TWO-STAGE COPPER(II) AND NICKEL(II) REMOVAL USING RUBBER WOOD SHAVINGS AND STRONTIUM ALGINATE IMMOBILIZED BACTERIA

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I wish to dedicate this thesis, with affection,
to Mak and Abah for their endless love, support,
and encouragement

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In the name of Allah, the Most Gracious, the Most Merciful

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ABSTRACT

The increasing level of heavy metals in aquatic system due to incomplete treatment of industrial wastewater by existing conventional methods is of environmental concern. Therefore, there has been an increasing interest on the possibility of using biological treatments such as biosorption in the abatement of heavy metal–contaminated wastewater. In this study, a two-stage process for Cu(II) and Ni(II) removal has been developed. The first stage involves biosorption of Cu(II) and Ni(II) ions by rubber wood shavings (RWS) followed by a polishing stage where the remaining metal ions exist in the wastewater will be adsorbed by dead bacterial biomass immobilized in alginate matrix (Strontium Alginate Immobilized Bacteria, SAIB) prior to discharge. *Acinetobacter haemolyticus, Acinetobacter calcoaceticus, Clavibacter agropyri* and *Cellulosimicrobium cellulans* were tested for growth in cheap carbon sources i.e. liquid pineapple waste (LPW) and brown sugar and tolerance towards Cu(II) and Ni(II). SAIB and NaOH–treated RWS were subjected to Cu(II) and Ni(II) removal studies where parameters such as contact time, adsorbent dosage and initial metal concentrations were optimized. Both LPW and brown sugar can serve as an alternative and cost-effective growth medium for large-scale cultivation of bacteria. Supplementation of brown sugar with tryptone gave the highest biomass yield. Bacterial growth in LPW were greatly affected after addition of metal especially Cu(II) indicating the toxic effect. *A. haemolyticus* was chosen for biosorption study as it is the most tolerant strain where it can resist up to 25 mg L^{-1} of Cu(II) and 500 mg L^{-1} of Ni(II). From the biosorption study using RWS and SAIB, contact time of 5 hours and initial metal concentration of 100 mg L^{-1} were optimized. Nevertheless, the adsorbent dosage for RWS and dried SAIB bead varies with values of 3% (w/v) and 0.025g respectively. The Cu(II) and Ni(II) adsorption using RWS and SAIB in single and mixed metal solution occurs as monolayer coverage as confirmed by Langmuir and Freundlich isothermal analysis. However, the Ni(II) adsorption by RWS in mixed metal solution cannot be modeled by both isotherms. FTIR and FESEM–EDAX analysis suggest the removal of Cu(II) and Ni(II) by *A. haemolyticus* was due to electrostatic interaction or complexation of the metal ions with the functional groups such as hydroxyl, carbonyl, amide and sulphamide present on the cell wall. The integration of biosorption processes using RWS and SAIB resulted in 77.8 % Cu(II) and 64.1 % Ni(II) removal from the electronic wastewater.

ABSTRAK

Peningkatan aras logam berat dalam sistem akuatik disebabkan oleh pengolahan air sisa industri yang tidak lengkap menggunakan kaedah rawatan konvensional sedia ada menimbulkan kerisauan terhadap alam sekitar. Justeru itu, terdapat peningkatan minat terhadap kemungkinan menggunakan rawatan biologi seperti biopenjerapan dalam usaha merawat air yang tercemar dengan logam berat. Dalam kajian ini, suatu proses dua – peringkat bagi tujuan penyingkiran Cu(II) dan Ni(II) telah dibangunkan. Peringkat pertama melibatkan biopenjerapan ion Cu(II) dan Ni(II) oleh habuk kayu getah (RWS) diikuti oleh peringkat penggilapan di mana saki baki ion-ion logam di dalam air sisa akan di jerap oleh biomas bakteria disekat gerak dalam matrik alginat sebelum disingkirkan. *Acinetobacter haemolyticus, Acinetobacter calcoaceticus, Clavibacter agropyri* dan *Cellulosimicrobium cellulans* telah diuji pertumbuhannya dalam sumber karbon murah seperti air sisa nenas (LPW) dan gula merah serta toleransi terhadap Cu(II) and Ni(II). Sel mati *A. haemolyticus* yang disekat gerak dalam matrik alginat (SAIB) dan habuk kayu getah yang dirawat menggunakan NaOH telah digunakan dalam kajian penyingkiran Cu(II) dan Ni(II) dimana parameter seperti masa interaksi, dos zat penjerap dan kepekatan awal logam telah dioptimumkan. LPW dan gula merah boleh berfungsi sebagai medium alternatif dan kos efektif untuk pertumbuhan bakteria berskala besar. Penambahan tripton ke dalam gula merah memberikan hasil biomass yang paling tinggi. Pertumbuhan bakteria di dalam LPW menunjukkan kesan selepas penambahan logam terutamanya Cu(II), menandakan kesan ketoksikannya. *A. haemolyticus* dipilih untuk kajian biopenjerapan kerana mempunyai tahap toleransi yang paling tinggi di mana ia mampu bertahan sehingga 25 mg L^{-1} Cu(II) dan 500 mg L^{-1} Ni(II). Daripada kajian biopenjerapan menggunakan RWS dan SAIB, masa interaksi 5 jam dan kepekatan awal logam 100 mg L^{-1} telah dioptimumkan. Namun begitu, dos zat penjerap bagi RWS dan manik kering SAIB adalah berbeza dengan nilai masing-masing iaitu 3% (w/v) dan 0.025g. Penjerapan Cu(II) dan Ni(II) oleh RWS dan SAIB dalam larutan tunggal dan larutan campuran berlaku secara ekalapisan yang disahkan melalui analisis isoterma Langmuir dan Freundlich. Walaubagaimanapun, penjerapan Ni(II) oleh RWS dalam larutan campuran tidak dapat dimodelkan menggunakan kedua-dua isoterma. FTIR dan FESEM-EDAX analisis mencadangkan penyingkiran Cu(II) dan Ni(II) oleh *A.haemolyticus* secara interaksi elektrostatik atau pengkompleksan ion-ion logam dengan kumpulan-kumpulan berfungsi seperti hidroksil, karbonil, amida dan sulfamida yang hadir pada dinding sel. Integrasi proses biopenjerapan menggunakan RWS dan SAIB telah memberikan penyingkiran sebanyak 77.8 % Cu(II) and 64.1 % Ni(II) daripada air sisa elektronik (EW).

