomass age was 2 days, contacting time was 100 minutes, and agitation rate was 150 rpm.

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1.1 Introduction:

The discharge of heavy metals into ecosystem has become a matter of concern over the last few decades. This toxic pollutant may be derived from mining operations, refining ores, sludge disposal, metal plating, or the manufacture of electrical equipments, paints, alloys, batteries, pesticide or preservatives (Ahalya *et al*., 2005).

The chemical processes that exist are not economical for removing a large volume of heavy metals from effluents. The conventional processes used for effluent treatment are chemical precipitation, lime coagulation, reverse osmosis and ion exchange. These processes are expensive and not eco-friendly. Further, the major disadvantage with conventional treatment techniques is the production of sludge (Gupta *et al*., 2000).

Thus, studies have been looking for cheaper, environment friendly and more effective methods to remediate heavy metal-contamination and reduce the growing public health risk.

Biosorption is proven to be quite effective at removing metal ions from contaminated solution in a low-cost and environment-friendly manner. The major advantages of biosorption over conventional treatment methods include low cost, high efficiency of metal removal from dilute solution, minimization of chemical and/or biological sludge, no additional nutrient requirement, and regeneration of biosorbent and the possibility of metal recovery (Vinoj Kumar and Kaladharan, 2006).

The physiological state of microorganism, the age of the cells, the availability of micronutrients during their growth and the environmental conditions during the biosorption process (such as pH, temperature, and presence of certain co-ions), are important parameters that affect the performance of a living biosorbent. The efficiency of metal concentration

on the biosorbent is also influenced by metal solution chemical features (Cossich *et al*., 2002)

Metal accumulation by microorganisms is either of energy driven active bioaccumulation or metabolism independent passive biosorption. The latter process of microbial metal removal by purely physico-chemical processes seems more appropriate for bioremediation with cation sequestration mainly regulated by the characteristics of the microorganism, the targeted metal and the solution microenvironment. Since, it is the chemical composition of the cell wall and other surface materials responsible for cation sequestration, cell viability or other metabolic activities that do not interfere with such characteristics effectively have no impact on biosorption. (Sar and D'Souza, 2001).

Both living and dead biomasses exhibit biosorption capacity, the performance of living biomass in binding metal ions depends not only on nutrient and environmental status, but also on cell age, also if living cells subjected to toxic effect of heavy metals reaching a certain level, resulting in cell death (Yan and Viraraghavan, 2000).

According to what introduced above, this study was aimed to:

- Isolation and identification of *Pseudomonas spp.* from different environments to be used in heavy metals (nickel, cobalt, lead and zinc) biosorption experiments.
- Studying the ability of different isolates of *Pseudomonas spp*. in heavy metals (nickel, cobalt, lead and zinc) biosorption and select the efficient isolate for this purpose.
- Studying the optimum conditions for heavy metals biosorption by the selected isolate.

1.2 Literature Review

1.2.1 Heavy metals pollution

Heavy metals release to the environment have been increasing continuously as a result of activities and technological development, posing a significant threat to the environment and public health because of their toxicity, accumulation in food chain and persistence in nature. Mining activities, agricultural runoff, industrial and domestic effluents are mainly responsible for the increase of metallic species released into the environment (Cossich *et al*., 2002).

Heavy metal(s) are widespread pollutants of great environment concern as they are non-degradable and thus persistent. It is well perceived that there is a permissible limit of each metal, which they are generally toxic and some are even hazardous (Gupta *et al*., 2000). The presence of heavy metals in the environment is of a major concern because of their toxicity, bioaccumulating, tendency, threat to human life and the environment (Horsfall and Spiff, 2005; Igwe and Abia, 2006) Lead, cadmium and mercury are examples of heavy metals that have been classified as priority pollutants by the U.S Environmental protection Agency (Beaugeard, 2001). Pollution is discharged into rivers and lakes and leaches into the soil and ground water, or is emitted into air as particulate matter. Heavy metals are critical in this regard because of their easy uptake into the food chain and because of bioaccumulation processes (Diagomanolin *et al*., 2004)

Metals are introduced into aquatic systems as a result of the weathering of soils and rocks, from volcanic eruptions, and from a variety of human activities involving the mining, processing, or use of metals and/or substances that contain metal contaminants (Ilhan *et al*., 2004). Heavy metals may enter the food chain in several ways. Small amounts are absorbed by organisms directly from the water through their gills and

other tissues. However, most of the pollutants found in aquatic organisms arrive there through the food chain. First, bacteria, and other small organisms absorb these materials. In turn, these are eaten by larger animals, eventually being eaten by people. Secondly, industrialization for our convenience has provided new ways of absorbing heavy metals (such as the manufacture of cans, also ceramic and enamelware are made from or coated/painted with pigments which contain heavy metals). If the finish on the food container is not perfect, heavy metals can potentially contaminate the food stored/placed in them (Beaugeard, 2001).

1.2.2 Heavy metals toxicity

Metals can be toxic to microbial population at sufficiently high concentrations. However, some metals such as silver, mercury, cadmium and copper are markedly more toxic even at very low levels (Igwe and Abia, 2006). Metals exert their toxic effects on microorganisms through one or more mechanisms (Nies, 1999). An extensive work is available on the effect of metals on general soil microbiological processes. The impact of metals on litter decomposition, methanogenesis, acidogenesis, nitrogen transformation, biomass generation, and enzymatic activity (Raina and Todd, 2003).

Heavy metals are found naturally in the Earth's crust, they are with density beyond $5g/cm^3$, which is five times higher than water. Of the 90 naturally occurring elements, 21 are non-metals, 16 are light-metals and the remaining 53 (with as included) are heavy-metals. Most heavy metals are transition elements with incompletely filled d orbital. This d orbital provides heavy-metal cations with the ability to form complex compounds which may or may not be redox-active. Thus, heavy-metal

cations play an important role as trace elements in sophisticated biochemical reactions (Nies, 1999).

Many metals are essential to biochemical processes, and others have found therapeutic uses in medicine (Soghoian *et al*., 2006). All metals whether essential or inessential can exhibit toxicity above certain threshold concentrations which for highly toxic metal species may be extremely low. The toxicity caused by heavy-metals is generally a result of strong coordinating abilities (Gadd, 1992). Other heavy-metals, such as cadmium were not recognized as poisonous until the early nineteenth century (Abdullah, 2006). So the health hazards presented by heavy metals depend on the level, and length of exposure. In general, exposures are divided into two classes: acute and chronic exposure. Acute exposure refers to contact with a large amount of the heavy-metal in a short period of time. In some cases the health effects are immediately apparent; in others the effects are delayed, while chronic exposure refers to contact with low levels of heavy-metal over a long period of time (Abdullah, 2006).

Heavy metals differ from other toxic substances in that they are neither created nor destroyed by human. As such, they are stable elements (meaning they cannot be metabolized by the body) and bio-accumulative (passed up the food chain to human). Heavy metal overload in the walls of coronary arteries will increase the risk of vascular blockage, also heavy metal overload in the adrenal glands reduce the production of hormones, and also its overload will lead to neurological diseases.

An example of these heavy metals is mercury in its organic form (methyl mercury) is a neurotoxin and nephrotoxin that inhibits sulfhydryl – dependent enzymes mitosis, and cell migration (Choi *et al*., 1981).

Descriptions of the harmful effects and application of some heavy metals can be described as follows:

1.2.2.1 Nickel

Nickel is a metallic chemical element with the symbol **Ni** and atomic number 28, the daily requirement for human was 300 - 700µg, foods naturally high in nickel include chocolate, soybeans, nuts, and oatmeal; it is a silvery-white metal that takes on a high polish. It belongs to the transition metals, and is hard and ductile. It occurs most usually in combination with sulfur and iron in pentlandite, with sulfur in millerite, with arsenic in the mineral nickeline, and with arsenic and sulfur in nickel glance(NPI).

Exposure to nickel metal and soluble compounds of nickel should not exceed 0.05 mg/cm³ in nickel equivalents per 40-hour work week. Nickel sulfide fume and dust is believed to be carcinogenic, and various other nickel compounds may be as well. Nickel carbonyl, [Ni $(CO)_4$], is an extremely toxic gas. The toxicity of metal carbonyls is a function of both the toxicity of a metal as well as the carbonyl's ability to give off highly toxic carbon monoxide gas, and this one is no exception (Kasprzak *et al*., 2003; Dunnick *et al*., 1995)

Nickel plays numerous roles in the biology of microorganisms and plants, though they were not recognized until the 1970s. In fact urease (an enzyme which assists in the hydrolysis of urea) contains nickel. The NiFe-hydrogenases contain nickel in addition to iron-sulfur clusters. Such [NiFe]-hydrogenases characteristically oxidise H_2. A nickel-tetrapyrrole coenzyme, F430, is present in the methyl coenzyme M reductase which powers methanogenic archaea. One of the carbon monoxide dehydrogenase enzymes consists of a Fe-Ni-S cluster (Jaouen, 2006). Other nickel-containing enzymes include a class of superoxide dismutase and a glyoxalase (Szilagyi *et al*., 2004).

1.2.2.2Cobalt

Cobalt is a silver or grey ferromagnetic element with the symbol **Co** and atomic number 27, the daily requirement was 10 - 20µg, naturally occurring cobalt uses are alloys, magnets, Catalysts and Electroplating. Cobalt is a hard, lustrous, silver-grey metal. It is found in various ores, and is used in the preparation of magnetic, wear-resistant, and high-strength alloys. Its compounds are used in the production of inks, paints, and varnishes (Flexner, 1993). Cobalt in small amounts is essential to many living organisms, including humans. Having 0.13 to 0.30 mg/kg of cobalt in soils markedly improves the health of grazing animals. Cobalt is a central component of the vitamin cobalamin, or vitamin B-12(Herbert, 1988). Toxicity may be caused through oxidant-based and free radical-based processes. Exposure to soluble cobalt leads to increased indices of oxidative stress, diminished levels of reduced glutathione, increased levels of oxidized glutathione, activation of the hexose monophosphate shunt, and free radical-induced DNA damage (Lewis *et al*., 1991).

1.2.2.3 Zinc

Zinc is an essential element, with the symbol **Zn,** the human daily requirement was 10 to 20 mg. (Lester, 1987). Zinc is an essential element in all living organisms. Nearly 200 zinc-containing enzymes have been identified, including many dehydrogenases, aldolases, peptidases, polymerases, and phosphatises (W H O).

Zinc tends to be less toxic than other heavy metals as shown in table (1-1). However, some symptoms of zinc toxicity are vomiting, dehydratation, electrolyte imbalance, stomach pain, nausea, lethargy, dizziness, and muscular incoordination. Last, the role of zinc as a carcinogen or carcinogen is unclear. Protein-rich foods, such as meat and

marine organisms, contain high concentrations of zinc (10–50 mg/kg wet weight), whereas grains, vegetables, and fruit are low in zinc (usually <5 mg/kg) (WHO) (Elinder *et al*., 1986).

Table (1-1) Ranking of Risks Associated with Various Metals (Volesky, 1990).

Relative Priority	Environmental Risk	Reserve Depletion	Combined Factors
High	Cd	Cd	Cd
	Pb	Pb	Pb
	Hg	Hg	Hg
	—	Zn	Zn
Medium	Cr	—	—
	Co	Co	Co
	Cu	Cu	Cu
	Ni	Ni	Ni
	Zn	—	—
Low	Al	—	Al

1.2.2.4 Lead

Lead exists in several oxidation states: 0, +1, +2 and +4 with the symbol **Pb**. However, the divalent form Pb2 is the stable ionic species in most of natural environments (EPA, 1979). Precipitation and sorption are the principal mechanisms controlling the distribution of lead in the aquatic environment. In the presence of inorganic ligands, lead can precipitate as a number of compounds including $PbSO_4$, $PbCO_3$, Pb (OH)2, PbS, and Pb3(P04)2. At 5< pH<7, most of the lead is precipitated and sorbed as sparingly soluble hydroxides (CCME, 1994). At 7<pH <9, $PbCO_3$ is the major species (EPA, 1985). Below about pH 5-6, the formation of cationic species prevents the formation of hydroxides (CCME, 1994). Lead is a metal that is toxic to humans, aquatic fauna and livestock; It has no known physiological function. Acute or classical lead poisoning in humans is manifested by anaemia, alimentary symptoms, wrist and foot drop, renal damage, and sometimes encephalopathy. Concentrations of lead greater than 0.8 mg/L in the blood are unequivocal evidence of clinical lead poisoning (Lester, 1987).

1.2.3 General methods for removing heavy metals

The commonly used procedures for removing metal ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange and reverse osmosis (Ahalya *et al*., 2003). The process description of each method is presented below.

Reverse Osmosis: It is a process in which heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by the dissolved solids in wastewater. The disadvantage of this method is that it is expensive.

Electrodialysis: In this process, the ionic components (heavy metals) are separated through the use of semi-permeable ion selective membranes. Application of an electrical potential between the two electrodes causes a migration of cations and anions towards respective electrodes. Because of the alternate spacing of cation and anion permeable membranes, cells of concentrated and dilute salts are formed. The disadvantage is the formation of metal hydroxides, which clog the membrane.

Ultrafiltration: They are pressure driven membrane operations that use porous membranes for the removal of heavy metals. The main disadvantage of this process is the generation of sludge.

Ion-exchange: In this process, metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. The disadvantages include: high cost and partial removal of certain ions.

Chemical Precipitation: Precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers. The large amount of sludge containing toxic compounds produced during the process is the main disadvantage.

Phytoremediation: Phytoremediation is the use of certain plants to clean up soil, sediment, and water contaminated with metals. The disadvantages include that it takes a long time for removal of metals and the regeneration of the plant for further biosorption is difficult.

Hence the disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal has made it imperative for a cost-effective treatment method that is capable of removing heavy metals from aqueous effluents.

1.2.4 Biosorption of Heavy Metals

The commonly used procedures for removing metal ions (table 1-2) from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction (Rich and Cherry, 1987), Hence the disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge high cost which encourage the researchers to look for new technologies with low cost (Ahalya *et al*., 2003).

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). The case of a sorption process involves a solid phase (sorbent) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, e.g. metal ions). So due to the higher 'affinity' of the sorbent for the sorbate species the latter is attracted into the solid and bound there by different mechanisms. This process takes place until equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in solution (Volesky, 2006).

Gupta and Rastogi (2007) approved that the biosorption is the effective method for the removal of heavy metal ions from wastewaters this is agreed with the results which are showing the sorption of Pb (II) from solutions by biomass of commonly available, filamentous green algae *Spirogyra sp*.

Sedum alfredii has been identified as a new Zn-hyperaccumulator plant native to China, in which root system is the main interface of material exchange between plants and their environment, and plays an important role in metal uptake and transport in plant (Li *et al*., 2006).

Even though the literature presents papers using bacteria such as *Staphylococcus saprophyticus* (Ilhan *et al*., 2004), algal cells (Cossich et al., 2005), filamentous fungi, *Aspergillus* spp. (Sen and Ghosh Dastidar, 2007) and other biomaterials (Lister and Line, 2001) Sang Yun and Volesky use the waste crab-shells (*Ucides cordatus cordatus)* for the biosorpion of chromium (VI) and vanadium (V). Tudury (2006) proved that the eggshell using for biosorption of many heavy metals such as Cd, Ni, Zn and Co.

Table (1-2) Different technologies for the removal of heavy metals from the industrial wastewater (Ahalya *et al*., 2003).

Technology	Concentration dependence	pH	Suspended solids	Effluent Concentration (mg/I)	Regeneration	Sludge Generation
Biosorption	**Yes**	**Yes**	**Yes**	**<1**	**Yes**	**No**
Hydroxide Precipitation	**No**	**No**	**yes**	**2-5**	**No**	**Yes**
Sulfide Precipitation	**No**	**No**	**Yes**	**<1**	**No**	**Yes**
Ion Exchange	**yes**	**Some**	**No**	**<1**	**Yes**	**Yes**

Continued

Evaporation	**yes**	**yes**	**yes**	**1-5**	**------**	**No**
Reverse Osmosis	**No**	**Some**	**No**	**1-5**	**No**	**No**
Adsorption	**Yes**	**Some**	**Yes**	**1-5**	**Yes**	**No**

1.2.5 Biosorbent types

Various types of microbial biomass have been used for their biosorptive potential, including bacteria (Ilhan *et al*., 2003), fungi (Sen and Ghosh Dastidar, 2007), marine and freshwater algae (Cossich *et al*., 2002). Studies using the mixed microbial biomass found in activated sludge have also been reported (Hammaini *et al*., 2006).