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LIST OF ABBREVIATIONS

Å	-	Ångström (1×10^{-10} metre)
A. calcoaceticus	-	*Acinetobacter calcoaceticus*
A. haemolyticus	-	*Acinetobacter haemolyticus*
AAS	-	Atomic Absorption Spectroscopy
ATTC	-	American Type Culture Collection
b	-	constant related to the affinity of binding site
C. agropyri	-	*Clavibacter agropyri*
C. cellulans	-	*Cellulosimicrobium cellulans*
$CaCl_2$	-	calcium chloride
C_{eq}	-	final (equilibrium) metal concentration (mg L^{-1})
CFU	-	colony forming unit
C_i	-	initial metal concentration
COD	-	chemical oxygen demand
Cr(VI)	-	chromium(VI)
Cu(II)	-	copper(II)
DNA	-	deoxyribonucleic acid
DSMZ	-	German collection of microorganisms and cell cultures
EDAX	-	Energy Dispersive X-ray
EDS	-	Electron Dispersive Spectroscopy
EW	-	electronic wastewater
FESEM	-	Field-Emission Scanning Electron Microscope
FTIR	-	Fourier-transform Infrared
g L^{-1}	-	gram per liter
H_2SO_4	-	sulphuric acid
HCl	-	hydrochloric acid

HNO_3	-	nitric acid
ICP-MS	-	Inductive Coupled Plasma-Mass Spectrometry
k	-	relative adsorption capacity
KBr	-	Potassium bromide
LPW	-	liquid pineapple waste
M	-	molar
mg g^{-1}	-	milligram per gram
mg L^{-1}	-	miligram per liter
MIC	-	minimum inhibitory concentration
mm	-	millimeter
mM	-	millimolar
n	-	affinity constant
NA	-	nutrient agar
NaOH	-	sodium hydroxide
NB	-	nutrient broth
Ni(II)	-	nickel(II)
ºC	-	degree Celsius
OD	-	optical density
OD_{410}	-	optical density at 410 nm
OD_{485}	-	optical density at 485 nm
OD_{600}	-	optical density at 600 nm
PIPES	-	Piperazine - N,N' - bis (2 - ethanesulfonic acid)
q_e	-	uptake at equilibrium
$q_{e,\ max}$	-	maximum uptake at equilibrium
RNA	-	ribonucleic acid
rpm	-	rotation per minute
RWS	-	rubber wood shaving
SA	-	strontium alginate
SAD	-	dried strontium alginate
SAIB	-	strontium alginate immobilized bacterial suspension
SAIBW	-	wet strontium alginate immobilized bacterial suspension
SAIP	-	strontium alginate immobilized bacterial pellet

SAIPD	-	dried strontium alginate immobilized bacterial suspension
SAIPD	-	dried strontium alginate immobilized bacterial pellet
SAIPW	-	wet strontium alginate immobilized bacterial pellet
SAW	-	wet strontium alginate
str.	-	stretching
USEPA	-	United States Environmental Protection Agency
v/v	-	volume per volume
w/v	-	weight per volume
w/w	-	weight per weight
XAFS	-	X-ray Absorption Fine Structure
XPS	-	X-ray Photoelectron Spectroscopy
μm	-	micrometer

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CHAPTER I

INTRODUCTION

1.1 The scenario of the electrical and electronics sector in Malaysia

Malaysia's electrical and electronics industry started some 30 years ago and has become one of Malaysia's leading industrial sector. From a handful of companies with less than 600 workers in 1970, the industry has today attained world–class capabilities and is the largest contributor to the country's manufacturing output, employment and exports. By 2003, around 360,048 job opportunities have been created by more than 900 companies (Electronics Industry Division and ICT & Electrical Industries Division, MIDA, 2004).

In 2007, gross output of the industry totalled RM197.1 billion (US$61.6 billion), while the industry's exports of electrical and electronics products amounted to RM266.3 billion (US$83.2 billion) or 58.9 per cent of Malaysia's manufactured exports and 44 per cent of Malaysia's total exports (Electronics Industry Division and ICT & Electrical Industries Division, MIDA, 2008).

Malaysia is also home to several worlds' leading, as well as local contract manufacturers, providing volume turnkey manufacturing, PCB design and layout, and product testing. Over 20 of the world's major electronics companies have established manufacturing operations in Malaysia including Motorola, Intel, AMD, Sony, NEC, BenQ, Alcatel, Philips, Siemens and Infineon.

Malaysia manufactures a wide range of electronic product including semiconductor, telecommunications equipment, computers, computer peripherals and printers, data storage devices, consumer electronic and passive component– capacitors, resistors and inductors where these industries generate large quantities of metals and other byproducts which needs to be treated before being released to the environment. Heavy metals in these electronic wastewaters have become a serious threat where its inappropriate disposal can endanger human health and other living organisms. These toxic metals which enter the environment are non-biodegradable and will ultimately accumulate in animal and human tissues through the food chain. Therefore, detoxification of these metals bearing wastewaters is a pressing environmental concern.

The conventional techniques to remove toxic metals e.g., ion exchange and precipitation, lack specificity and are ineffective at low concentrations of metal ions. These physico-chemical procedures have significant disadvantages such as incomplete metal removal, high reagent and energy requirements and generate toxic sludge or other byproducts. As environmental regulations become more stringent on the quality of effluent discharged into natural waterways and municipal treatment facilities, electronic component manufacturers must employ effective treatment methods to meet these strict discharge requirements. In light of the above, microorganisms and waste materials has emerged as an alternative for developing economic and eco-friendly wastewater bioremediation process.

1.1.1 The Printed Circuit Board Industry

The printed circuit board (PCB) is the platform upon which microelectronic components such as semiconductor chips and capacitors are mounted, provides conductive connections between components. Once considered low technology known as printed wiring boards, the PCB is evolving into a high technology product. PCB manufacturing is highly complicated, requiring large equipment investments and over 50 process steps. Printed circuit boards are found in virtually all electronics products (LaDou, 2006).

Printed circuit board can be classified into three basic types according to its layers: single-sided, double-sided and multi-layered. Layer count is a common method of categorization because it relates to overall technology level where higher layer counts require more sophisticated technology. Single sided boards are those with a conductive pattern on one side only. Double-sided boards have conductive patterns on both faces. Multilayer boards may have from two to 40 alternating layers of conductors and insulating materials bonded together. The type of board produced depends on the spatial and density requirement, and on the complexity of the circuitry (USEPA, 1990).

1.1.1.1 Printed circuit board manufacturing process

Production methods that had been employed by the industry to produce printed circuit boards include subtractive processes and additive processes. Because of the limitations of the additive processes, the subtractive method is currently the most widely used, although it can produce more metal wastes than additive methods. The conductive circuit is generally copper, although aluminum, nickel, chrome, and other metals are sometimes used. The metal is fixed to a non-conductive base

through use of adhesives, pressure/heat bonding, and sometimes screws. Base materials include pressed epoxy paper, epoxy glass resins, teflon-glass, and many other materials (USEPA, 1990). The manufacturing process of the PCB is illustrated in Figure 1.1.

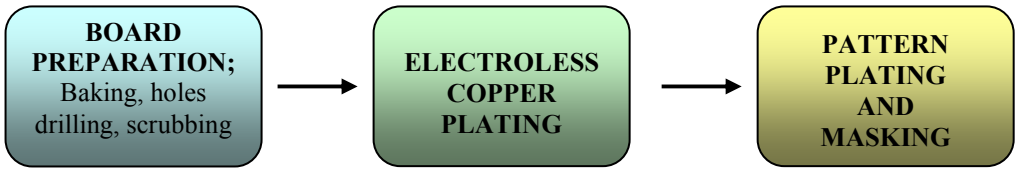


Figure 1.1: The process sequence of Printed Circuit Board Manufacturing

Production of PCB begins with a sheet of plastic or glass epoxy laminated with a thin layer of copper foil. Some of the PCB manufacturers often purchase panels of board that are already copper clad from independent laminators. The PCBs are then baked to ensure that the copper laminated boards are completely cured. Holes are then drilled through the boards using an automated drilling machine. The holes are used to mount electronic components on the board and to provide a conductive circuit from one layer of the board to the other. The drilling operation results in burrs being formed on one or both sides of the panel. Following drilling, the board is scrubbed to remove fine copper particles left by the drill. The rinse water from the scrubber unit can be a significant source of copper waste. In the scrubber, the copper is in the particulate form and can be removed by filtration or centrifugation (USEPA, 1990).

After being scrubbed, the board is thoroughly cleaned and etched to promote good adhesion before it is plated with an additional layer of copper. The smooth copper-clad board is subsequently electroless-plated with copper to provide a conducting layer through the drilled holes for circuit connection between the copper-clad board surfaces. Electroless plating involves the catalytic reduction of a metallic

ion in an aqueous solution containing a reducing agent, resulting in deposition without the use of external electrical energy (USEPA, 1990).

Electroless plating with copper provides a uniform but very thin conducting layer over the entire surface that has little mechanical strength. It is used initially to deposit metal on the drilled holes. Electroplating is required to build up the thickness and strength of the conducting layers. Pattern plating is one method of building up conducting layer thickness and is the most common type of subtractive process used. It consists of electroplating only the insides of the holes and circuit patterns. A layer of resist consists of tin lead solder or pure tin is deposited, using screen or photolithography techniques, in areas where electroplated conducting material is not desired. The plating resist is later stripped off to expose the copper foil which is not part of the final circuit pattern (USEPA, 1990).

The area where the resist has not been deposited constitutes the circuit pattern. These areas receive several electrodeposition layers. Tin or lead plating is one of the layers deposited, and it functions as another resist layer, allowing copper foil in the non-circuit areas to be etched away without the circuit pattern being damaged. Ammonia-based etching solutions are most widely used. An alternative to ammonia etching is sulfuric acid/hydrogen peroxide etching solutions. This latter etchant is continuously replenished by adding concentrated peroxide and acid as the copper concentration increases to about 80 g L^{-1}. At this concentration, the solution is cooled to precipitate out copper sulfate. After replenishing with peroxide and acid, the etchant is reused. Disadvantages of sulfuric acid-peroxide etching solution are that it is relatively slow when compared to ammonia, and controlling temperature can be difficult. The circuit pattern then receives final electroplated layers of metal such as nickel and gold.