Some biomass types are very effective in accumulating heavy metals. Availability is a major factor to be taken into account to select biomass for clean-up purposes. The economy of environmental remediation dictates that the biomass must come from nature or even has to be a waste material (Vieira and Volesky, 2000). Ilhan *et al*. (2003) stated that non-viable microbial biomass frequently exhibits a higher affinity for metal ions compared with viable biomass probably due to the absence of competing protons produced during metabolism. Cabuk *et al*. (2004) notice that the use of dead cells offers the following advantages over live cells: the metal removal system is not subject to toxicity limitations, there is no requirement for growth media and nutrients, the biosorbed metal ions can be easily desorbed and biomass can be re used, and dead

biomass-based treatment systems can be subjected to traditional adsorption models in use. As a result, the use of dead fungal biomass has been preferred in numerous studies on biosorption of toxic metal ions from aqueous solutions.

Some microbial derivatives such as excreted metabolites, polysaccharides or cell wall constituents have also been used as biosorbents.

1.2.5.1 Algae

It has been work on the Bengal gram husk *Cicer arientinum* for the removal of Cr (VI) ions from aqueous solutions. Bengal gram husk (Bgh) *Cicer arientinum* is a milling agrowaste available in plenty in a tropical country like India. Also, the protein content in bgh is less than 5%, which is advantageous over the protein rich algal and fungal biomass, since proteinious materials are likely to putrefy under moist conditions (Ahalya *et al*., 2005).

Lead and cadmium have been effectively removed from very dilute solutions by the dried biomass of some ubiquitous species of brown marine algae such as *Ascophyllum* and *Sargassum,* which accumulate more than 30% of biomass dry weight in the metal (Volesky and Holan, 1995). Non-living *Sargassum natans,* a brown seaweed, showed extraordinary uptake capacity for gold with 420 mg Au/g dry biomass (Kuyucak and Volesky, 1988).

Volesky and Holan (1995) focus on the composition of marine algae polysaccharide structures, which seem instrumental in metal uptake and binding.

1.2.5.2 Yeasts and fungi

Mycelia of the industrial steroid transforming fungi *Rhizopus* and *Absidia* are excellent biosorbents for lead, cadmium, copper, zinc, and uranium and also bind other heavy metals up to 25% of the biomass dry weight (Volesky and Holan, 1995). As shown in figure (1-1) Fungal cell walls and their components have a major role in biosorption and also take up suspended metal particulates and colloids (Ilhan *et al*., 2004).

Rhizopus arrhizus, a filamentous fungus containing, chitin, a naturally occurring material, and chitosan, the deacetylated form of chitin, chitin and chitosan as a main cell wall component. Which they were shown to have good metal- sequestering properties (Sag and Aktay, 2002)

Yan and Viraraghavan (2000) investigated the effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii*. Kapoor and Viraraghavan (1999) studied the effect of pretreatment of *Aspergillus niger* biomass on biosorption of lead, cadmium, copper and nickel.

Non-living samples of *Saccharromyces cerevisiae* sequestered uranium, zinc and cadmium to a greater extent than the corresponding living biomass. Dead fungal biomass of *Rizopus arrhizus* exhibited the highest uptake of uranium with 180 mg U/g dry biomass while dead yeasts *Saccharromyces cerevisiae* removed about 157 mg U/g dry biomass (Kuyucak and Volesky, 1988).

1.2.5.3 Bacteria

Bacterial surfaces are typically anionic and, therefore, interact with metal cations, Gram positive walls consist of a variety of hetero- and homopolymers which in combination produce an electronegative charge density though out the wall fabric at neutral pH. Beveridge and Koval (1981) indicated that teichoic and teichuronic acids are the prime sites of metal binding in *Bacillus licheniformis* walls.

Strandberg *et al*., (1981) stated that the uranium accumulation by *Pseudomonas aeruginosa* occurred intracellullary and was extremely rapid (<10s), and no environmental parameter could be detected.

Matthews and Doyle (1979) reported that the carboxyl groups of peptidoglycans in the cell walls of *Bacillus subtilis* are the primary sites of divalent metal complexation.

Hussein *et al*., (2004) work on the biosorption of heavy metal by the different *Pseudomonas* species, most of the metal ions were sequestered very fast from solutions within the first 10 minutes and almost no increase in the level of bound metals have been occurred after this time interval.

It should be stressed here that both living and dead cells are capable of uptake and accumulation of heavy metals. However a dead biomass is preferred to a viable one for the following reasons:

(1) The biosorption process is often executed in harsh environmental conditions.

(2) There is no requirement for maintenance and nutrition, and the biosorbents can be stored for a long time without affecting their performance.

(3) There is no toxic effect of metals on dead microorganisms, so their sorptive capacity is not affected.

(4) In many cases, sorption by dead biomass is more efficient than sorption by living biomass (Tsezos, 1990).

1.2.6 Biosorption and bioaccumulation

Microorganisms have been adapted a number of mechanisms to remove and tolerate heavy metals from their surrounded. There are two modes of metal uptake; Biosorption and bioaccumulation. Microbial biomass can passively bind large amounts of metal(s), a phenomenon

commonly referred to as biosorption (Tsezos and Volesky, 1982; Macaskie and Dean, 1985). Biosorption is possible by both living and nonliving biomass; while, bioaccumulation is mediated only by living biomass (Garnham *et al*., 1992). Biosorption is a rapid phenomenon of a passive metal sequestration by the non-growing biomass. Biosorption mainly involves cell surface complexation, ion exchange and micro precipitation (Volesky, 1987). The uptake of heavy metals by biomass is usually classified into three categories:

(1) Cell surface binding.

(2) Intracellular accumulation.

(3) Extracellular accumulation.

Being metabolism independent, the cell surface binding can occur in either living or inactivated microorganisms, whereas the intracellular and extracellular accumulation of metals are usually energy-driven processes, and thus can take place only in living cells (Ilhan *et al*., 2004).

Different microbes have been found to vary in their affinity for different heavy metal(s) and hence differ in their metal binding capacities. Some biomass (es) exhibit preference for certain heavy metal(s) whereas others do not show any specific binding and are broad range (Greene and Darnall, 1990).

Metal binding appears to be at least a two-step process where the first step involves a stoichiometric interaction between the metal and the reactive chemical groups in the cell wall and the second step is an inorganic deposition of increased amounts of metal(s).

According to Crist *et al*. (1981), the potential metal bind in groups in photoautotroph's (eukaryotic algal cell) is carboxylate, amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl. Of these, amine and imidazoles are positively charged when protonated and may build negatively charged metal complexes (Greene and Darnall, 1990)

Cell walls of gram-negative bacteria are somewhat thinner than the gram-positive ones and are also not heavily cross-linked. They have an outer membrane which is composed of an outer layer of lipopolysaccharide (LPS), phospholipids and Proteins (Remacle, 1990)

Gourdon *et al*. (1990) compared the Cd^{2+} biosorption capacities of gram-positive and gram-negative bacteria. Glycoprotein present on the outer side of gram-positive bacterial cell walls have been suggested to have more potential binding sites for Cd^{2+} than the phospholipids and LPS.

In *Bacillus subtilis*, teichoic acid and in *Bacillus licheniformis*, teichoic acid and teichouronic acid were found to be the prime sites for metal binding.

The phosphoryl groups of the LPS and phospholipids have been demonstrated to be the most probable binding sites for metal cations in the *E. coli* outer membrane (Ferris and Beveridge, 1984).

Avery unique mechanism of metal uptake was reported in *Citrobacter sp*. This species showed a very high U^{6+}, Cd^{2+}, Cu^{2+} and Pb^{2+} removal from solution supplemented with glycerol 2-phosphate. The mechanism of uptake involved a phosphatase-mediated cleavage of glycerol 2-phosphate to release HPO_4^{2-} which precipitated metal on the surface as insoluble metal phosphate (Macaskie and Dean, 1985).

The mechanisms by which microorganisms remove metals from solutions are:

(I) Extracellular accumulation/precipitation.
(II) Cell-surface sorption or complexation.
(III) Intracellular accumulation.

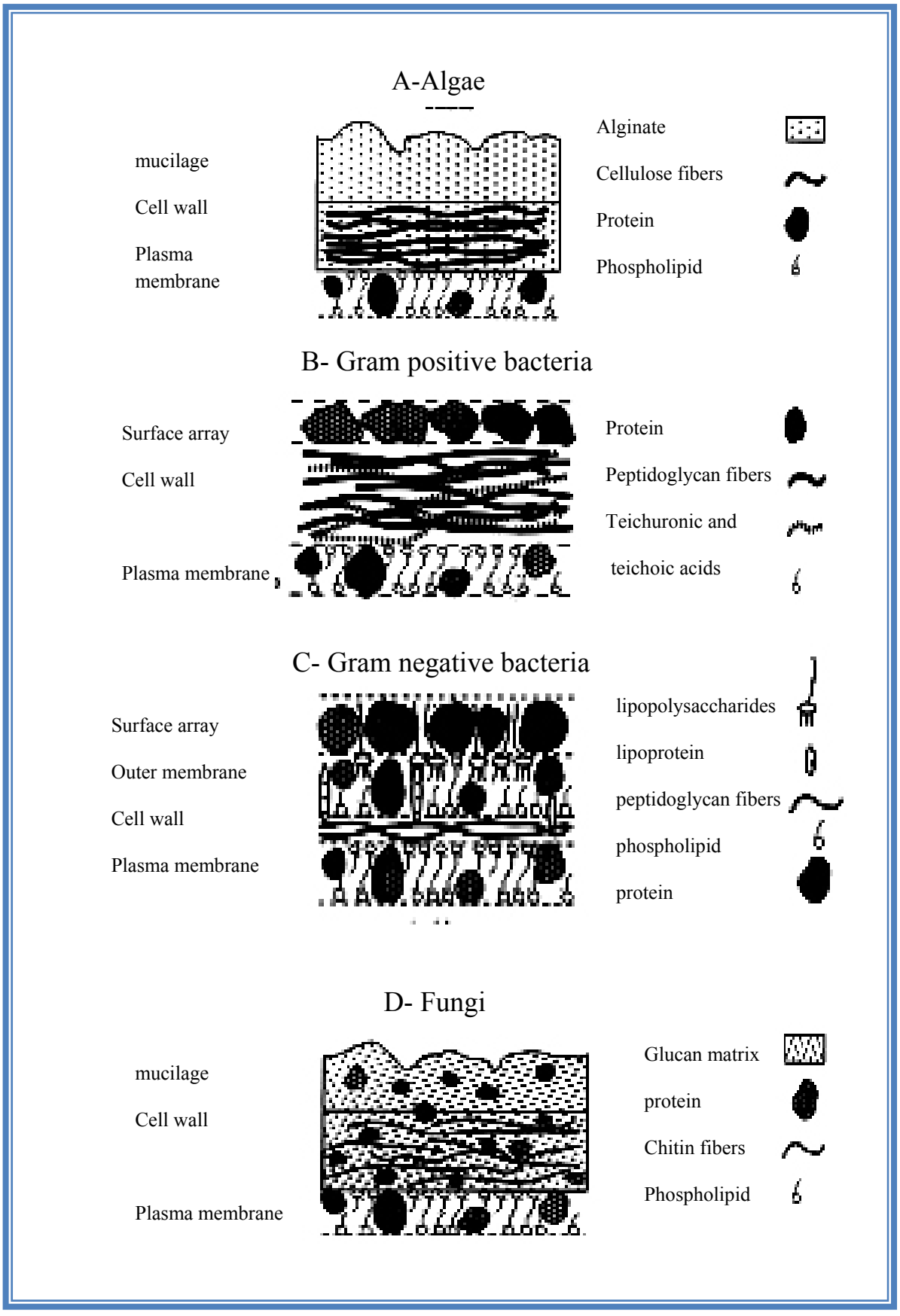


Figure (1-1): Cell wall structures of some microbial biosorbent.

Among these mechanisms, process (I) may be facilitated by using viable microorganisms, process (II) can occur with alive or dead microorganisms, while the process (III) requires microbial activity. (Muraleedharan *et al*., 1991).

Fourest and Volesky (1990) have studied the involvement sulfonate groups and alginate in biosorption by a dry biomass of *Sargassum fluitans*. The mode of interaction between metal species and microbial cell components may be simple adsorption, ion exchange, electrostatic interaction, complexation, precipitation, and crystallization. (Crist *et al*., 1994).

The brown seaweed *Sargassum* sp. is mainly constituted by the polysaccharide alginate, usually calcium and sodium alginates, thus with a high potential for the accumulation of heavy metals, as compared to other algal genera (Da Costa and de Franca, 1996).

Microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Shumate and Strandberg, 1985) biosorptive process are more applicable than the bioaccumulative processes , because living systems (active uptake) often require the addition of nutrients and hence increase biological oxygen demand (BOD) or chemical oxygen demand (COD) in the effluent. In addition, maintenance of healthy microbial population is difficult due to metal toxicity and other unsuitable environmental factors. (Brown and Lester, 1982). It is well-known that marine algal biomass contains carboxyl groups capable of binding Ni (II) through cation-exchange (Holan and Volesky, 1994) In the case of the *Ecklonia* biomass; the functional group related to the interaction between protons and metal ions is a carboxyl group.

1.2.7 Factors affecting biosorption

There is more than one variable affecting the biosorption process, such as temperature, pH, agitation rate and metal concentration.

1.2.7.1 pH

The biosorption of metal ions strongly depend on pH of the aqueous phase, it seems that for the majority of biosorbents, the optimum pH is slightly acid to around neutral (5.0 -7.0) **(**Kiff and Little, 1986). According to the study by Guibal *et al*. (1992) on the uranium biosorption by *Mucor miehei,* pH imposes its influence on metal or cell wall chemistry. The biosorption capability of *Ganoderma lucidum* at pH 6 was much higher than at pH 4 (Matheickal *et al*., 1991).

Tsezos and Volesky (1981) thought that acid pH in solution will decrease biomass uptake of metals via competition at the binding site between metal ions and H_2O. Kiff and Little (1986) reported on the increase in the biosorption of cadmium on *A. oryzae* with an increase in pH. A study by Lewis and Kiff (1988) also showed that in using *Rhizopus arrhizus,* its uptake capacity was decreased by acidic pH, low temperature, and presence of competing cations, and optimum pH was 6 to 9. The biosorption of Ni, Zn, Cd, and Pb by *Penicillium digitatum* was found to be highly pH-sensitive and was severely inhibited when pH was below 3 (Gdun *et al*., 1987). Brady *et al*. (1994) reported that the optimal pH for biosorption of Zn on *Saccharomyces cerevisiue* biomass was 7.5 even though biosorption occurred above pH 4.

Ross and Townsley (1986) found that at lower pH, removal of copper by *Penicillium spinulosum* was reduced. *Rhizopus nigricuns* had significantly low sorption capacity of lead at pH values below 3; at pH above 4, more lead biosorption was expected to take place (Zhang *et al*.,

1998). In addition, it was also found that at higher pH insoluble lead hydroxide started to precipitate. Tobin and Roux (1998) also reported that at initial pH values of 5.5 and 7.0, significant precipitation effects occurred in the removal of chromium by *Muccor meihi***.**

It is a fact that pH of the solution influences both the ionic forms of metals and the ligands for binding of metals at the cell surface. Which strongly affecting the biosorption, also it has often been accepted that many biosorbents have high uptake capacities for heavy metals at high pH (Volesky, 1990; Kaewsarn, 2000). The maximum capacity for biosorption for both Ni2+and Pb2+ was observed at pH 4 by *Phanerochaete chrysosporium* (Ceribasi and Yetis, 2000). The copper uptake by *Ulva reticulata* increased with increasing pH, to the maximum near pH 5.5, then decreased at higher pH value. In general very little or no copper uptake was observed at pH less than 2.0. Working over pH 6.0 was avoided to prevent the possible precipitation of copper hydroxide (Vijayaraghavan *et al*., 2004).

The highest nickel adsorption was obtained from *Bacillus subtilis* at pH 8.0 while *Enterobacter agglomerans* gave the highest value at pH 7.0 (Kaewchai and Prasertsan, 2002).It was stated that the optimum pH values for chromium, lead and copper biosorption was found to be 2.0, 4.5 and 3.5 respectively(Ilhan *et al*., 2004). The initial pH of the solution had a significant effect on uranium accumulation. At pH 6.5, maximum accumulation of uranium was obtained (86% within the first 5 min) and at higher and lower pH, the amount of uranium accumulation was less (Malekzadeh et al, 1996). Biosorption of Cr (VI) by *Trichoderma viride* was pH dependent and the maximum adsorption was observed at pH 2.0 (Bishnoi *et al*., 2007).