1.1.1.2 Printed circuit board waste description

The PCB industry makes use of large number of chemicals throughout the various stages in the process. A direct discharge of the solution containing hazardous chemicals and solutions containing contaminants into the sewer system is not permitted by regulations imposed by the local administration. This is because some chemicals cannot be mixed together as they produce hazardous reactions whereas others may generate toxic fumes or generate violent heat. The resultant wastes could be metal bearing or non-metal bearing. The high concentration of heavy metals such as Cu, Fe, Cr etc., in the effluent is very harmful to any biological process.

Wastes are generated from the following principal operations that are common to the manufacturer of all types of printed circuit board; cleaning and surface preparation, catalyst application and electroless plating, pattern printing and masking, electroplating and etching (USEPA, 1990).

Typical waste streams generated from the operations in the PCB manufacturing industry are listed in Table 1.1.

Table 1.1: Types of waste streams generated in the Printed Circuit Board Manufacturing Industry

Waste Source	Waste Stream Description	Waste Stream Composition
Cleaning/Surface preparation	1. Airborne particulates 2. Acid fumes/organic vapors 3. Spent acid/alkaline solution 4. Spent halogenated solvents 5. Waste rinse water	Board materials, sanding materials, metals, fluoride, acids, halogenated solvents, alkali.
Catalyst application/ Electroless plating	1. Spent electroless copper bath 2. Spent catalyst solution 3. Spent acid solution 4. Waste rinse water	Acids, stannic oxide, palladium, complexed metals, chelating agents.
Pattern printing/masking	1. Spent developing solution 2. Spent resist removal solution 3. Spent acid solution 4. Waste rinse water	Vinyl polymers, chlorinated hydrocarbons, organic solvents, alkali.
Electroplating	1. Spent plating bath 2. Waste rinse water	Copper, nickel, tin, tin/lead, gold, fluoride, cyanide, sulfate.
Etching	1. Spent etchant 2. Waste rinse water	Ammonia, chromium, copper, iron, acids.

1.1.1.3 Current treatment employed by the PCB industry

In order to protect an increasingly aware public, every civic authority adopts stringent regulations related to the disposal of effected effluent and sludge. These regulations are generally mandated and there are severe penalties for non-compliance and disregard for discharged requirement.

The effluent quality of any discharge from an industrial treatment process must meet the minimum requirements of the Environmental Quality Act 1974 and the limits set down by the Environmental Quality (Sewage Industrial Effluent Regulations, 1979) which is presented in Table 1.2.

Table 1.2: Discharge limit of sewage and industrial effluents

THIRD SCHEDULE

ENVIRONMENTAL QUALITY ACT, 1974

ENVIRONMENTAL QUALITY (SEWAGE AND INDUSTRIAL EFFLUENTS) REGULATIONS, 1979

[Regulation 8(1), 8(2), 8(3)]

PARAMETER LIMITS OF EFFLUENT OF STANDARDS A AND B

Parameter (1)	*Unit* (2)	*Standard* A (3)	*Standard* B (4)
(i) Temperature	°C	40	40
(ii) pH Value	—	6.0-9.0	5.5-9.0
(iii) BOD_5 at 20°C	mg/l	20	50
(iv) COD	mg/l	50	100
(v) Suspended Solids	mg/l	50	100
(vi) Mercury	mg/l	0.005	0.05
(vii) Cadmium	mg/l	0.01	0.02
(viii) Chromium, Hexavalent	mg/l	0.05	0.05
(ix) Arsenic	mg/l	0.05	0.10
(x) Cyanide	mg/l	0.05	0.10
(xi) Lead	mg/l	0.10	0.5
(xii) Chromium, Trivalent	mg/l	0.20	1.0
(xiii) Copper	mg/l	0.20	1.0
(xiv) Manganese	mg/l	0.20	1.0
(xv) Nickel	mg/l	0.20	1.0
(xvi) Tin	mg/l	0.20	1.0
(xvii) Zinc	mg/l	2.0	2.0
(xviii) Boron	mg/l	1.0	4.0
(xix) Iron (Fe)	mg/l	1.0	5.0
(xx) Phenol	mg/l	0.001	1.0
(xxi) Free Chlorine	mg/l	1.0	2.0
(xxii) Sulphide	mg/l	0.50	0.50
(xxiii) Oil and Grease	mg/l	Not Detectable	10.0

The industry employed various treatment processes in order to meet the requirement. Khandpur (2006) listed down wastewater treatment processes used in the electronic industry especially in the printed board manufacturing companies i.e. recycling of water, filtration, precipitation, evaporative recovery and ion exchange. Among the treatments, precipitation is the most popular and employed by the industry.

In order to neutralize the spent chemicals, it is necessary to reduce the volume of toxic materials and to get a minimum quantity of highly concentrated and water insoluble sludge. The basic process involved in heavy metals precipitation is shown in Figure 1.2.