1.2.7.2 Temperature

Temperature is greatly affecting the biosorption. Decreasing temperature below 24ºC decreased fungal growth and enzymatic activity. Furthermore, increasing the temperature up to about 40°C decreased the fungal growth and consequently the chromium removal extent. Nouri Sepehr *et al*., (2005) proved that the maximum biomass growth and chromium removal rate was achieved at 30ºC. The data obtained showed that adsorption of metal ion by the *Caladium bicolor* biomass increased with increase in temperature which is typical for the biosorption of most metal ions from their solution (Ho, 2003). Singleton and Simmons (1995) stated that temperature increase from 4°C to 55°C decreased silver biosorption by *Saccharomyces cerevisiae.* While Horsfall *et al*., (2005) stated that the adsorption of metal ion by the *Caladium bicolor* biomass increased with the increase in temperature, they also mentioned that the decrease in adsorption with increasing temperature, suggest weak adsorption interaction between biomass surface and the metal ion, which supports physisorption

1.2.7.3 Initial metal concentration

Generally, biosorption capacities of *Phanerochaete chrysosporium* for Ni and Pb increased with increasing initial metal concentration (Ceribasi and Yetis, 2000). The maximum adsorption was observed for Cr^{6+}, $Pb^{2+}$and Cu^{2+}, at the initial concentrations of 193.66 mg Cr^{2+}/l; 100 mg Pb^{2+}/l and 105 mg Cu^{2+}/l and under these conditions the biosorption values were found to be 88.66 mg Cr^{6+}/l; 100 mg Pb^{2+}/l and 44.94 mg Cu^{2+}/l, respectively(Ilhan *et al*., 2004).

It was found that the amount of uranium taken up by the cells increased with increase in concentration of uranium. No increase in

uptake of uranium was observed at concentrations greater than 200 mg /L (Malekzadeh *et al*., 1996).

The presence and concentration of organics may hinder the biosorption process. For example, probably because of binding of chromium with organics such as proteins, bacteria, or tannins in solution, *Saccaromyces cerevisiae* was not effective in removing chromium from tannery wastewater (Brady *et al*., 1994). Thus, it can be implied that some biosorbents are applicable to wastewater with relatively low concentrations of organics. Padmavathy *et al*., (2002) proved that the nickel sorption capacity by baker yeast increased by increasing initial metal concentration.

1.2.7.4 Physiological state

Physiological state is also affect the biosorption according to Safarikova *et al*., (2004) who mentioned that yeast cells which are at first magnetically modified and then heated exhibit higher adsorption capacity than yeast cells heated and subsequently magnetically modified. Cabuk *et al*., (2004) showed that the live biomass of *Aspergillus versicolor* had the highest biosorbent activity for lead ions among the species studied such as *Metarrhizium anisopliae* var. *anisopliae* and *Penicillium verrucosum* live biomass. Also Cabuk *et al*., (2004) noticed that an increase in biosorption of lead ions as a result of pretreatment could be due to an exposure of active metal binding sites embedded in the cell wall or chemical modifications of the cell wall components.

1.2.7.5 Agitation rate

It was found that copper uptake by *Sargassum* sp increases with the increase in shaking rate (q = 2.7 mg/g in absence of agitation and 3.8 mg/g at 50 rpm), and that the adsorption capacity of algae remained

constant at 4.3 mg/g for agitation rates greater than 100 rpm (Antunes *et al*., 2003).

In order to study the effect of shaking velocity on chromium removal rate by *Aspergillus oryzae*, this parameter was varied in the range of 50 and 250 rpm. The maximum amount of biomass growth and chromium removal rate occurred at 150 rpm. With increasing shaking velocity, biomass growth was stationary (Nouri Sepehr *et al*., 2005).

1.2.7.6 Time of biosorption

Ahalya *et al*., (2006) stated that the sorption of iron by Bengal gram husk biomass was very rapid, in which the equilibrium was achieved with in 15 min. Bishnoi *et al* (2007) stated that the adsorption equilibrium was reached in 90 min. for Cr (VI) with *Trichoderma viride*. Park *et al*. (2005) stated that the concentration of Cr (VI) decreased with increasing contact time.

1.2.8 Mechanisms of biosorption

The mechanisms involved in the process may include ion exchange, coordination, complexation, chelation, adsorption and microprecipitation (Guibal *et al*., 1992; Fourest and Roux, 1992). These may take place even when cells are metabolically inactive such as when they are killed by physical or chemical methods (Brady *et al*., 1994). Neither an active membrane transport mechanism nor metabolic energy is required in the process. A non-directed physicochemical reaction dominates the process (Gadd, 1992).

Binding of metals to cell surface components and intracellular accumulation were identified as two mechanisms in biosorption of metals by live microorganism (Gadd and Grifiths, 1978). The intracellular accumulation or metabolic processes may result in the accumulation of

relatively large amounts of metal but these processes are slow and are mostly dependent on nutrient and environmental conditions (Brierley *et al*., 1985). Surface and wall binding is a passive process and takes place with both live and dead biomass.

This non-metabolic surface binding is very rapid, often less than a few minutes (Khovrychev, 1973). As shown in the figure (1-2) this kind of metal uptake occurs by ion exchange processes involving specific chemical sites on the cell wall (Hancock, 1986).

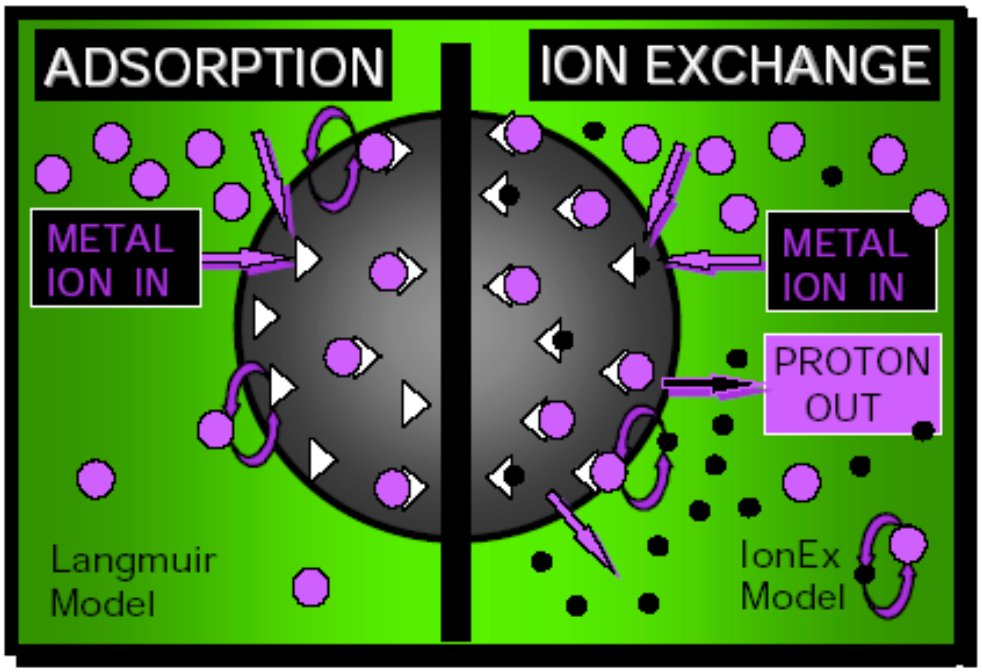


Figure (1-2): Adsorption is different from of ion exchange.

In the zinc uptake by live *Neocosmospora vasinficta,* Paton and Budd (1972) found that the uptake involved two phases: a rapid established phase or adsorption to negatively charged groups in the hyphal surface-membrane and a slowly established phase of transport into the cytoplasm. Khalid *et al*. (1993) suggested that in the biosorption of uranium on live *Trichoderma harriantrm* two phase adsorption phenomenon were

involved: the first one was due to rapid physical sorption, and the second was due to structural change and surface transformation.

Copper biosorption by live yeast *Saccaromyces cerevisae* was found to be also biphasic and consisted of an initial, rapid surface binding, followed by a second, slower intracellular uptake. The intracellular uptake could account for 23% of total copper uptake (Huang et al., 1990).

Muraleedharan and Venkobachar (1994) reported that in metal uptake by *Ganoderma lucidum,* metal uptake was at the cell wall and structural polysaccharides probably were the main sites of interaction. Kiff and Little (1986) explained that the majority of biosorption of cadmium on live *Aspergillus oryzae* was accounted for by surface adsorption and intercellular accumulation was of little significance.

Tsezos and Volesky (1982) suggested that surface binding was the key to the rapid establishment of uptake equilibrium in biosorption of uranium and thorium by dead *Rhizopus arrhizus.* Huang *et al*. (1990) showed that copper biosorption by dead yeast *Saccaromyces cerevisae* was achieved via surface binding, while cadmium and lead biosorption by the Iive yeast was found to be also via only surface binding.

Ross and Townsley (1986) suggested that the uptake of Cu, Cd and Zn by *Penicillium. spinulosum* and *Aspergillus niger* mycelium was a non-metabolic process, It is likely that extensive binding of metal ions to external parts of cells would mask the metabolism-dependent transport of smaller amounts of metals into the cells under some conditions. However, Paton and Budd (1972) reported uptake of zinc by mycelium of *Neocosmosporu vasinfecta* was a metabolism-dependent, so was copper bioasorption by protoplasts of *P, ochro-chloren* (Gadd and white, 1985).

Biosorption using *Rhizopus arrhizzus* was found to be independent of the ionic charge or electrostatic strength and was linearly influenced by

the ionic radius (Tobin *et al*., 1984). However, *Penicillium* biomass biosorption was inversely related to the ionic radius (Galun *et al*., 1987).

According to the results of cadmium adsorption by 12 species of fungi, Huang *et al*. (1988) showed that the removal of cadmium was realized by adsorption rather than precipitation, at least in the pH region less than 8. Studies on uranium and thorium binding by *Rhizzopus arrhizus* indicated that binding was not a simple adsorption but a complex process (Tsezos and Velosky, 1982).

Chitin component of fungal biomass was reported to be the major factor contributing to the metal removal capacity (Strangberg, 1981). However, Tien and Huang (1988) reported that both the polysaccharide and the protein component played an equal role in metal adsorption. Chitin content showed little difference among various fungal species, averaging 30% (w/w) (Huang *et al*., 1988).

As mentioned earlier, surface or cell wall binding involves specific chemical sites on the cell wall. Dead biomass may be more efficient because the cell walls may be raptured, exposing more surface binding sites (Gadd, 1990). There are many potential ligands including carboxylate, amine, phosphate, hydroxyl, sulfhydryl, and other functional groups in biomass (Beveridge and Koval, 1981).

Sigg (1987) suggested that amino, phosphate, sulfhydryl, carboxyl or hydroxy groups were major potential adsorption sites of microbial surface for metal ions. A significant portion of the metal uptake may be due to coordination of the functional groups in the biomass to the metal ions. Tobin *et al*., (1984) suggested that any site could have several different functional groups participating to various extents in binding the metal ions. Biosorption of heavy metals on different fungi may involve

different functional groups to varying extents (Kapoor and Viraraghavan, 1998).

The polymeric structure of biomass surfaces consists primarily of proteins, carbohydrates, nucleic acid and lipid, and is of a negative charge because of the ionization of organic groups such as carboxylic, aliphatic, aromatic and amino groups and inorganic groups such as hydroxyl and sulphate groups (Hughes and Poole, 1989). Bux and Kasan (1994) suggested that the principal driving force for biosorption of metal ions was the net negative surface charge of the biomass. The higher the biomass electronegativity the mater the attraction and adsorption of heavy metal ions. Therefore, pH will affect the performance of these functional groups. Through investigation of metal binding by *Rhizzopus arrhizus,* Tobin *et al*., (1984) found that at pH 4, amines would be positively charged and may not interact with metal ions; many of the carboxylate groups would be neutral. Most of the phosphate groups have a negative charge above pH 3.0, thus continue to the meal binding (Beveridge and Murray, 1980). Hydroxyl and amine groups are weak bases and could form weak bonds with metal ions; therefore, most of the sites of metal binding may contain carboxylate or phosphate ligands or both (Tobin *et al.,* 1984). At pH 4 few functional groups in the biomass are of negative charges. Hunt (1986) recommended that enhancement of hydroxyde concentration between pH 6.0 and 7.0 could activate additionna1 binding sites in the hyphal walls such as phosphate monoesters groups of sugars or phospholipids. Kapoor and Viraraghavan (1997) showed that both carboxyl and amine groups played an important role in biosorption of lead, cadmium and copper on *Aspergillus niger*. Gardea-Torresdey *et al*., (1996) reported that in the biosorption of copper on inactive *Mucor romii,* the carboxyl group could be one of the mechanisms involved. Lead adsorption capacity of *Rhizopus nigricans* is related to the free amino

group of the chitosan monomer (Zhang *et al*., 1998). Farkas (1980) reported that up to 90% of dry fungal biomass consisted of amino- or no amino-polysaccharides. With an electron pair available for coordination, the amino group behaves like a strong Lewis base to coordinate metal ions.

The function of specific chemical sites is driven by the ion exchange process. In other words, ion exchange was considered to be a major mechanism of metal uptake (see figure 1.2). In biosorption of copper on *Gunudenna Iuciduni,* Ca and H ions were released (Muraleedharan and Venkobachar, 1994). Kapoor and Viraraghavan (1998) found that biosorption of metal ions on *Aspergillus niger* released K, Cd, H and Mg ions, indicating an ion exchange mechanism.

2. Materials and Methods

2.1 Materials:

2.1.1 Equipment and apparatus:

The following equipments and apparatus were used throughout the study:-

	Apparatus	Company(Origin)
1	Atomic absorption flame emission spectrophotometer	Shimadzu (Japan)
2	Autoclave	Express (Germany)
3	Centrifuge	Hermle (Germany)
4	Hot plate magnetic stirrer	Gallenkamp (England)
5	Incubator	Sanyo (Japan)
6	Compound Light microscope	Olympus (Japan)
7	Micro-pipettes	Witey (Germany)
8	Oven	Sanyo (Japan)
9	pH-meter	Mettler Toledo
10	Shaking incubator	GFL (Germany)
11	Vortex	Stuart scientific
12	Distillator	Gallenkamp
13	UV lamp	Vilber Lourmat (France)

2.1.2 Chemicals:

The following chemicals were used in this study:

Material	Company(Origin)
Peptone	Oxoid (England)
$MgCl_2$	BDH (England)
K_2SO_4	Sigma (USA)
Cetrimide	Merck (Switsland)
Glycerol	BDH
$MgSO_4.7H_2O$	BDH
Tryptone	Sigma
Yeast extract	BDH
NaCl	BDH
Glucose	Fluka (Germany)
Gelatin	Difco (USA)
Beef extract	Difco
Tetramethyl-p-phenlenediamine Dihydrochloride	BDH
H_2O_2	Difco
$NiCl_2.6H_2O$	BDH
Safranin	Fluka
Crystal violet	Fluka

2.1.3 Culture media:

2.1.3.1 Ready to made media:

2.1.3.1.1. Nutrient agar (Oxoid- England).

2.1.3.1.2. Nutrient broth (Oxoid- England).

2.1.3.1. 3. Brain heart infusion broth. (HiMedia Laboratories –India)

All these media were prepared according to the instructions of manufacturer. pH was adjusted to 7 and sterilized by autoclaving.

2.1.3.2 Synthetic media

2.1.3.2 .1. Certrimide agar medium (Stolp and Gudkari, 1984):

This medium was consisted of the following components:

Component	Weight (g)
Peptone	20
$MgCl_2$	4.5
K_2SO_4	10
Cetrimide	0.3
Agar	15

All the components were dissolved in 900 ml distilled water, pH was adjusted to 7, and then volume was completed to 1000ml and sterilized by autoclaving.

2.1.3.2 .2 King A medium (Starr, *et al.*, 1981)

This medium was consisted of the following components

Component	Weight (g)
Peptone	20
$MgCl_2$	1.4
K_2SO_4	10
Glycerol	10 ml
Agar	15

All the components were dissolved in 900 ml distilled water, pH was adjusted to 7, and then volume was completed to 1000ml and sterilized by autoclaving.

2.1.3.2.3King B medium (Starr, *et al.,* 1981)

This medium was consisted of the following components

Component	Weight (g)
Peptone	20
$MgSO_4.7H_2O$	3.5
K_2SO_4	15
Glycerol	10 ml
Agar	15

All the components were dissolved in 900 ml distilled water, pH was adjusted to 7, and then volume was completed to 1000ml and sterilized by autoclaving.

2.1.3.2.4 Luria – Bertani broth(Maniatis *et al*, 1982)

This medium was consisted of the following components:

Component	Weight (g)
Tryptone	10
Yeast extract	5
NaCl	5
Glucose	10

All ingredients were dissolved in 900ml distilled water, pH was adjusted to 7, and then volume was completed to 1000ml and sterilized by autoclaving.

2.1.3.2.5. Gelatin agar (Cown, 1979)

This medium was consisted of the following components:

Component	Weight (g)
Gelatin	4
K_2HPO_4	0.5
KH_2PO_4	0.5
Glucose	0.05

All the components were dissolved in 900 ml distilled water, pH was adjusted to 7, and then volume was completed to 1000ml and sterilized by autoclaving.