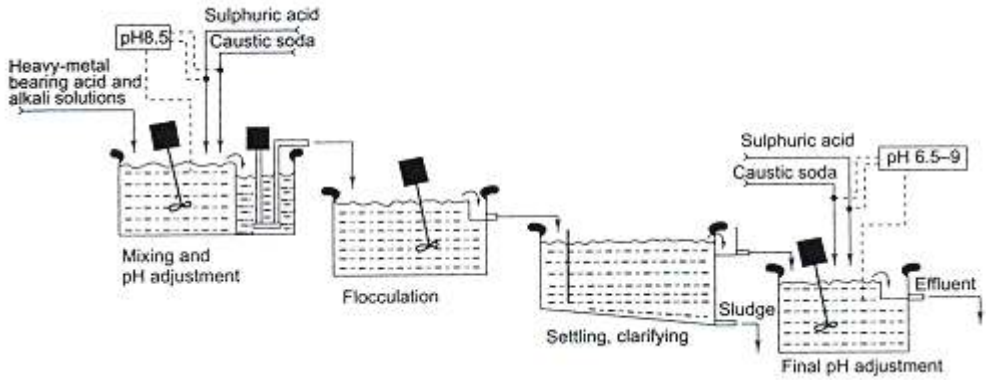


Figure 1.2: Principle of heavy metal precipitation (Khandpur, 2006)

In principle, the pH of the solution is raised to a certain level where the heavy metals like Cu, Ni, Pb, Fe or Cr get precipitated as water insoluble hydroxides. The sludge from the salting tank can be further concentrated by filtration. The hydroxide formation or actual neutralization takes place immediately after the critical pH value is reached. However the flocculation and settling of the hydroxides may require several hours. The pH range in which precipitation occurs is not the same for all metals.

The sludge so formed can be processed to separate some chemicals which can be used again. This approach is gaining popularity because of the possibility of recovering heavy metals and other materials. Alternatively, if it is not economical to follow the metal recovery route, sludge can be buried in selected dumping sites where no ground water can be affected or it can be subjected to incineration.

Ion exchange is one of the highly recommended treatments for rinsing water. Special resins are available which selectively fix one or few ions of the percolating liquid and replace them with H^+ or other non-polluting ions. Apart from that, most resins can be regenerated when exhausted. The rinsing water is passed through two de-ionizing beds for cations and anions. This process makes use of the fact that most of water soluble chemicals used in the PCB production are ionized in water forming anion and cation. However this treatment is expensive and not economical to treat large volume of wastewater.

1.2 Metal and its role

Metal ions involves in all aspects of life. Some metal cations such as K^+ and Mg^{2+} exist as bulk intracellular species known as macronutrient whilst Na^+, Ca^{2+} and a number of transition elements such as Zn, Mn, Fe, Cu, Co, Mo are essential metals required at trace or ultra trace levels. The transition elements have unpaired electrons that allow participation in redox reactions involving one electron loss (oxidation) or gain (reduction). These metal ions are known as highly effective catalysts of a range of biochemical processes and in some cases able to trigger, moderate or inhibit reactions (Hughes and Poole, 1989). It can be said that these elements are beneficial and have nutritional values at low dosages but become inhibitory to toxic with an increase in concentration beyond those necessary for their biological functions (Fraga, 2005). The threshold toxic concentrations differ for metal and are governed primarily by the chemistry of each metal in question and associated physiological

effects (SenGupta, 2002). Therefore, it is important to address the issue of increment of metal ion in the environment as they can cause adverse effect to the human and other living things. In order to do that, the origin, chemistry and how they can cause toxicity need to be fully understood.

1.2.1 Copper essentiality and toxicity

Copper has the atomic number 29 with electron configuration of Ar-$4s^1 3d^{10}$ and atomic mass 63, and belongs to Group IB of the Periodic Table along with silver and gold. It occurs either in metallic form, or in compounds of Cu(I) and Cu(II). In the two ionic forms of copper, Cu(I) with Ar-$3d^{10}$ and Cu(II) with Ar-$3d^9$, one electron is borrowed from the 4s orbital by the 3d orbitals to produce five completely filled 3d orbitals. With 9 or 10 d-electrons, no octagonal complex of copper is possible, because at least one of the two anti-bindings orbitals has to be filled with an electron pair. Due to its electronic state with one borrowed electron, copper has a character comparable to radicals and easily interact with radicals especially with molecular oxygen forming reactive species such as H_2O_2, O_2^- and OH. Due to this radicallic character, copper can be toxic but are concentration dependant (Nies, 1999).

Copper is malleable, ductile, and an extremely good conductor of both heat and electricity. The production of copper has increased over the last decades and due to this, copper quantities in the environment have expanded. Copper and its compound are used in everyday lives from electrical to medicinal and agricultural industries. As copper is an excellent conductor of electricity, one of its main industrial usage is for the production of cable, wire and electrical products for both the electrical and building industries. The construction industry also account for copper's second largest usage in the production of pipes for plumbing, heating, roofing and ventilating as well as building wire and sheet metal facings. On the other

hand, copper compounds have been used extensively in agriculture as insecticides and fungicides to control animal and plant diseases. Fertilizers are also often supplemented with copper compound to increase soil fertility and boost up crop's growth. The most commonly used compound of copper is copper sulfate. Many copper compounds can be recognized by their blue-green color (ASTDR, 2005a).

Copper is an essential element for all known living organisms including humans and other animals at low levels of intake. Therefore, plants and animals must absorb some copper from eating, drinking, and breathing. At much higher levels, toxic effects can occur (ASTDR, 2005a). Copper is one of the seven well known micronutrients (Zn, Cu, Mn, Fe, B, Mo and Cl) required for good health. In human, an estimated safe and adequate uptake is approximately 5mg/day where 2 mg/day is directly absorbed through gastrointestinal tract and transferred to the blood and distributed to all tissues and organ (Cousins, 1985). Likewise, Bradl (2005) reported that a daily dietry intake of <2 mg Cu per day for adults may suffice. Copper is assimilated and incorporated into specific proteins, where it performs its biological role as a prosthetic group in a wide variety of metalloenzymes with specific functions. The metal is a cofactor for at least 30 enzymes, reflecting the complexity of its metabolism. In blood plasma, copper is linked mainly in albumin and ceruloplasmin (Vassiliev *et al.,* 2005), and excreted through the bile (Scott and Turnlund, 1994).The most common function of copper in biological systems is electron transfer associated with oxidative enzymes and energy capture as listed in Table 1.3.

Table 1.3: Example of copper-containing enzymes and their function

Enzyme/Protein	Biological function
Amine oxidase	Removal of hormones
Ascorbate oxidase	Ascorbate oxidation
Ceruloplasmin	Oxidation of Fe(II) to Fe(III)
Cytochrome oxidase	Electron transport
Dopamine β-monooxigenase	In the pathway of catecholamines formation
Galactose oxidase	Oxidation of primary alcohols to aldehyde
Lysyl oxidase	Cross-linking of collagen elastin
Monooxigenase	Required for melanin formation
Tyrosinase	Oxidation of phenols
Cu-Zn-SOD	Oxidative defense
Peptide-α-amidating monooxigenase	Involved in pituitary peptide hormone maturation

A malfunction of the copper homeostasis system is involved in the genetic disorders of copper homeostasis such as in Menkes' and Wilson's diseases. Menke's disease is a fatal X-linked genetic disorder causing copper deficiency that leads to progressive neurodegeneration and death in children. Likewise, Wilson's disease is an autosomal recessive disorder causing accumulation and toxicity of copper in the liver and brain which leads to progressive hepatic and neurological damage (Sarkar, 2007). Copper is also potentially involved in some neurodegenerative diseases such as familial amyotrophic lateral sclerosis (Kramer *et al.,* 2003), Alzheimer's disease (Friedlich *et al.,* 2003) and prion diseases (Sassoon and Brown, 2003; Sorensen, 2001). Breathing high levels of copper can cause irritation of the nose and throat. Ingesting high levels of copper can cause nausea, vomiting, and diarrhea. Intentionally high intakes of copper can cause liver and kidney damage and even death. EPA does not classify copper as a human carcinogen because there are no adequate human or animal cancer studies. The EPA has determined that drinking water should not contain more than 1.3 mg copper per liter of water (1.3 mg/L) (ASTDR, 2005a).

1.2.2 Nickel essentiality and toxicity

Nickel has the atomic number of 28 with electronic configuration of Ar-$4s^2 3d^8$ and atomic weight 58.7. It belongs to the iron–cobalt group (group VIII) of the Periodic Table, and is a silvery-white, hard, malleable, ductile, ferromagnetic metal. It normally occurs in oxidation states 0 and II. The oxidation states of I and III can exist under certain conditions, but are not stable in aqueous solutions. The most common nickel species which are mostly water – soluble compounds is Ni(II) and have characteristic green colour (Bradl, 2005). Nickel and its compounds also have no characteristic odor or taste. From the electronic configuration, the first six of the eight d-electrons of nickel reside in the three non – binding d-orbitals which are thus completely filled. The remaining two electrons occupy the two anti binding d-orbitals which make the octagonal nickel complexes very weak due to the half bound ligand to the complex. This contributes to the catalytical properties of nickel complexes.

Nickel is mainly used in electroplating, alloy production, Ni-Cd and Ni-metal hydride batteries, electronic components, colourant and catalyst for hydrogenation of fats and methanation. The largest application of nickel is in stainless steel and several alloy production such as nichrome, alnico and permaloy, and nickel is therefore found in large variety of products, i.e. automobiles, batteries, coins, jewellery, surgical implants, kitchen appliances and utensils (Bradl, 2005). Nickel is also used as a protective and ornamental coating for metals susceptible to corrosion, particularly iron and steel.

Nickel's essentiality in higher organisms is still questionable. No enzymes or cofactors that include nickel are known in higher organisms (Denkhaus and Salnikow, 2002). However, nickel-containing enzymes are well known in the bacterial world. Currently, seven microbial nickel-containing enzymes have been identified including urease, hydrogenase, CO-dehydrogenase, methylcoenzyme M

reductase, Ni-superoxide dismutase, glyoxylase I, and *cis–trans* isomerase. Table 1.4 summarized the function of these enzymes.

Table 1.4: Example of nickel-containing enzymes and their function

Enzyme/Protein	**Biological function**
Urease	Hydrolisis of urea into CO_2 and ammonia
Hydrogenase	Split molecular hydrogen into proton and electron
CO-dehydrogenase	Oxidation of CO to CO_2
Methylcoenzyme M reductase	Convert methyl group to methane
Ni-superoxide dismutase	Dismutation of superoxide into O_2 and H_2O_2
Glyoxylase I	Isomerization of hemithioacetal adducts
Cis-trans-isomerase	Isomerisation of geometric isomers

However, nickel at high doses in certain forms is toxic to both man and animals. The most common harmful health effect of nickel in humans is an allergic reaction when in contact to items containing nickel. Once a person is sensitized to nickel, further contact with the metal may produce a reaction such as skin rash at the site of contact. In some sensitized people, dermatitis (a type of skin rash) may develop in an area of the skin that is away from the site of contact. The most serious harmful health effects from exposure to nickel, such as chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus, have occurred in people who have breathed dust containing certain nickel compounds while working in nickel refineries or nickel processing plants. Exposure to high levels of nickel compounds that dissolve easily in water (soluble) may also result in cancer when nickel compounds that are hard to dissolve (less soluble) are present, or when other chemicals that can produce cancer are present. The International Agency for Research on Cancer (IARC) has determined that some nickel compounds are carcinogenic to humans and that metallic nickel may possibly be carcinogenic to humans. EPA recommends that drinking water levels for nickel should not be more than 0.1 mg per liter (ASTDR, 2005b).