2.1.4 Reagents

- Oxidase reagent

This reagent was prepared by dissolving 1g of tetra methyl -p-phenlenediamine dihydrochloride in 100ml distilled water and kept in dark bottle at 4°C until use.

- Catalase reagent

It was prepared to be consist of 3% H_2O_2 in distilled water.

2.1.5 Solutions:

2.1.5.1 Nickel stock solution (100mg/L):

Stock solution of nickel (Ni^{2+}) was prepared by dissolving 100 mg of $NiCl_2.6H_2O$ in 1L of deionized distilled water and kept in tightly closed bottle at 4C°.

2.1.5.2 Cobalt stock solution (100mg/L):

Stock solution of cobalt (Co^{2+}) was prepared by dissolving 100mg of $CoCl_2.6H_2O$ in 1L of deionized distilled water and kept in tightly closed bottle at 4C°.

2.1.5.3 Zinc stock solution (100mg/L):

Stock solution of zinc (Zn^{2+}) was prepared by dissolving 100mg of $ZnCl_2.6H_2O$ in 1L of deionized distilled water and kept in tightly closed bottle at 4C°.

2.1.5.4 Lead stock solution (100mg/L):

Stock solution of lead (Pb^{2+}) was prepared by dissolving 100mg of $PbCl_2$ in 1L of deionized distilled water and kept in tightly closed bottle at 4C°.

2.2 Methods:

2.2.1 Sterilization methods

2.2.1.1 Autoclaving: Culture media were sterilized by autoclaving at 121°C for 15 min. and 15 Ib/In.

2.2.1.2 Oven sterilization: Glasswares in the oven were sterilized at 200°C for 2 hours.

2.2.2 Isolation of *Pseudomonas spp.*

2.2.2.1 Collection of samples

Eighty-five samples from different environments including soil, waste water, sewage and fresh water were collected during the period from October 2006 to January 2007. These samples were used to isolate *Pseudomonas spp.*

2.2.2.2 Samples preparation

In order to isolate *Pseudomonas* sp. from the collected samples, one gram of each soil sample was added to 9 ml of sterilized D.W in sterile test tubes, mixed vigorously, and let to stand for few minutes. Aliquots (1ml) was taken from each of waste water, sewage, and fresh water samples and add it to 9ml of sterilized D.W in sterile test tubes, mixed well, then serial dilutions were made.

2.2.3 Isolation of *Pseudomonas spp.*

A portion of 100 microliter of suitable dilution was taken from each sample, spreaded on nutrient agar plates, and incubated at 28º C for 24 hr. The resultant colonies were then transferred to cetrimide agar plats, and incubated at 28° C for 48hrs, then single colonies were transferred

separately and put on King A and King B plates, to study the ability of these isolates in production of pyoverdin and pyocianin pigments (Holt, *et al.,* 1994).

2.2.4 Identification of the locally isolated *Pseudomonas* spp.

Locally isolates of *Pseudomonas spp*. from different environmental samples were identified according to their morphological, physiological characteristics and biochemical tests and as follows:

2.2.4.1 Morphological and physiological characteristics:

As a primary step for identification of *Pseudomonas spp*., suspected local isolates were examined after staining, according to their shape, gram reaction, size, production of pigments, and mucoidal growth properties were studied after incubation on nutrient agar plates at 28º C for 24 hrs. (Palleroni, 1985).

2.2.4.2 Biochemical tests

In order to identify the locally isolated *Pseudomonas* spp., all isolates were subjected to further identification using some biochemical tests (Bradbury, 1986).

2.2.4.2.1 Catalase test

This test was performed according to Atlas *et al* (1985) by adding few drops of 3%hydrogen peroxide solution to the surface of colonies grown previously on nutrient agar plates. Production of gas bubbles indicates a positive result.

2.2.4.2.2 Oxidase test

This test was performed according to atlas *et al* (1985) by miostening whattman filter paper with few drops of tetramethyl-p-phenylenediamine dihydrochloride solution, and then a loopful of each

isolate grown previously on nutrient agar plate was smeared on moistened filter paper using a sterile wooden stick. Development of a violet to purple color within 10 seconds indicates a positive result.

2.2.4.2.3 Gelatin hydrolysis test

A gelatin medium in test tubes was inoculated with the bacterial isolates and incubated at 37C° for 24 hour, and then test tubes were placed in a refrigerator for 30 minutes. Liquefaction of the media indicates a positive result (Atlas *et al*., 1995).

2.2.4.2.4 Growth on king A

Single colony of the each isolates streaked on king A agar medium and incubated at 28º C for 24 hrs. to examine the isolates ability in pyocyanin pigment production.

2.2.4.2.5 Growth on king B

Single colony of the each isolates streaked on king A agar medium and incubated at 28º C for 24 hrs. then the plates were exposed to U.V light, fluorescent growth indicates appositive result**.**

2.2.4.2.6 Growth at 4 C and 41 C

Ability of the bacterial isolates to grow at 4°C and 41º C wasµl studied according to Palleroni (1985), by inoculating 50 ml of nutrient broth with 50 microliter of freshly prepared culture of different isolates, then incubated at 4 º C and 41 º C, separately. Appearance of growth indicates a positive result.

2.2.5 Maintenance of *Pseudomonas* isolates:

- **Short -term storage**

Bacterial isolates were maintained on Luria –Bertani agar plates for weekly work .The plates were tightly warped in Para-film and stored at 4°C.

- **Medium – term storage**

Bacterial isolates were maintained for few months by stabbing nutrient agar medium in a screw capped tubes and stored at 4°C.

2.2.6 Screening the locally isolated *Pseudomonas* isolates for metal ion tolerance

Pseudomonas isolates were screened for metal tolerance according to plate diffusion method described by Abbas and Edwards (1989). In which square plates (100mm× 100mm) containing nutrient agar, as shown in fig. (2.1). Using a hot knife a trough (0.2 mm× 0.8mm)was made along one side of the plate (approx . 10 mm× 80 mm) , after which 0.2 ml of 0.1 g/ml of the appropriate metal was added . Plates were incubated overnight at 28° C to allow diffusions of the metal into the agar, by which time, a concentration gradient of metal had been established. Bacterial isolates were then streaked in lines at right angle to the troughs and right up it.

The plates were then incubated at 28° C for 24 h. After incubation, the tolerance of each strain was determined by measuring the leading edge of growing colony. Metal tolerance was expressed as a percentage of the total measured distance available for growth on the agar.

Growth (%) = A / 80 *100 (Sarhan, 2006).

A: the distance of bacterial growth (mm)

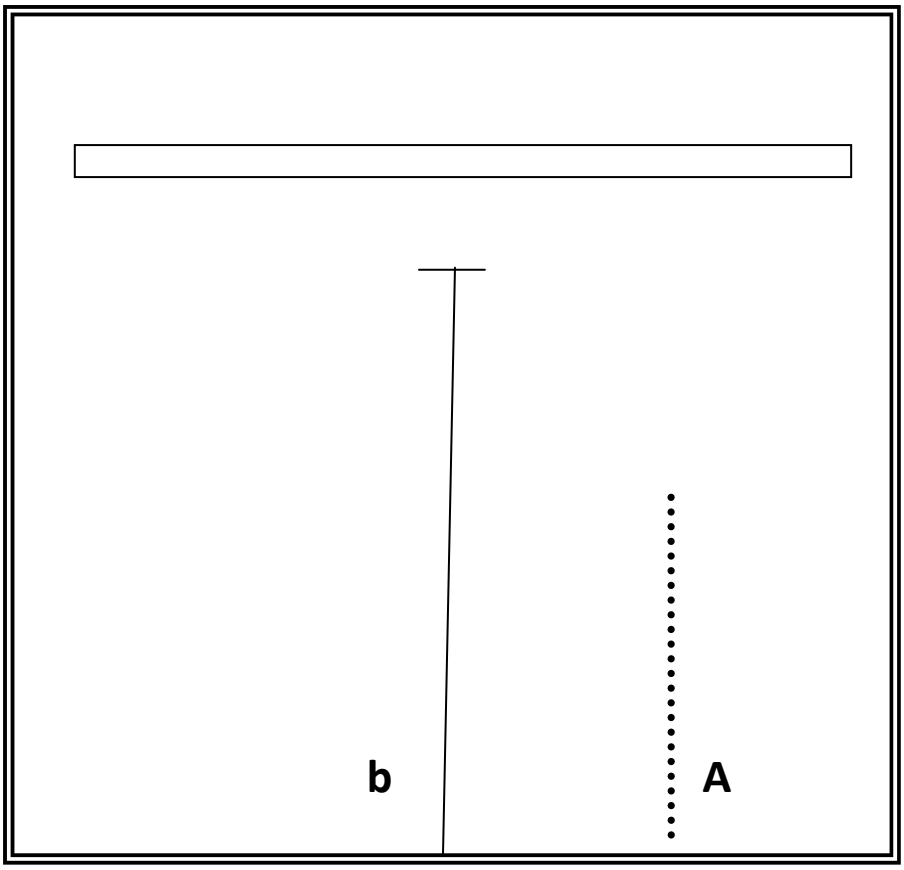


Figure (2-1) Diagram for actual dimensions of agar plate, showing the distance of growth (A), and the total available distance for growth on the agar (b).

2.2.7 Preparation of the biosorbent biomass:

For biosorption experiments, *Pseudomonas* isolates were grown in 500 ml Erlenmeyer flasks, each flask containing 100 ml of Brain heart infusion broth. Flasks were then incubated in an orbital shaker incubator (150 rpm) at 28 °C for 48 h. bacterial cells were then harvested by centrifugation at 6000 rpm for 20 min, washed twice with distilled water then resuspended in deionized distilled water. The dry weight of 1ml

from this suspension was estimated after drying at 90º C in the oven until their weight be constant.

2.2.8 Batch Biosorption experiment

Biosorption experiment for Ni^{+2} was performed in batches as described by Vecchio *et al*., (1998). A known amount of bacterial biomass was added to each Erlenmeyer flask containing selected concentration of Ni^{+2} stock solution in duplicate. Before adding biomass, pH of the metal solutions was adjusted to the desired values using 0.1 N NaOH. All flasks were shaken at 100 rpm in a rotary shaker at 28° C for 30 min unless otherwise stated. The sorption phase was terminated by centrifugation at 6.000 × g for 20 min to separate the flocculated biomass from the metal sorbed. The amount of metal sorbed by the biomass was determined by measuring the residual metal concentration in the supernatant using atomic absorption flame emission spectrophotometer.

The biosorption of metal was determined according to following equation:

$Q= (Ci – Cf)/x$ (Kogej and Pavko, 2001)

Where:

Q= Ni^{2+} uptake (mg Ni^{2+}/g biomass. Dry. Wt) is the amount of Ni^{2+}ions adsorbed on the biosorbent.

Ci= initial Ni^{2+} concentration (mg /L)

Cf= final (residual Ni^{2+} concentration (mg/L)

X= concentration of biomass (g dry wt/L)

2.2.9 Determining factors influencing biosorption of Ni^{+2} (Sarhan, 2006).

2.2.9.1 Effect of biomass age

This is done by the batch bisorobtion experiment when biomass was harvested in various interval times (1, 2, 3 , 4 , 5 ,6 days) then used for biosorption of Ni^{2+} solution .The optimum biomass age was used in the following steps.

2.2.9.2 Effect of biomass concentration

To determine the effect of biomass concentration on Ni²+ biosorption, concentration of Ni²+ was kept constant, while the biomass concentration was varied to 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 (g/l)

The optimum biomass concentration was used in the following experiments.

2.2.9.3 Effect of solution pH

To determine the optimum pH for Ni^{2+} biorption, Ni^{2+} solutions were adjusted to different pH values, (4, 5, 6, 7, 8, and 9).

2.2.9.4 Effect of temperature

This is done to determine the effect of temperature on Ni^{2+} biosorption with optimal pH and biomass concentration. The incubation temperatures used were 20, 25, 30, 35, 40 and 45°C.

2.2.9.5 Effect of initial concentration

Different metal concentrations were used to determine the capacity of biomass for metal sorption. The biomass concentration was kept constant, while Ni^{2+} concentrations were (20, 30, 40, 50, 60 and 75) mg/ 1.

2.2.9.6 Effect of physiologeical state of the cells

Different methods were used to inhibit the biological activity of the cells. In this experiment, boiling the biomass in a water bath at 100° C for 10 min was used. Biomass cells were treated with each of 20 ml alcohol

(60%) and with chloroform, 2N HCl and 2N NaOH for 1h. at room temperature (Kurek *et al.* , 1981) . After that the cells were washed twice with distilled water and collected by centrifugation at 6000 x g for 10 min. The dead biomass then was used for batch biosorption experiments.

2.2.9.7 Effect of contacting time

Biomass concentration and metal concentration kept constant for optimal pH, while the contacting times were varied (20, 40, 60, 80, 100, 120) min.

2.2.9.8 Effect of agitation rate

The biomass concentration in this experiment was 175 mg/350 ml, while the initial metal concentrations kept constant at optimal pH, various rates of agitation (50, 75, 100, 125, 150, 175, and 200 rpm/min) were used.

3. Results and Discussion

3.1 Isolation of *Pseudomonas sp.*

Results indicated in table (3-1) showed that 85 isolates were obtained from different environmental sources; only 42 of these were able to grow on cetrimide agar medium. This may referred that all the 42 isolates may belong to *Pseudomonas* sp. because cetrimide agar medium is a selective medium for isolation Pseudomonas.

Table (3-1) Bacterial isolates from different environmental samples.

Source of sample	No. of isolates	No. Grown on cetrimide agar
Soil	**33**	**17**
Fresh water	**19**	**5**
Waste water	**11**	**9**
Sewage	**22**	**11**
Total	**85**	**42**

These 42 isolates were further identified according to the morphological and physiological characteristics, and biochemical tests.

3.2 Identification of bacterial isolates

3.2.1 Morphological and physiological characteristic

Local isolates that were able to grow on cetrimide agar plates, may be suspected as Pseudomonas. They were further identified according to their cultural and morphological characteristics. For the former, colonies of each isolate were plated on nutrient agar medium show different morphological characteristics of *Pseudomonas sp.* mucoidal growth, smooth in shape with flat edges and elevated center, whitish or creamy in color.

Microscopical examination of each isolates showed that they are all having single cells, non- spore forming, Gram negative and rode shape. All these characteristics are similar to those of *Pseudomonas* sp. according to (Palleroni, 1985).

3.2.2 Biochemical tests

Biochemical tests were used to ensure that all the 42 isolates were *Pseudomonas sp*. Results indicated in table (3-2) showed that all of these isolates gave a positive result for catalase and oxidase tests; catalase is used to detect the presence of catalase enzymes due to the decomposition of hydrogen peroxide to release oxygen and water. Catalase enzyme is present in most cytochrome -containing aerobic and facultative anaerobic bacteria. While oxidase test is usually perform for identifying *Pseudomonas* sp. based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and α-naphthol. Also the 42 isolates gave positive results for gelatinase and pyocianin production on king A medium. All these isolates have the ability to grow in a range of temperature between 4-41°C. From these results it can be concluded that 42

isolates were belonging to *Pseudomonas sp.* as mentioned by (Palleroni, 1985).

3.2 Screening for heavy metals tolerance

Several studies proved that Pseudomonas have the ability to adsorb heavy metals and tolerate heavy metals. Plate diffusion method is qualitative assessments of the effects of heavy metals on growth. The results in the table (3-3) showed that the most resistant isolate of pseudomonas P36 for nickel while the most sensitive isolate was P2. The most resistant for cobalt was the isolate named P37 while the most sensitive was the isolate P6. Lead resistant isolate of pseudomonas was named P41, while the most sensitive. Finally the resistant isolate for zinc was P36, while the most sensitive was P14. While for other isolates the tolerance percent range from 12.5 to 73%. All these values obtained at a concentration 0.1g/ml. Also other results for these isolates are examined at different concentrations such as 0.05g/ml, 0.06g/ml, 0.07g/ml, 0.08g/ml, 0.14g/ml, 0.18g/ml and 0.2g/ml. the results associated with these different concentrations varied from heavy growth for these isolates to absence of bacterial growth. Therefore, the greater the distance of the colony from the trough edge, the greater the inhibition exerted by the metal as it was shown in figure (3-1).

Metal ion solution dispersion in the plate

The length of bacterial growth

P42 P36 P8 P38 P28

Figure (3-1): Growth of *Pseudomonas* isolates in presence of heavy metal by plate diffusion method

The result obtained from plate diffusion method regarding the 42 *Pseudomonas* isolates tolerance to nickel, cobalt, Lead, and zinc are shown in table (3-3). Some of the isolates showed high tolerance to one or more of the heavy metals, while the others showed less tolerance. The most tolerant isolate to Ni^{2+} and Zn^{2+} was P36, while for Co^{2+} was P37, and for Pb^{2+} was P41. On the other hand results obtained declared that the most sensitive (non tolerant) isolates for Ni^{2+} was P2, while for Pb^{2+}, Co^{2+} , Zn^{2+} were P6,P6,and P14.

According to these results, isolateP36 was chosen for the optimization of biosorption experiments. The plate diffusion method was used to give a rapid detection and qualitative estimation of isolates tolerance (Abbas And Edwards, 1989). By using such method was able to identify effect of the heavy metals on a number of *Pseudomonas* isolates. Other potential applications of this method include a rapid visual indication of the effects of different metal concentrations on sporulation and on any pigments produced as secondary metabolites produced by some species of streptomycetes (Abbas And Edwards, 1989).

Table (3-3) Metals tolerance of *Pseudomonas* isolates obtained by the plate diffusion method.

Isolate	Bacterial growth (%) for each heavy metal tolerance			
	Nickel (0.1g/ml)	Cobalt (0.1g/ml)	Lead (0.1g/ml)	Zinc (0.1g/ml)
P1	27.5	56.25	28.2	68.7
P2	18.75	31.2	70	58.7
P3	62.5	56.25	66.2	62.5
P4	50	56.2	38.7	68.7
P5	65	57.5	28.7	53.2
P6	58.7	12.5	14.5	58.7
P7	53.7	67.5	42.5	56.2
P8	70	58.75	48.7	34.2

P9	**40**	**53.7**	**52.5**	**45.5**
P10	**26.25**	**22.5**	**35**	**56.2**
P11	**50**	**40**	**73.7**	**58.7**
P12	**67.5**	**57.5**	**28.7**	**54.7**
P13	**65**	**57.5**	**12.5**	
P14	**43.7**	**31.25**	**76.2**	**12.5**
P15	**55**	**25**	**38.7**	**65**
P16	**45.7**	**57.5**	**33.7**	**37.2**
P17	**58.5**	**63.7**	**53.7**	**55.7**
P18	**23.5**	**57.5**	**30**	**46.7**
P19	**65.7**	**40**	**68.7**	**57.7**
P20	**29.5**	**55**	**47.5**	**53.7**
P21	**55**	**46.2**	**67.5**	**63.6**
P22	**33.7**	**31.2**	**20**	**34.5**
P23	**43.7**	**57.5**	**52.5**	**56.8**
P24	**53.7**	**55**	**70**	**43.6**
P25	**50**	**58.7**	**70**	**62.5**
P26	**27.5**	**18.7**	**67.5**	**48.7**
P27	**53**	**57.5**	**41.2**	**56.8**
P28	**37.5**	**50**	**28.7**	**60**
P29	**28.5**	**21.2**	**57.5**	**57.5**
P30	**50**	**18.7**	**63.7**	**58.7**
P31	**52.5**	**43.7**	**35**	**67.5**

Continued

P32	**52.5**	**33.75**	**45**	**58.7**
P33	**55**	**56.2**	**12.5**	**34.5**
P34	**41.2**	**16.2**	**34.5**	**63.7**
P35	**52.5**	**25**	**42.5**	**62.5**
P36	**73.7**	**55**	**28.7**	
P37	**43.7**	**71.2**	**57.5**	**23.7**
P38	**70**	**56.2**	**22.5**	**51.2**
P39	**58.7**	**46.2**	**46.2**	**54.6**
P40	**51.2**	**45.2**	**72.5**	**43.7**
P41	**60**	**57.5**	**78.7**	**58.5**
P42	**62.5**	**46.2**	**28.7**	**58.7**

Continued

3.3 Optimum conditions for Ni^{+2} biosorption *Pseudomonas* isolate P36.

To identify the optimum conditions effective for the removal of metal ions from aqueous solutions, it is necessary to have information about the effect of system parameters under varying process conditions. Biosorption of metal ions is strongly influenced by the local physical and chemical environment (Sarhan, 2006).

The most important parameters affect on biosorption include the biomass age, biomass concentration, initial metal concentration, pH of the solution, contact time and temperature. *Pseudomoas* sp. (P36) was used as a model system to determine the optimum biosorption process (as it is

representing the relatively sorbent isolate) providing a useful technological application to bioremediate against heavy metal pollution.

3.3.1 Effect of physiological state on Ni biosorption:

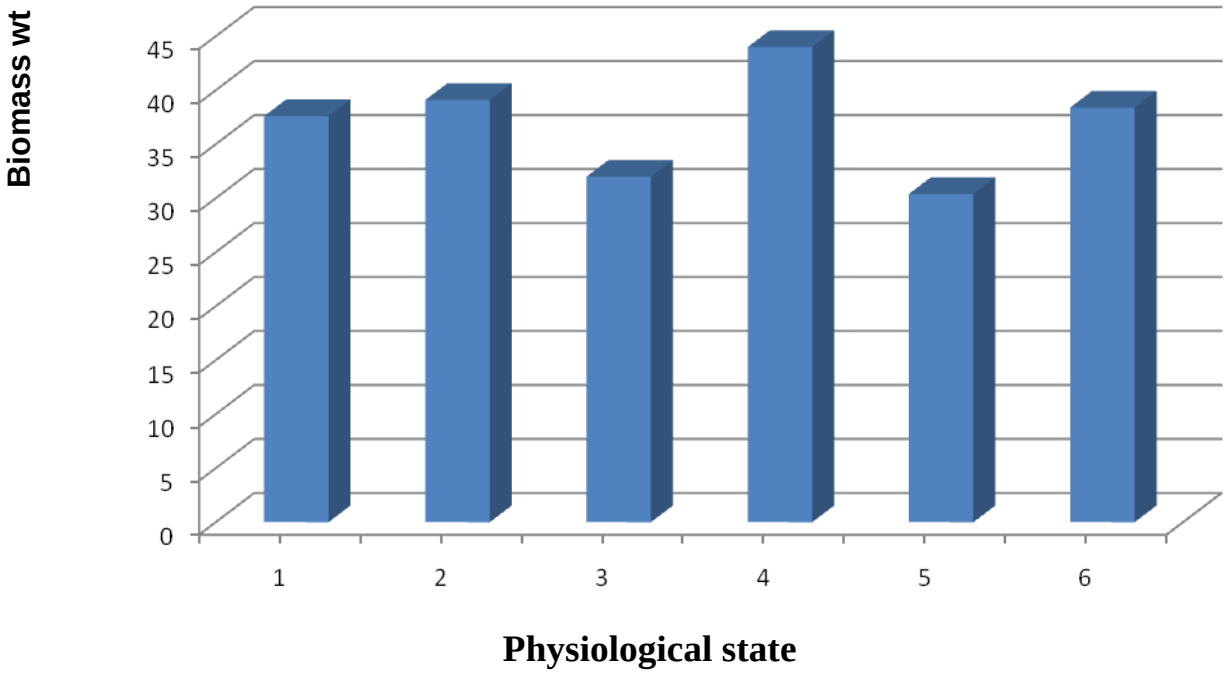
In order to investigate the effect of changing physical state on the biosorption capacity, dead biomass of *Pseudomonas* sp. P36 was treated with several chemical agents including 2N NaOH, 2N HCl, 60% of alcohol and chloroform, in addition to the treatment by heat in water bath at 100C°.

Results associated with the pretreatment of the biomass were indicated in figure (3-2), showed highest biosorption capacity of nickel 44 mg/g for biomass treated with NaOH, for heating at 39.1 mg/g, biomass treated with alcohol the biosorption capacity result was 38.4 $mg.g^{-1}$, while for untreated biomass 37.6 mg/g, also the biosorption of biomass treated with chloroform was 32 mg/g, finally the lowest biosorption of biomass treated 2N HCl was 30 mg/g.

The optimum value was achieved by NaOH treatment, this may be due to ability of the alkali destruct the autolytic enzymes that cause putrefaction of biomass and remove lipids and proteins masking the reactive sites (Yan and Viraraghavan, 2000). Or maybe, to the removal of certain polysaccharides from the cell wall by alkali treatment which generates more accessible spaces within the β-glucan chitin skeleton, thus allowing more zinc ions to be sequestered by this structure (Luef *et al*., 1991). Also, the increase in metal uptake maybe due to exposure of active metal binding sites embedded in the cell wall, or chemical modifications

of the cell wall components (Cabuk *et al*, 2004). Ilhan *et al*, (2004) stated that the increase in metal biosorption after pretreating the biomass could be due to removal of surface impurities and exposure of available binding sites for metal biosorption. While the decrease in metal uptake after pretreatment with acid solution may be related to the mass loss after acid pretreatment (Abdullah, 2006).

Treatment of *Pseudomonas* P36 with 2Nof sodium hydroxide was applied in the next experiment of nickel biosorption.



1. Untreated biomass. 2. Heat treated biomass. 3. Chloroform treated biomass. 4. NaOH treated biomass. 5. Hcl treated biomass. 6. Alcohol treated biomass.

Figure (3-2): Effect of physiological state of the local *Pseudomonas* isolate P36 biomass on nickel ions sorption.

3.3.2 Effect of pH:

The effect of pH on Ni^{-2} sorption by the local *Pseudomonas* isolate P36 was examined in the pH range 4.0- 9.0. Results mentioned in fig. (3-3) showed that the biosorption of Ni^{-2} ions from the aqueous solution was affected by pH of the solution, and that Ni^{-2} sorption capacity increased with the increase of pH medium. Highest Ni^{-2} biosorbed (36 mg/g) was obtained at initial pH value of 6.0, while the lowest (25 mg/g) was at pH 4.0. This maybe to the increase in pH which resulted in increasing the negative charge on the surface of the cell favoring electrochemical attraction and adsorption of metal (Kaewchai and Prasertsan, 2002). While at low pH, the concentration of proton is high, so metal binding sites become positively charged and metal cations making protons compete for binding sites, which results in lower uptake of metal (Ray *et al*., 2005).

Padmavathy *et al* (2002) stated that the maximum adsorption capacity of nickel by deactivated yeast was achieved at pH 6.75. This could be to the fact that pH influences the binding of metal ions by protonated yeast indicating that there is an interaction of biomass binding sites with the protons. With increase in pH, the sorption uptake increases as the degree of ionization of negative groups present on the biomass would increase.

The decrease in removal of metal ions with increase in pH is due to the decreased availability of hydrogen ions for the protonation of the cell wall functional groups, thus reducing the interaction between the metal ions and the available binding sites (Sen and Dastidar, 2006).

Optimum pH 6.0 for nickel biosorption was fixed in the next experiments of biosorption.

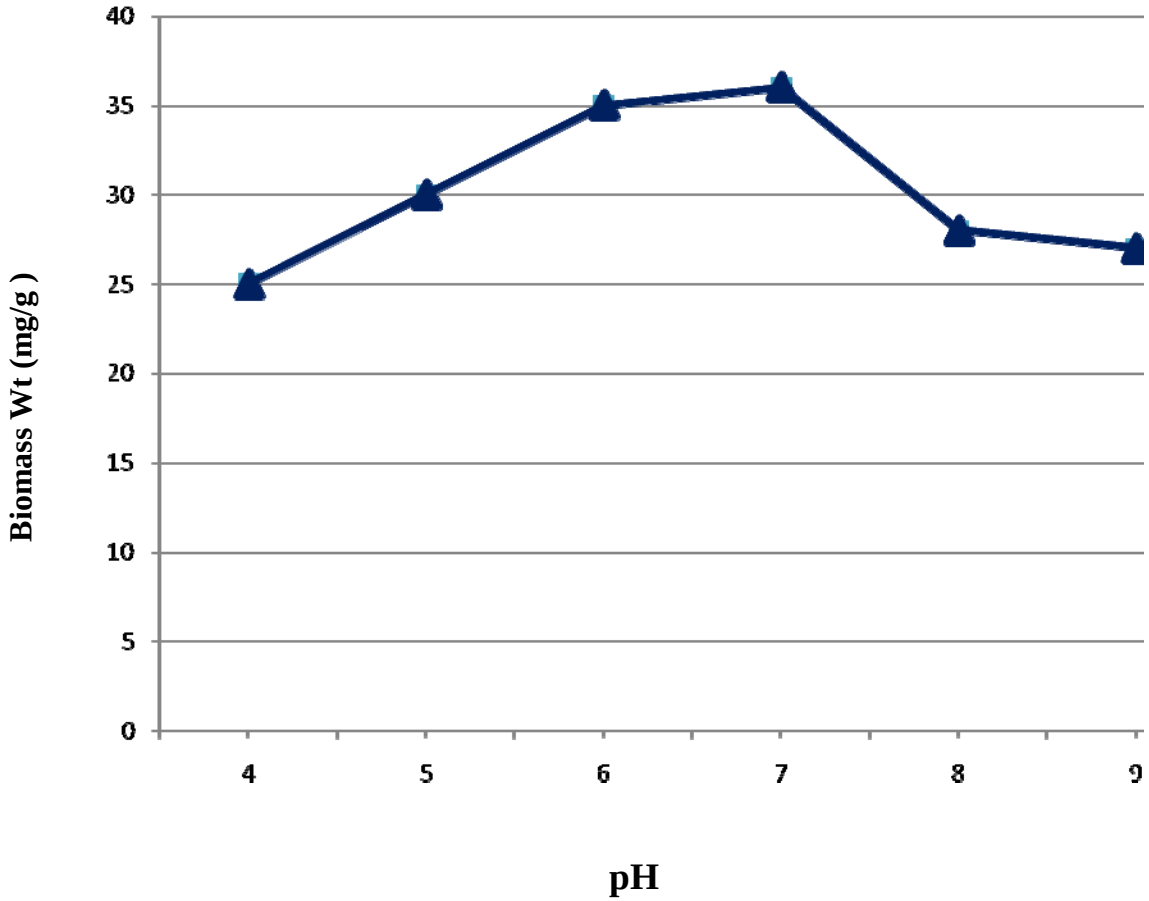


Fig (3-3): Effect of biosorption pH medium on Ni^{2+}biosorption by the locally isolated *Pseudomonas* P 36.

3.3.3 Effect of temperature

Results of metal sorption experiment carried out at different temperature values ranging from 20-45 C°. As shown in Fig. (3-4), results showed that the temperature changes were sharply affected uptake of Ni^{-2} by *Pseudomonas* isolate P36. If the main mechanism is physical adsorption, effectiveness of the process should increase with decreasing temperature as physical adsorption is exothermic process. But the main mechanism is chemisorptions, the effectiveness is increased with increasing temperature. The effect of temperature on biosorption is related to groups involved to metal biosorption. It has been found that the formation of coordination complexes between transition metal cations

and carboxylate ligands is endothermic, whereas amine ligand complex formation is exothermic (Singh *et al*., 2002).

Horsfall *et al.,* (2005)Also it has been mentioned that the decrease in adsorption with increasing temperature, is due to weak adsorption interaction between biomass surface and the metal ion, which supports physisorption. Decreasing temperature below 24ºC decreased growth and consequently the enzymatic activity, while increasing the temperature up to about 40°C decreased the bacterial growth and consequently the metal removal extent (Nouri Sepehr *et al*, 2005).

Figure (3-4): Effect of temperature on biosorption of Ni (II) by the locally isolated *Pseudomonas* P36.

3.3.4 Effect of biomass concentration

The effect of biomass concentration of *Pseudomonas* isolates P36 on Ni^{-2} biosorption was studied. Results mentioned in Fig. (3.5) showed that biomass concentration was strongly affected the amount of nickel removed from aqueous solution (Sarhan, 2006). When the biomass increased to 0.5 g/l, Ni^{-2} biosorption increased to 35.4 $mg.g^{-1}$ biomass. The increase in Ni^{-2} biosorption at biomass of 0.5 g /l may be due to the increase in the external surface area of the biosorbents available for adsorption, It is suggested that at lower biomass concentration for a given metal concentration, the metal/biosorbent ratio is enhanced and thus metal uptake is increased, as long as the biosorbent is not saturated (Tsekova and Petrov, 2002). Decreasing metal uptake at higher biosorbent dosage can be explained by considering a partial cells aggregation taking place at high biosorbent concentrations causing a decrease in number of the active sites (Esposito *et al.,* 2001; and Abdullah, 2006).

Chen and Pan (2005) discover that the adsorption capacity decreased at higher cell concentration. This may be due to the higher biomass concentration, where the cells can provide more space for the adsorption process. Also decreasing the rate of elimination of metal ion could be attributed to the biomass granules, which are agglomerated (Ray *et al.,* 2005)

The reduction of the uptake with raising the biomass concentration is attributed to electrostatic interaction between functional groups of the cell surface. Reduction in biomass concentration of the suspension at a given metal concentration enhances the metal/biosorbent ratio, and thus increases metal uptake per gram of biosorbent as long as the latter is not saturated (Ianis *et al*, 2006). The overall enhancement in the initial rate of

metal removal with an increase in biomass may be explained on the basis of an increase in the number of active sites available for adsorption (Prasenjit and Sumathi, 2005).