1.3 Alternative technology for heavy metal removal

The chemical processes that exist today are not economical and safe enough to remove heavy metals from industrial effluent. Therefore, there is an urgent need for other low cost innovative clean-up technology. Biological treatments arouse great interest because of their lower impact to the environment with respect to the chemical treatments. Microorganisms and biological waste material have been intensively studied by researchers in the past decades due to their promising ability to be used as natural adsorbents.

1.3.1 Biological waste material as adsorbent

Various natural materials and waste from the industrial and agricultural sectors can be used as adsorbent in the abatement of metal-contaminated wastewater. The utilization of these waste is gaining serious attention because it represents unused resources and in many cases present serious disposal problem. By converting these wastes material into value added product or process, it would benefit both the environment and the industry via solving the waste disposal problem, contaminated wastewater and the opening of new market potential for the industry that generates the waste. Table 1.5 shows the different types of natural adsorbents that had been used for metal removal.

Table 1.5: The different types of adsorbent used for metal removal

Natural adsorbent	Metal ion	Form	Reference
Wheat shell (byproducts of wheat bread production industries)	Cu	Dried powder	Basci *et al.* (2004)
Rice husk (agricultural waste obtained from rice mill)	Fe, Zn and Cu	Native form	Chockalingam and Subramaniam (2006)
Cone biomass of *Thuja orientalis*	Cu	Dried powder	Nuhoglu and Oguz (2003)
Rice husk, maize cobs and sawdust	Pb	Grinded particle	Abdel-Ghani *et al.* (2007)
Grass	Pb	Grinded dried particle	Lu *et al.* (2009)
Sawdust	Cu(II)	Native form	Ajmal *et al.* (1998)
Barley straw	Cu and Pb	Powdered form	Pehlivan *et al.* (2009)
Waste tea leaves	Ni(II), Pb(II), Fe(II) and Zn(II)		Ahluwalia and Goyal (2005)

Among the low cost adsorbents mentioned, sawdust is the most promising adsorbent because it is not only abundant but it has been proven to be effective to remove many types of contaminants such as dyes, oil, salt, heavy metals and etc (Janoš *et al.,* 2009). Sawdust from various plants were investigated for their adsorption capacity i.e. maple sawdust for removal of Cu(II) and Pb(II) (Yu *et al.,* 2001), maple wood sawdust for removal of Cu(II) (Rahman and Islam, 2008), maple sawdust for removal of Ni(II) (Shukla *et al.,* 2005), red fir sawdust for removal of divalent copper and hexavalent chromium (Bryant *et al.,* 1992), mango tree sawdust for removal of Cu(II) (Ajmal *et al.,* 1998) and rubber wood sawdust for the removal of Cr(VI) (Zakaria *et al.,* 2007).

Sawdust is an agricultural waste that mainly contains lignin and cellulose as major constituents and may also include hemicellulose, extractives, lipids, proteins, simple sugars, starches, water, hydrocarbons, ash and other compounds. These compounds comprise a variety of functional groups i.e. acetamido groups, carbonyl,

hydroxyl, phenolic, structural polysaccharides, amido, amino, sulphydryl carboxyl groups of alcohol and esters which can contribute to the binding process (Demirbas, 2008; Sud *et al.,* 2008). It has been speculated that ion exchange and hydrogen bonding may be the principle mechanism for heavy metals ion removal (Ajmal *et al.,* 1998).

1.3.2 Microorganism as biosorbent

Microbial biomass can passively bind large amounts of metals, a phenomenon commonly referred to as biosorption. Biosorption is a property of certain type of inactive, non-living microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solution. Biomass exhibits this property, acting just as chemical substance i.e. as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria, which was found responsible for this phenomenon (Volesky, 1990). This property arise from the interaction between toxic metal ions and the functional groups such as carboxylate, hydroxyl, sulphate, phosphate and amino groups present on the cell wall surface, mainly composed of polysaccharides, proteins and lipids.

Different non-living biomass types have been used to adsorb heavy metal ions in aqueous solutions. Non-living biomass of algae, aquatic ferns and seaweed, waste biomass originating from plants, mycelial wastes from fermentation industries or bacteria easily obtained from environment are potential biosorbent for removal of heavy metal from aqueous solution and wastewater. The efficiency of the biosorbent depends on the capacity, affinity and specificity between the biosorbent and the metal ions (Ahluwalia *et al.*, 2006). Among the microorganisms available, fungal biomass offers the advantage of having a high percentage of cell wall material which shows excellent metal-binding properties. Marine algae are preferred due to the bulk availability of their biomass from water bodies (Gupta *et al.*, 2000). Bacteria also can

make excellent biosorbents because of their high-surface area-to-volume ratios and high content of potential chemosorption sites such as on techoic acid in their cell walls (Beveridge, 1989).