The optimum biomass concentration (0.5 g/l) was fixed at for the next experiments of optimazation.

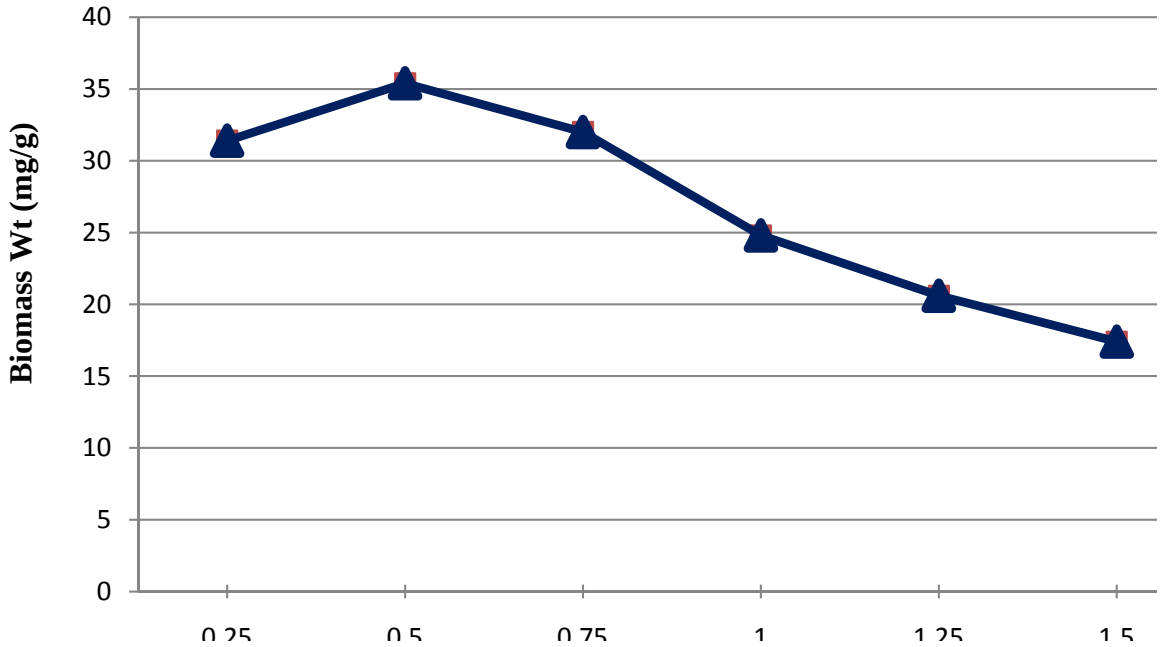


Figure (3-5): Effect of biomass concentration on Ni^{2+} biosorption by the locally isolated *Pseudomonas* P36.

3.3.5 Effect of biomass age:

As a characteristic of life cycle of many bacteria including *Pseudomonas* that exhibit morphological changes, cell age may have an effect on Ni^{-2} binding sites (Sarhan, 2006). Hence, samples were taken from different ages of *Pseudomonas* P36 cultures and their uptakes to Ni^{-2}were examined. Effect of biomass age of *Pseudomonas* isolate P36 on Ni^{-2} biosorption is shown in Figure (3-6). The amount of metal biosorbed from the solution reached a maximum value when biomass of two days old was used.

The age of biomass is sometimes a governing factor in biosorption, since it can have some effect on the cell wall characteristics, i.e. the groups involved in metal binding and hence , on the mechanism of uptake (Singh et al., 2002). Sen and Dastisar (2007) indicates that the specific chromium removal increase with increase biomass age reaching a maximum at 36 h when they inter the stationary phase, and then decreased with further increase in biomass age.

Optimum biomass age (two days biomass) was used in the next experiments of biosorption.

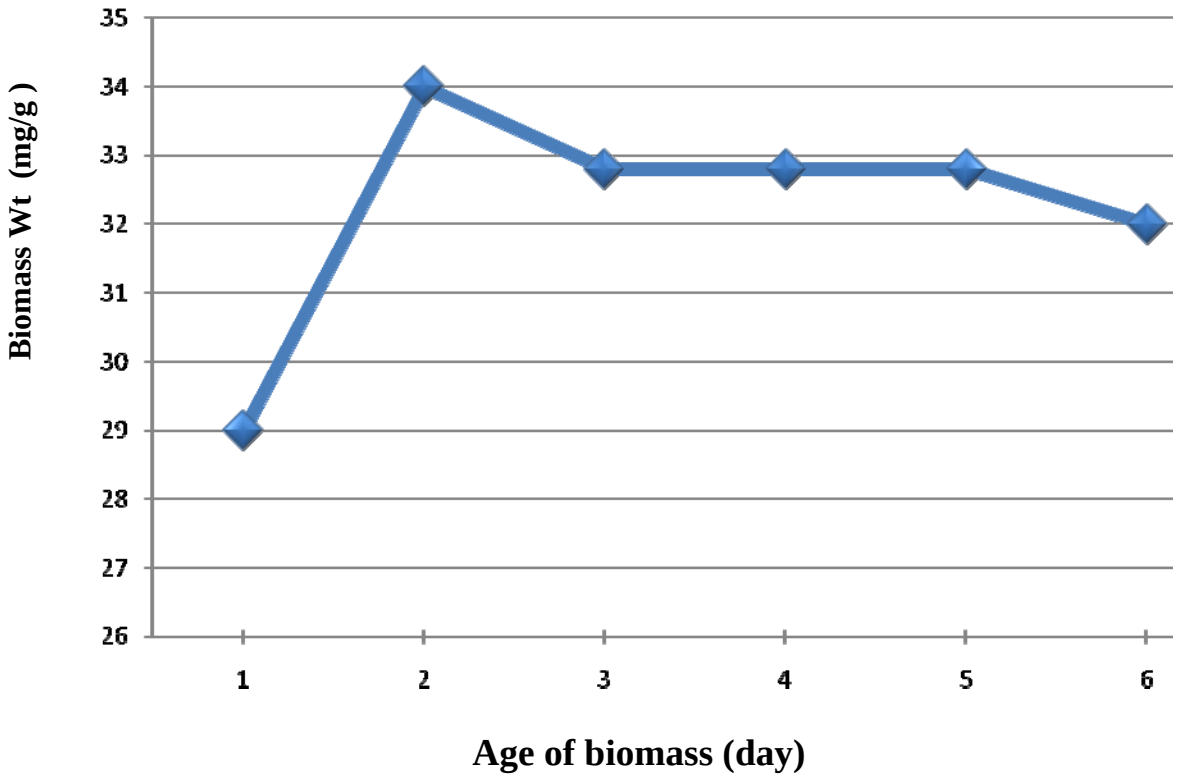


Figure (3-6): Effect of locally isolated *Pseudomonas* P36 biomass age on biosorption of nickel ions.

3.3.6 Effect of initial metal concentration

Effect of initial Ni^{-2} concentration on biosorption by dead biomass of *Pseudomonas* P36 was studied. Results mentioned in Figure (3-7), showed that the percentage of nickel biosorption decreased progressively with increasing the metal concentration. This is due to the increase in the number of ions competing for the available binding sites in the biomass and also due to the lack of binding sites for the complexation of Ni ions at higher concentration levels (Ahalya *et al.*, 2006).

Ray *et al.* (2005) stated that the amount of adsorption of metal ion per unit mass of biosorbent increased with increasing the initial metal ion concentration, but percent adsorption decreased with increasing the initial heavy metal concentration due to rapid saturation of metal binding sites of the biosorbent. But Mahvi *et al* (2007) found that adsorption of chromium by *Platanus orientalis* caused increases with increasing initial concentration of Cr (VI) until reached to 20mg/L. These results may be explained by an increase in the number of metal ions competing for the available binding sites in the adsorbent for complexation of Cr (VI) ion at higher concentration levels.

Figure (3-7): Effect of initial metal concentration on nickel ion biosorption by the locally isolated *Pseudomonas* P36.

3.3.7 Effect of contacting time:

Contacting time is one of the important parameters for an economical wastewater treatment system. Adsorption of nickel ions increased can shown in figure(3-8) after 80 minutes of contact between the dead biomass of locally isolated *Pseudomonas* P36 and nickel ions when the optimum time of contact, gave a maximum biosorption of nickel ions of (32.5) mg/g biomass. After that the contact time became almost constant for120 min. The initial rapid phase is related to passive physical adsorption or ion exchange at cell surface, while the subsequent slower phase may involve active metabolism-dependent transport of metal ion across the cell membrane into the cytoplasm in the presence of salt and other biosorption mechanisms containing microprecipitation, complexation etc.(Abdullah, 2006). Chen and Pan (2005) proved that the uptake of metal ions can be divided into two stages: rapid and slow. In the 'rapid' stage, metal ions are adsorbed onto the surface of microorganism, while in the 'slow' stage, metal ions transport across the cell membrane into the cytoplasm.

Rapidity of the process of biosorption seems to be consistent with the mechanism of passive adsorption to the cells rather than being metabolically active process (Singh *et al*., 2002). Optimum contacting time (80 min.) between the dead biomass and heavy metal ions was fixed in the next experiments of optimization.

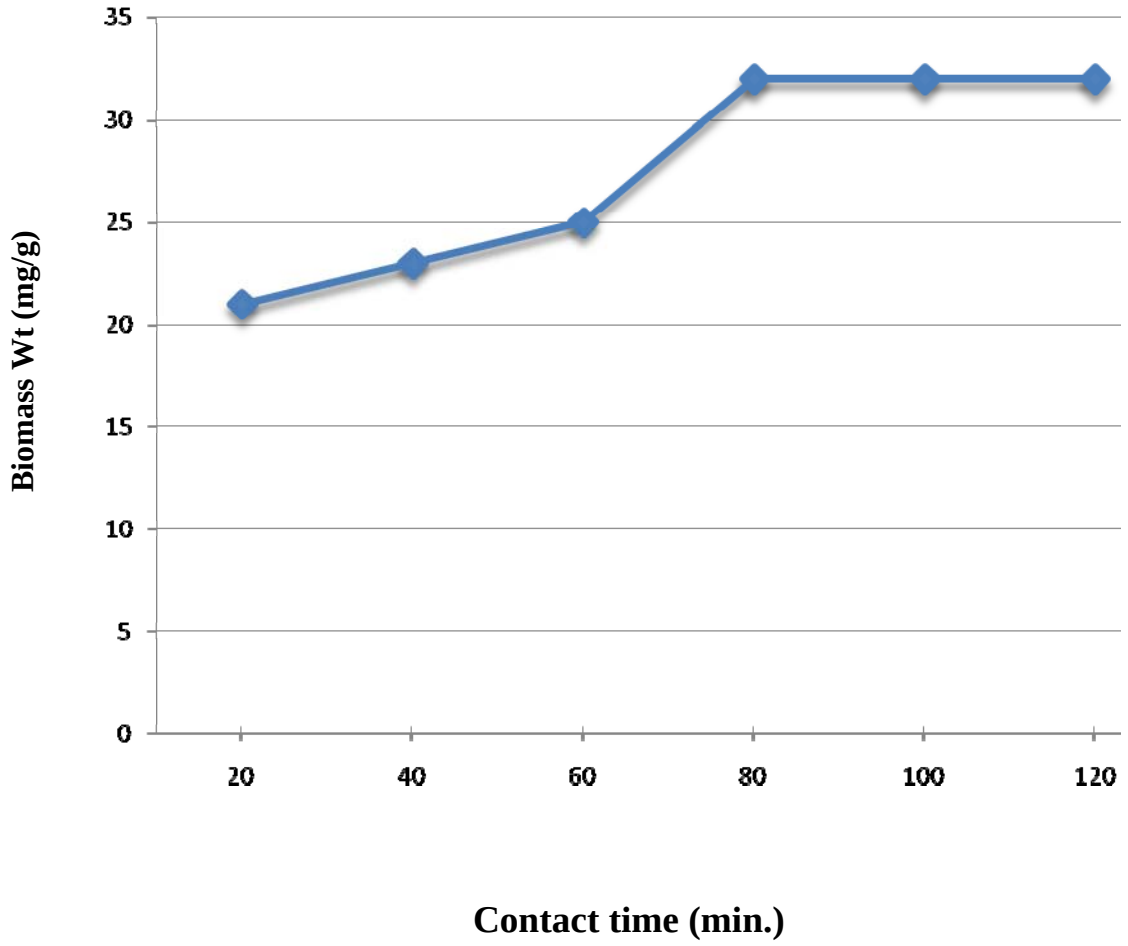


Figure (3-8): Effect of contact time on biosorption capacity by the locally isolated *Pseudomonas* P36.

3.3.8 Effect of agitation rate

Effect of the shaking rate on nickel removal by biomass of locally isolated *Pseudomonas* isolate P36 was examined. Results mentioned in figure (3-9) showed that the optimum shaking rate was at 150 rpm. In general, results indicate that a moderate speed of 150 rpm was optimum as it facilitates proper contact between the metal ions in solution, as well as the biomass binding sites and thereby promoting effective transfer of sorbate ions to the sorbent sites. This may be due to decreasing shaking

velocity below 150 rpm caused the cell growth and metal removal to decrease (because aeration rate was insufficient for bacterial growth) but when exceeding 150 rpm, bacterial growth and metal removal rate decreased. The reason is that when shaking velocity and agitation is very high, bacterial cells are not capable to use the nutrient and transfer oxygen (Nouri Sepehr *et al.*, 2005).

Also the moderate speed are optimum as it facilitates proper contact between the metal ions in solution and the biomass binding sites and thereby promoting effective transfer of sorbate ions to the sorbent sites (Ahalya *et al.*, 2006).

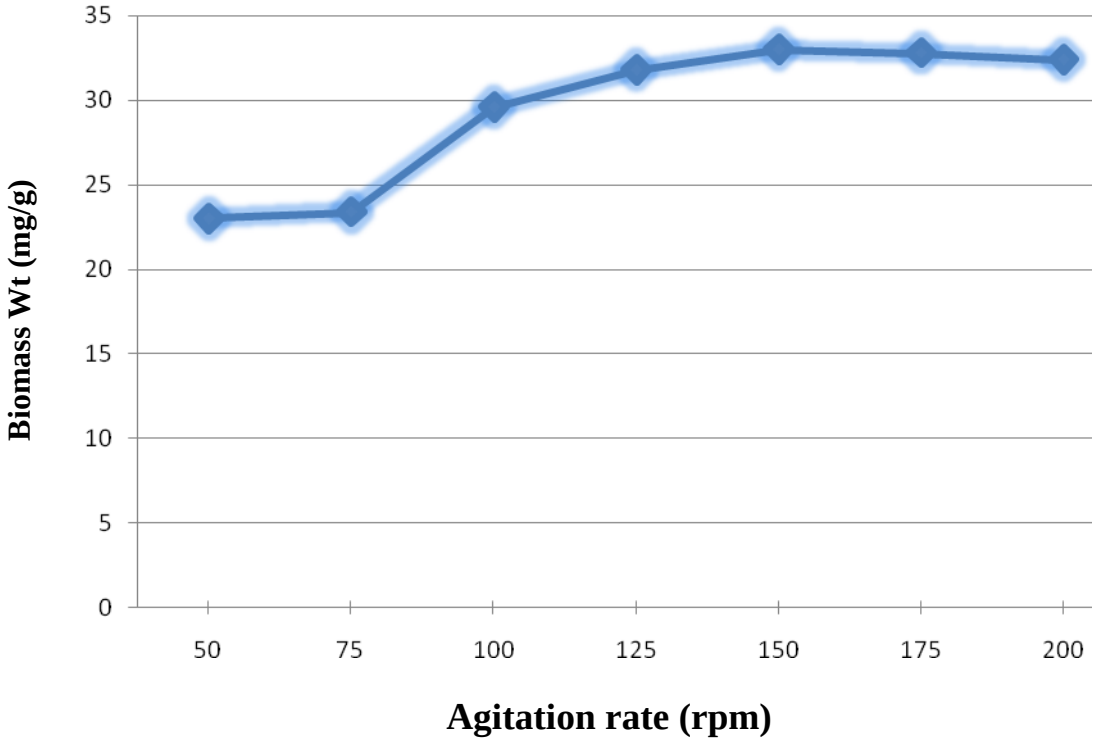


Figure (3-9): Effect of agitation rate on biosorption of nickel ions by dead biomass of locally isolated *Pseudomonas* P36.

Conclusions:

1- *Pseudomonas* spp. Isolated from different environments are efficient in heavy metal ion biosorption.
2- Treating dead biomass of one *Pseudomonas* isolate with 2N NaOH increased the capacity of biosorption.
3- pH (7.0) and moderate temperature (25º C) was the optimum for nickel ion biosorption by dead biomass of *Pseudomonas* P36.
4- Maximum capacity for nickel ion biosorption by dead biomass of *Pseudomonas* P36 was achieved when the biomass concentration was 0.5g/l, and initial nickel concentration was 20 mg/L.
5- Two days age of biomass was the optimum for nickel ion biosorption by dead biomass of *Pseudomonas* P36
6- Optimum contact time between nickel ions and dead biomass of *Pseudomonas* P36 was achieved after 80 min. at 150 rpm. .

Recommendations:

1. Studying the optimum conditions for other heavy metal ions by locally isolated *Pseudomonas* P36.
2. Using the locally isolated *Pseudomonas* P36 in treatment of industrial and sewage water contaminated with nickel ions.
3. complete identification of locally isolated *Pseudomonas* P36.
4. Studying biosorption kinetics using the locally isolated *Pseudomonas* P36

References:

- Abbas, A. And Edwards, C., (1989). Effects of Metals on a Range of *Streptomyces* Species. Applied and Environmental Microbiology, 55. 8 :2030-2035.
- Abdullah, N.H.L.B., (2006). Chromium (VI) biosorption studies using non- living microorganisms. A thesis for Master of Science, chemistry. University of technology, Malaysia.
- Ahalya, N., Ramachandra, T.V., and Kanamadi, R.D., (2003). Biosorption of Heavy Metals. Research Journal of Chemistry and Environment, 7, 4.
- Ahalya, N; Kanamadi R.D., and Ramachandra T.V. (2006). Biosorption of iron (III) from aqueous solutions using the husk of *Cicer arientintum*. Indian Journal of Chemical Technology.13:122-127.
- Ahalya, N; Ramachandra T.V., and Kanamadi R D. (2005). Biosorption of chromium (VI) from aqueous solutions by the husk of Bengal gram *Cicer arientinum*. Electronic Journal of Biotechnology ISSN: 0717-3458.
- Antunes , W.M.; Luna , A.S.; Henriques , C.A.; and da Costa , A.C.A.(2003). An evaluation of copper biosorption by brown seaweed under optimized conditions. Electronic Journal of Biotechnology, 6 . 3.
- Atlas, R.M., Brow, A.E., Dobra, K.W. and Miller, L. (1985). *Experimental Microbiology: Fundamentals and Applications*. Collier Macmillan publisher. London.
- Baig, T.H; Garcia, A.E; K. Tiemann, K.J; and Gardea-Torresdey, J.L. (1999). Adsorption of heavy metal ions by the biomass of *Solanum, (Elaeagnifolium)* (Silverleaf nightshade). Department of Chemistry and Environmental Sciences and Engineering, University of Texas at El Paso, Conference on Hazardous Waste Research.

- Beaugeard,M.,(2001). Biosorption of heavy metals by red algae *Palmaria palmata* .Thesis for Master of Science, McGill University, Montreal.
- Beveridge, T. J. and Koval, S. F. (1981). Binding of metals to cell envelops of *Escherichia coli* K- 12. Applied Environmental Microbiology, 42:325-335.
- Beveridge, T.J., Forsberg, C.W., and Doyle, R.J. (1982). Major Sites of Metal Binding in *Bacillus licheniformis* Walls. Journal of Bacteriology. Vol.150 (3):1438-1448.
- Beveridge, T.J., Murray, R.G.E., (1980). Sites of metal deposition in the cell wall of *Bacillus subtilis*. Journal of Bacteriology. 141:876–87.
- Bishnoi, N. R; Kumar, R; and Bishnoi, K. (2007). Biosorption of Cr (VI) with *Trichoderma viride* immobilized fungal biomass and cell free Ca-alginate beads. Indian Journal of Experimental Biology.vol.45:657- 664.
- Brown, M.J., and lester. J.N., (1982). Role of Bacterial Extracellular Polymers in Metal Uptake in Pure Bacterial Culture and Activated Sludge- VII, Effects of Mean Cell Retention Time. 1:1549-1560.
- Bux, F., and Kasan, H.C., (1004). Comparision of selected methods for relative assessment of surface charge on waste sludge biomass. *Water SA,* 20 (1). 73-76.
- Brady, D., Stoll, A. and Duncan, J. R. (1994). Biosorption of heavy metals cations by non-viable yeast biomass. Environmental Technology .15:429-438.
- Briedey, J. A., Brierley, C. L, and Goyak, G. M. (1985). A new wastewater treatment and metal recovery technology. In Fundamental and Applied Biohydrometallurgy, eds. R. W. Lawrence, M. R, Branion, and H. G. Ebner, Elsevier, Amsterdam, pp. 291-303.

- Brierley, C.L., Brierley, J.A., Davidson, M.S. (1985). Applied microbial processes for metals recovery and removal from wastewater. In: Beveridge, T.J., Doyle, R.J., editors. Metal ions and bacteria. New York: Wiley. p. 359–382.
- Cabuk, A; Ilhan, S; Fuluk, C; and Aliskan, F. (2004). Pb^{2+} Biosorption by Pretreated Fungal Biomass. Turk .J. Biol.29: 23-28.
- CCME. 1994. Canadian water quality Guidelines. Biotechnology Research Institute, Montreal, Canada.
- Ceribasi, I.H. and Yetis, U. (2001). Biosorption of Ni(II) and Pb(II) by *Phanerochaete chrysosporium* from a binary metal system – Kinetics. Environmental Engineering Department, Ankara. ISSN 0378-4738, Vol. 27 No. 1.
- Chen, H. and Pan, S. (2004). Bioremediation potential of Spirulina: toxicity and biosorption studies of lead. J. Zhejiang Univ. Sci. 6(3): 171–174.
- Cossich, E.S.; Tavares, C.R.G.; Ravagnani, T.M.K. (2002). Biosorption of chromium (III) by *Sargassum* sp. biomass. Electronic Journal of Biotechnology. Vol.5 No.2.
- Crist, R.H., Oberholser, K., Shank, N., Nguyen, M. (1981). Nature of bonding between metallic ions and algal cell walls. Environmental Science Technology. 15: 1212-1217.
- Crist, R.H., Martin, J.R., Carr, D., Watson, J.R., Clarke, H.J. and Crist, D.R. (1994). Interaction of metals and protons with algae. 4. Ion exchange vs adsorption models and a reassessment of Scatchard plots; ion-exchange rates and equilibria compared with calcium alginate. Environmental Science Technology. 28:859-1866.
- Da Costa, A.C.A; Tavares, A.P.M and de Franca, F.P. (2001). The release of light metals from brown seaweed (*Sargassum* sp.) during zinc biosorption in a continuous system. Electronic Journal of Biotechnology Vol.4 No.3, ISSN: 0717-3458.

- Da Costa, A.C.A. and de Franca, F.P. (1996). Cadmium uptake by biosorbent seaweeds: Adsorption isotherms and some process conditions. Separation Science and Technology 31:2373-2393.

- Diagomanolin, V., Farhang, M., Ghazi-Khansari, M., and Jafarzadeh, N. (2004).Heavy metals (Ni, Cr, Cu) in the Karoon waterway river, Iran. Toxicology Letters 151, 63–68.

- Dunnick, JK. Elwell, MR. Radovsky, AE. Benson, JM. Hahn, FF. Nikula, KJ. Barr, EB. and Hobbs, CH.(1995). Comparative Carcinogenic Effects of Nickel Subsulfide, Nickel Oxide, or Nickel Sulfate Hexahydrate Chronic Exposures in the Lung. Cancer Research. 15; 55(22):5251-6.

- Elinder CG. Zinc. In: Friberg L, Nordberg GF, Vouk VB, (1986). eds. Handbook on the toxicology of metals, 2nd ed. Amsterdam, Elsevier Science Publishers:664-679.

- Elmaci, Yonar, A.; Taner; Ozengin, and Nihan (2007). Biosorption characteristics of copper (II), chromium (III), nickel (II), and lead (II) from aqueous solutions by *Chara* sp. and *Cladophora* sp. Water Environ. Res., 79, 1000.

- EPA. (1979). Lead in water related environmental of 192 priority pollutant, vol 1. Introduction, technical background, metal and inorganics, pesticides, polychlorinated biphenils. Office of Water Planning and Standards, U S Environmental Protection Agency, Washington, D.C.

- EPA. 1985. Water quality assessment: a screening procedure for toxic and environmental pollutant in surface and ground water, part 1, revised EPA.

- Esposito, A.; Pagnanelli, F.; Lodi, A.; Solisio, C. and Veglió, F. (2001). Biosorption of heavy metals by *Sphaerotilus natan*s: an equilibrium study at different pH and biomass concentrations. Hydrometallurgy 60:129-141.

- Farkas, V. (1980). Biosynthesis of ce11 walls of fungi. Microbiology Review. 43:117-141.

- Ferris, F. G. and Beveridge, T. J. (1984). Binding of a paramagnetic metal cation to *Escherichia coli* K-12 outer-membrane vesicles. *FEMS Microbiol. Lett*., 24, 43.

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

- Fourest, E., and Volesky, B. (1990). Contribution of sulfonate groups and alignate to heavy metal biosorption by the dry biomass of *Sargassum fluitance*. Environmental Science Technology 20 237-25.

- Gadd, G. M. and White, C. (1985). Copper uptake by *Penicillium ochro-chloron*: influence of pH on toxicity and demonstration of energy-dependent copper influx using protoplasts. Journal of General Microbiology. 131:1875-1879.

- Gadd, G.M., (1992). Microbial control of heavy metal pollution. In: Fry JC, Gadd, G.M., Herbert, R.A., Jones, C.W., Watson-Craik, I.A., editors. Microbial control of pollution. Cambridge: Cambridge University Press.

- Gadd, G. M. (1 990). Biosorption. Journal of Chemistry and Industry, 2nd July. 421-426.

- Gadd, G.M. and Grifiths, A.J. (1978) Microorganisms and heavy metal toxicity. Microbial Ecology. 4:303-317.

- Galun, M., Galun, E., Siegel, B. Z., Keller, P., Lehr, H. and Siegel, S. M. (1987). Removal of metal ions from aqueous solutions by *Penicillium* biomass: kinetic and uptake parameters. Crater, Air, and Soil Pollution, 33:359-371.

- Gardea-Torresdey,J.L., Gonzalez, J.H., Tiemann, K.J., Rodriguiz, O. (1996). Biosorption of cadmium, chromium, lead and zinc by biomass of Medicago sativa (alfalfa), en HSRC/WERC Joint Conference on the environment. Albuquerque (New Mexico), USA.

- Garnham, G.W., Codd, G.A. and Gadd, G.M. (1992). Accumulation of zirconium by microalgae and Cyanobacteria. Journal Applied Microbiology and Biotechnology. Vol. 39, No. 4-5.

- 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. Biotechnol. Lett. 12, 839–842.

- Greene, B., Darnall, D.W., (1990). Microbial oxygenic photoautotrophs (cyanobacteria and algae) for metal-ion binding. In: Ehrilch, H.L., Brierly, C.L. (Eds.), Microbial Mineral Recovery. McGraw Hill, New York.

- Guibal, E., Milot, C. and Roussy, J. (1999). Molybdate sorption by cross-linked chitosan beads: dynamic studies. Water Environment Research. 71: 10-17.

- Guibal, E., Roulph, C. and Le Cloirec, P. (1992). Uranium biosorption by a filamentous fungus *Mucor miehei* pH effect on mechanisms and performances of uptakes, Water Research, 26:1139-1 145.

- Guibal, E.; Roulph, C.; le Cloirec, P. (1992). Uranium biosorption by a filamentous fungus *Mucor miehei* pH effect on mechanisms and performances of uptake. Water Research. 26:1139-1145.

- Gupta, R., Ahuja, P., Khan, S., Saxena, R.K., and Mohapatra, H. (2000). Microbial biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions. Current science, Vol. 78, No. 8.

- Gupta, V.K. and Rastogi , A.(2007). Biosorption of lead from aqueous solutions by green algae *Spirogyra* species: Kinetics and equilibrium studies. Journal of Hazardous Materials.Vol.152 (1):407-414.

- Hammaini, A., Gonzalez, F., Ballester, A., Blazquez, M.L., and Munoz, J.A. (2006). Biosorption of heavy metals by activated sludge and their desorption characteristics. Elsevier Publisher.

- Holan, Z.R., Volesky, B. (1994). Biosorption of Pb and Ni by biomass of marine algae. Biotechnology Bioengineering. 43:1001–1009.

- Holt, J. G. (ed.). (1994). Bergey's manual of determinative bacteriology. Williams and Wilkins, Baltimore, Md.

- Hancock, 1. C. (1986). Trace metal removal from aqueous solution. in Trace Meta1 Removal From Aqueous Solution, eds. R. Thompson, Special Publication No. 61, The Royal Society of Chemistry, London, U.K., pp. 25-43.

- Horsfall, M. Jnr and Spiff, A.I. (2005). Effects of temperature on the sorption of Pb^{2+} and Cd^{2+} from aqueous solution by *Caladium bicolor* (Wild Cocoyam) biomass. Electronic journal of biotechnology.Vol.8.No.2.

- Ho, Y.S., (2003). Removal of copper ions from aqueous solution by tree fern. Water Research. 37: 2323-2330.

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

- Huang, Chihpin, Huang, C. P. and Morehart, A. L. (1990). The removal of Cu (I1) from dilute aqueous solutions by *Saccharomyces Cerevisiae*. Water Research, 24:433-439.

- Hughes, M.N., and Poole, R.K., (1989). Metals and Microorganisms. Chapman and Hall, London. 10 pp.

- Hunt, S. (1986). Diversity of biopolymer structure and its potential for ion binding applications. In Immobilization of Ions by Biosorption, eds. H. Eccles and S. Hunt, Horwood, Chichester, pp. 15-46.

- Hussein, H. Ibrahim, S.F. Kandeel, K. Moawad, H.(2004). Biosorption of heavy metals from waste water using *Pseudomonas* sp. Electronic Journal of Biotechnology.Vol.7 No.1.

- Igwe, J. C., and Abia A.A. (2006). A bioseparation process for removing heavy metals from waste water using biosorbents. African Journal of Biotechnology Vol. 5 (12), pp. 1167-1179.
- Ilhan, S., Nurbas, M., Bakhsh, N., Kilicarslan, S., and Ozdag, H. (2004).Removal of Chromium, lead and copper ions from industrial waste waters by *Staphylococcus saprophyticus*. Turkish electronic journal of biotechnology.Vol.2, 51- 57.
- Ilhan, S; Cabuk , A; Filik, C; and Caliskan , F.(2005). Effect of pretreatment on biosorption of heavy metals by fungal biomass. Trakya Univ. J. Sci, 5(1): 11-17.
- Jaouen, G., (2006). Bioorganometallics: Biomolecules, Labeling, Medicine; Wiley-VCH: Weinheim.
- Kaewchai, S. and Prasertsan, P. (2002). Biosorption of heavy metal by thermotolerant polymer-producing bacterial cells and the bioflocculant. Songklanakarin J. Sci. Technol. 24(3): 421-430.
- Kaewsarn, P., (2002). Biosorption of copper (II) from aqueous solutions by pre-treated biomass of marine algae *Padina* spp. Chemospphere. 47:1081-1085.
- Kapoor, A. Viraraghavan, T. and Cullimore, D. R. (1999). Removal of heavy metals using fungus *Aspergillus niger*. Bioresource Technology. 70:95-104.
- Kapoor, A., Viraraghavan, T. (1997). Nitrate removal from drinking water. Journal of Environmental Engineering, 123(4):371-380.
- Kapoor, A., Viraraghavan, T. (1998). Removal of heavy metals from aqeoues solutions using immobilized fungal biomass in contieous mode, Water Research, 32(6):1986-1977.
- Kasprzak, KS. Sunderman, FW. And Salnikow, J. K.(2003). Nickel carcinogenesis. Mutation Research; 533(1-2):67-97.
- Khovrychev, M. P. (1973). Adsorption of copper ions by cells of *Cadida utilis*, Microbiology, 42:755-749.

- Kiff, R.J., and Little, D.R. (1986). Biosorption of heavy metals by immobilized fungal biomass. In: Hunt EH, editor. Immobilization of ions by biosorption. Chichster, UK: Ellis Horwood. p. 219.

- Kim, T.Y; Park, S.K; Cho, S.Y; Kim, H.B; Kang, Y.; Kim, S.D; and Kim, S.J.(2005) Adsorption of Heavy Metals by Brewery Biomass. Korean J. Chem. Eng., 22(1), 91-98.

- Khalid, A. M., Shemsi, A. M., Akhtar, K. and Anwar, M. A. (1993). Uranium biosorption by trichodema haczianum entraped in polyster foam beads. In: Biohydrometallurgicul Technologies, Vol. II, eds. A. E. Toma, M. L. Apel and C. L. Brierley, The Minerials, Metals and Materials Society. pp. 309-317.

- Kogej, A., and Pavko, A. (2001). Laboratory experiments of lead biosorption by self- immobilized *Rhizopus nigricans* pellets in the batch stirred tank reactor and the packed bed column. Chem.Biochem.Eng. Q. 15: 75-79.

- Kumar, V. and Kaladharan, P. (2006). Biosorption of metals from contaminated water using seaweed. Fishery Environment Management Division, Central Marine Fisheries Research Institute, Cochin 682 018, India. Vol. 90, NO. 9.

- Kurek, E., Czaban, J., and Bollag, J.M. (1981). Sorption of cadmium by microorganisms in competition with other soil constituents. Applied and Environmental Microbiology. 43: 1011-1015.

- Kuyucak, N., and Volesky, B., (1988).Biosorbents for recovery of metals from industrial solutions. Biotechnology Letters Vol. 0 No. 2:137-142.

- Lester, J.N. (1987). Heavy metals in waste waters and sludge treatment processes. CRC Press, Boca Raton FL.

- Lewis, D., and Kiff, R.G., (1988). The removal of heavy metals from aqueous effluents by immobilized fungal biomass. Environmental Technology Letters. 9:991–998.

- Li, T.Q., Yang, X.E, Meng, F.H., and Lu, L.L., (2006). Zinc adsorption and desorption characteristics in root cell wall involving zinc hyperaccumulation in *Sedum alfredii* Hance. Journal of Zhejiang University Science.Vol. 8(2):111-115.
- Lister, S.K. and Line, M.A. (2001). Potential utilization of sewage sludge and paper mill waste for biosorption of metals from polluted waterways. Bioresource Technology. 79:35-39.
- Luef, E., Prey, T., Hubicek, C.P., (1991). Biosorption of zinc by fungal mycelial wastes. Applied Microbial Biotechnology. 34: 688–692.
- Macaskie, L.E., and Dean, A.C.R. (1985). Strontium accumulation by immobilized cells of a *Citrobacter sp*. *Biotechnol. Lett*., **7**, 457–462.
- Mahvi , A.H; Nabizadeh , R.; Gholami , F. and Khairi , A.(2007) Adsorption of chromium from wastewater by *Platanus orientalis* leaves. Iranian Journal of Environmental Health Science and Engineering.4: 191-196.
- Maniatis, T., Fritsch, E.F., and Sambrook, J., (1982). Molecular cloning; a laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Matthews, T. H., R. J. Doyle, and U. N. Streips. 1979. Contribution of peptidoglycan to the binding of metal ions by the cell wall of *Bacillus subtilis.* Current Microbiology. 3:51-53.
- Matheickal, J. T., Iyengar, L**,** and Venkobachar, C.(1991). Sorption and desorption of Cu (II) by *Ganoderma Lucidum*. Water Pollution Research Journal of Canada.26:187-200.
- Muraleedharan, T.R., Iyengar L, Venkobachar C (1991). Biosorption: an attractive alternative for metal removal and recovery. Current Science. 61:379-385.

- Muraleedharan, T**.** R. and Venkobachar, L. 1. (1994). Further insight into the mechanism of biosorption of heavy metals by *Ganoderma Lucidum.* Environmental Technologv.15:1015-1027

- Nies , D.H.(1999). Microbial heavy metal resistance: Application Microbiology and Biotechnology. 51: 730-750.

- Nouri Sepehr , M.; Nasseri , S. Mazaheri Assadi , M. and Yaghmaian , K.(2005). Chromium bioremoval from tannery industries effluent by *Asperillus oryzae.* Iranian journal of environmental health science and engineering. 2 (4) :273-279

- Padmavathy, V.; Vasudevan , P.and Dhingra , S.C.(2002). Biosorption of nickel (II) ions on Baker's yeast. Process biochemistry .38:1389-1395.

- Palleroni, N.J. (1985). Biology of *Pseudomonas* and *Xanthomonas*, in *Biology of Industrial Microorganisms*, (A.L. Demain and N.A. Solomon, eds), pp. 27-56, Benjamin/ Cummings, Menlo Park, Calif.

- Park, D; Yun, Y.S; Yim, K.H; and Park J.M (2005). Effect of Ni (II) on the reduction of Cr (VI) by *Ecklonia* biomass. Bioresource Technology.

- Paton. W.H.N. and Budd, K. (1972). Zinc uptake in *Neocosmospora vasinfecta*. Journal of general microbiology.72:173-184.

- Prasenjit, B. and Sumathi, S. (2005). Uptake of chromium by *Aspergillus foetidus*. J .Mater Cycles Waste Manag. 7:88–92

- Raina, M. M.; and Todd, S.R. (2003). Impact of metals on the biodegradation of organic pollutants. National Institute of Environmental Health Sciences.

- Remacle, J. (1990). The cell wall and metal binding. In: Volesky B (ed). Biosorption of heavy metals. CRC Press, Boca Raton, FL, pp: 83–92.

- Rich, G., and Cherry, K., (1987) Hazardous Waste Treatment Technologies, Pudvan Publishers, New York

- Ross, 1**.** S. and Townsley, C. C. (1986). The uptake of heavy metals by filamentous fungi**.** In Immobilization of Ions by Biosorption, eds. H. Eccles and S. Hunt, Ellis Horwood Lid. Publishers, Chichester. pp. 49-58.

- Sag, Y. and Aktay, Y. (2002). Kinetic studies on sorption of Cr (VI) and Cu (II) ions by chitin, chitosan and *Rhizopus arrhizus.* Biochemical engineering Journal. Vol.12:143-153.

- Sar, P., and D'Souza, S. F. (2001). Radionuclide biosorption by bacterial biomass. Nuclear Agriculture and Biotechnology Division. Bhabha Atomic Research Centre.

- Sarhan, O.A., (2006). Biosorption of some heavy metal ions by local isolate of *Zoogloea ramigera.* A thesis of Master of Science, Biology Department. University of Baghdad.

- Sang Yun, Y., Niu, H., Volesky, B., (2001). The effect of impurities on metal biosorption. Department of Chemical Engineering, McGill University 3610 University Street, Montreal, Canada.

- Sen, M. and Ghosh Dastidar, M. (2007). Biosorption of Chromium (VI) by Resting Cells of *Aspergillus sp*. Iran .J. Environ. Health. Sci. Eng. 4:1. 9-12.

- Sigg, L**.** (1987). Surface chemical aspects of the distribution and fate of metal ions in lakes. In Aquatic surface chemistry, eds. Moo-Young Murray, Pergamon Press, New York, pp**.** 235-247.

- Singh, V.P., (2002). Biotransformations: Bioremedaition Technology for Health and Environmental Protection. Published by Elsevier, pages 634.

- Singleton' I. and Simmons, P.(1995). Factors affecting silver biosorption by an industrial strain of *Saccharomyces cerevisiae*. Journal of chemical technology and biotechnology Vol. 65 Issue 1:21 – 28.

- Shumate, H.S.E., Strangberg, G.W.(1985). Accumulation of metals by microbial cells, applications and regulations of biotechnology in industry, agriculture and medicine, Vol. (4), Pergamon Press, New York, USA.

- Soghoian, S., Sinert, R.,and Ferner, D.J., (2006). Toxicity, Heavy Metals. Found on http://www.emedicine.com/emerg/topic237.htm# section ~ introduction.

- Srandberg, G.W. Shumate, S.E. and Parrott, J.R. (1981). Microbial cells as biosorbents for heavy metals: Accumulation of uranium by *Saccharomyces cereviaie* and *Pseudomonas aeruginosa* . Applied and Environmental Microbiology.Vol.41 (1):237-245.

- Starr, M.P., (1981). The genus *Xanthomonas*: the prokaryotes (M.P. Starr et al., eds). Pp. 742-763.

- Szilagyi, R. K. Bryngelson, P. A.; Maroney, M. J.; Hedman, B.; Hodgson, K. O., and Solomon, E. I.(2004) S K-Edge X-ray Absorption Spectroscopic Investigation of the Ni-Containing Superoxide Dismutase Active Site: New Structural Insight into the Mechanism. Journal of the American Chemical Society, vol. 126, 3018-3019.

- Tien, C. T. and Huang, C. P. (1988). Surface complex formation between heavy metal ions and sludge particulate. Journal of environmental engineering, ASCE.

- Tobin, J. M., Cooper, D. G. and Neufeld, R. J. (1984).Uptake of metal ions by *Rhizopus arrhizus* biomass. Applied environmental microbiology, 47, and 82 1-824.

- Tobin, J.M., and Roux, J.C., (1998). Mucor biosorbent for chromium removal from tanning effluent, Water Research, 32(5):1407-1416.

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

- Tsezos, M. and Velosky, B. (1981). Biosorption of uranium and thorium. Biotechnology and bioengineering. 23(3):583-604.

- Tudury, H.A.P. (2006). The use of hatchery residual biosorbent for removal of heavy metals from wastewater. Thesis of Master of Science University of Maryland.

- Vecchio , A., Finoli, C., Simine, D.D., and Andereoni, V. (1998). Heavy metal biosorption by bacterial cells. Fresenius J. Anal. Chem. 361 :338-342.

- Vieira, R.H.S.F., and Volesky, V., (2000). Biosorption: a solution to pollution. International Microbiology.Vol. 3:17–24.

- Vijayaraghavan , K.; Jegan , J.R.; Palanivelu ,K.;and Velan , M.(2004). Copper removal from aqueous solution by marine green alga *Ulva reticulata*. Electronic journal of biotechnology. ISSN: 0717-3458.Vol.7, No.1.

- Volesky, B. (1987). Biosorbents for metal recovery. Trends Biotechnol, 5:96–101.

- Volesky, B. (1990). Removal and recovery of heavy metals by biosroption, en biosorption of heavy metals, Volesky, B., CRC Press, Boca Raton (Florida),USA.

- Volesky, B., Holan Z.R., (1995). Biosorption of heavy metal. Biotechnol.

- Wang, L.C., Michiels, C.P., Dawson, C.S., Kitisakkul, S., Baross, A.J., Keasling, D.J. and Clark, D. (1997). Cadmium removal by a new strain of *Pseudomonas aeruginosa* in aerobic culture. Application Environmental Microbiology. 63(10): 4075-4078.

- World health organization, WHO, (1996). Guidelines for drinking – water quality. Vol. 2. Geneva.

- Yan, G. and Viraraghavan , T.(2000). Effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii*. Water SA Vol. 26 No.

- Zhang, L., Zhao, L., Yu, Y., Chen, C. (1998). Removal of lead from aqueous solution by non-living *Rhizopus nigricans*. Water Research.32:1437–44.

Table (3-2) Biochemical tests of the locally isolated Pseudomonas

Isolate No	Gelatinase	Oxidase	Catalase	Pyocianin Production	King B	King A	Growth at 41
P1	+	+	+	+	+	+	+
P2	+	+	+	+	+	+	+
P3	+	+	+	+	+	-	+
P4	+	+	+	+	+	+	+
P	+	+	+	+	-	+	+
P6	+	+	+	+	+	+	-
P7	+	+	+	+	+	+	+
P8	+	+	+	+	+	-	+
P9	+	+	+	+	+	+	+
P10	+	+	+	+	+	-	+
P11	+	+	+	+	+	-	-
P12	+	+	+	+	-	+	+
P13	+	+	+	+	-	+	+
P14	+	+	+	+	+	+	-
P15	+	+	+	+	+	+	-
P16	+	+	+	+	+	+	+
P17	+	+	+	+	+	-	+
P18	+	+	+	+	+	-	+
P19	+	+	+	+	+	+	+
P20	+	+	+	+	+	+	+
P21	+	+	+	+	-	+	+

Isolate No	Gelatinase	Oxidase	Catalase	Pyocianin Production	King B	King A	Growth at 41
P22	+	+	+	+	+	-	+
P23	+	+	+	+	+	+	-
P24	+	+	+	+	+	+	+
P25	+	+	+	+	+	-	+
P26	+	+	+	+	-	+	+
P27	+	+	+	+	+	+	-
P28	+	+	+	+	+	+	+
P29	+	+	+	+	+	-	-
P30	+	+	+	+	+	+	-
P31	+	+	+	+	+	+	+
P32	+	+	+	+	+	+	-
P33	+	+	+	+	-	+	+
P34	+	+	+	+	-	-	+
P35	+	+	+	+	+	+	-
P36	+	+	+	+	-	+	-
P37	+	+	+	+	+	+	+
P38	+	+	+	+	+	-	+
P39	+	+	+	+	+	-	+
P40	+	+	+	+	+	+	-
P41	+	+	+	+	+	-	+
P42	+	+	+	+	+	+	+

وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الاحيائية

الامتزاز الحيوي للعناصر الثقيلة بوساطة بكتيريا *Pseudomonas spp.* المعزولة محلياً

رسالة مقدمة الى
كلية العلوم في جامعة النهرين
كاستكمال جزئي لمتطلبات نيل درجة ماجستير علوم
في التقانة الاحيائية

من قبل
اسراء عبد محمد غانم
بكالوريوس علوم تقانة احيائية (جامعة النهرين ٢٠٠٣)

ايلول **رمضان**

٢٠٠٨ **١٤٢٩**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

إِنَّ فِي خَلْقِ السَّمَاوَاتِ وَالْأَرْضِ وَاخْتِلَافِ اللَّيْلِ وَالنَّهَارِ

وَالْفُلْكِ الَّتِي تَجْرِي فِي الْبَحْرِ بِمَا يَنْفَعُ النَّاسَ وَمَا أَنْزَلَ اللَّهُ مِنَ

السَّمَاءِ مِنْ مَاءٍ فَأَحْيَا بِهِ الْأَرْضَ بَعْدَ مَوْتِهَا وَبَثَّ فِيهَا مِنْ كُلِّ

دَابَّةٍ وَتَصْرِيفِ الرِّيَاحِ وَالسَّحَابِ الْمُسَخَّرِ بَيْنَ السَّمَاءِ وَالْأَرْضِ

لَآيَاتٍ لِقَوْمٍ يَعْقِلُونَ

صدق الله العظيم

سورة البقرة

الاية: ١٦٤

الاهداء ...

الى الروح الطاهرة التي أفنت ذاتها من اجلنا... أبي

الى القلب العظيم الذي لايزال ينبض بالعطاء ... أمي

الى أساتذتي الافاضل ... ومنار الطريق

الى عائلتي ..... أحبتي

اهدي لكم جهدي المتواضع

إسراء

الخلاصة

جمعت ١١٠ عينة من مختلف المصادر البيئية (مياه المجاري ، التربة ، المياه العذبة) من مواقع مختلفة من محافظة، بغداد وقد تم الحصول على ٨٥ عزلة شخصت، ٤٢ منها يشك على انها *Pseudomonas* spp وذلك بعد التحري عن قابليتها على النمو على وسط الستريمايد. وبعد اجراء الفحوصات الكيموحيوية على هذه العزلات (٤٢ عزلة)،ومن الاختبارات الكيموحيوية هي اختبار الكاتليز و اختبار الاوكسيديز و اختبار التحلل الجيلاتيني، وايضا قابلية هذه العزلات على النمو على وسطي king A, king B لاختبار قابليتها على انتاج الصبغات، حيث اظهرت النتائج نتيجة موجبة لكل من اختبارات الكاتليز، الاوكسيديز، التحلل الجيلاتيني، اما بالنسبة لاختبار نمو العزلات على وسطي king A, king B فان النتائج كانت متفاوتة بين النتائج الموجبة والسالبة. اختبرت قابلية العزلات المشخصة على مقاومة المعادن الثقيلة (نيكل ، كوبلت ، رصاص و نحاس) بواسطة plate diffusion method. عزلات ال *pseudomonas* اكتسبت عدة انماط من المقاومة اعتمادا على اختلاف التراكيز الخاصة بالمعدن، حيث اظهرت النتائج ان العزلة *pseudomonas* spp. P36 كانت هي الاكثر مقاومة للنيكل والنحاس، بينما العزلة رقم ٣٧(P37) هي الاكثر مقاومة للكوبلت و العزلة رقم ٤١ (P41) هي الاكثر مقاومة للرصاص.

بعد ذلك درست الظروف المثلى لعملية الامتزاز الحيوي حيث كان افضل امتزاز للخلايا عند الاس الهيدروجيني ٧ وافضل درجة الحرارة ٢٥م□، وافضل تركيز كتلة حية كان ٠,٥غم /لتر ، وتركيز اولي للعنصر الثقيل (النيكل) كان ٢٠ ملغم/لتر، بينما كان افضل عمر للكتلة الحية هو ٢ يوم، فيما كان الوقت المتطلب لاتمام الامتزاز الحيوي هو ١٠٠ دقيقة، بينما عدد دورات التحريك المثلى كان ١٥٠ دورة في الدقيقة.

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