1.3.2.1 Mechanism of metal uptake by microorganism

The understanding of the mechanism by which microorganisms accumulate metal is crucial to the development of the microbial processes for metal concentration, removal and recovery from aqueous solutions. The mechanisms associated with metal sorption by microorganism include extracellular and intracellular metal binding. Both of these mechanisms are complex and depend on the metal ion and the biological system. The former mechanism is applicable to living organisms where the metal uptake is in active form. Metal ions are slowly transported into the cell through cell membrane and this process is also known as bioaccumulation.

Biosorption involve passively rapid metal ions uptake due to surface binding on the cell wall of non-living biomass. The actual attachment of the metal ions on the cellular surface may include physical adsorption, microprecipitation, ion exchange, complexation chelation, coordination and chemical adsorption. Due to complexity of the biomaterial used, it is possible that some of these mechanisms are acting simultaneously to varying degree depending on biosorbent and solution environment (Volesky, 2003). Cellular surfaces of microbial biomass mainly composed of polysaccharides, protein and lipids which offer cationic and anionic exchange sites such as the carboxyl, hydroxyl, sulphate, phosphate, amine and amide (Veglio and Beolchini, 1997). The net charge on the cell wall depends on the isoelectric point and the extent to which these sites are occupied by anions and cations. However cell walls are assumed to carry a net negative charge.

When the metal–biomass interaction mechanisms are understood, optimizing biosorption process on the molecular level can be carried out. This includes manipulation of the sorbent to increase its selectivity, uptake capacity and rate at which the metal being adsorbed.

1.3.2.2 Kinetic of biosorption

Kinetic of biosorption is the rate at which contaminants are removed from aqueous solution (Calero *et al.,* 2009). This removal rate control the residence time of the sorbate (metal ion) in the solid–solution interface (Febrianto *et al.,* 2009). The kinetic study is important to determine the efficacy of adsorption and to describe the mechanism through which the biosorption takes place. There are few factors determine the rate of metal ion uptake by biomass which includes mass transport and chemical reaction processes (Febrianto *et al.,* 2009; Iqbal and Saeed, 2007). Firstly is mass transfer or transportation of substances from liquid phase to solid surface either externally or internally. Intraparticle mass transfer has been established to be the rate controlling step and can be determined after the effect of external mass transfer was eliminated. External mass transfer resistance is proportional to the thickness of the stationary fluid layer or film which surrounds the biomass particle. The thickness of the layer can be controlled by agitation in the bulk solution. Generally, strong stirring decreases the film thickness and eventually eliminate film resistance (Volesky, 2003). Immobilization also might contribute to the increase in mass transfer. The metal ions have to traverse deeper into the biosorbent's pores and bind to the binding sites inside.

Another influencing factor that determine biosorption rate is particle size. Particle size is related to the diffusion distance that the metal ion must travel through for the case of a spherical biosorbent particle. Therefore, decrease in particle diameter normally reduces the diffusion distance and will accelerate adsorption rate.

Therefore by exploiting the particle size as well as the shape of the biosorbent, the rate of biosorption can be increased. The matrix structure of the biosorbent is also another factor that may have impact on biosorption rate. For example, by increasing the mechanical strength of the biosorbent through cross-linking, the mass transfer resistance and diffusion would also increases. *Sargassum* biomass reinforced by formaldehyde cross-linking showed lower removal rate compared to native *Sargassum* biomass (Yang and Volesky, 1999).

The sorption rate can be divided into two stages as reported by most researchers. The first stage was fast and rapid which can take few minutes followed by a relatively slower second stage until equilibrium is attained after few hours. Kaeswarn (2002) observed that 90% of soluble Cu(II) were removed from metal solution within 15 minutes of agitation by marine algae *Padina* sp. A slower rate of uptake was observed to about 30 minutes and no further adsorption were observed beyond this period. Uptake of Pb(II) by the yeast *Saccharomyces cerevisiae* immobilized on cone biomass of *Pinus niagra* was rapid in the first 30 minutes and reached equilibrium after 120 minutes (Cabuk *et al.,* 2007). A rapid biosorption process is a significant parameter for large–scale application in the industry. Generally, biosorption of metal ions consist of three continuous processes. The metal ions diffuse across the particle to fluid film from the bulk solution before entering the biosorbent. Then, they diffuse further towards the binding sites through the gel phase of the biomass. Finally, the metal ions react with the chemical group on the binding sites (Yang and Volesky, 1999). In the initial stage, the biomass surfaces may be relatively free of metal ions and the metal ions arriving at the biomass surfaces may attach instantly to the surfaces sites. Hence, the adsorption rate may be dominated by the number of metal ions diffused from bulk solution to the biomass surface. In the later stage, other factors start to play a role in controlling the adsorption process. Since most of the adsorption sites have been occupied, the remaining metal ions in the solution have to find the available sites before attachment can occur. Therefore, the later stage showed decrease in rate and would probably transit from initial diffusion-controlled process to a final attachment-controlled process (Li and Bai, 2005).

For quantitative description of the biosorption process dynamics it is necessary to develop a mathematical model capable of reflecting the toxic metal ions concentration change with the contact time. Various kinetic models were proposed to test the experimental data including pseudo-first, pseudo second order and intraparticle diffusion to examine the controlling mechanism involved in the biosorption of metal. The pseudo-first and second-order kinetic models are the most well-liked model by researchers. The pseudo-first-order Lagergren model is generally expressed as below (Equation 1.1):

$$\ln\left(q_{eq} - q_t\right) = \ln q_{eq} - K_1 t \qquad \text{(Eq. 1.1)}$$

Where q_{eq} (mg g^{-1}) is the mass of metal adsorbed at equilibrium, q_t (mg g^{-1}) is the mass of metal adsorbed at time t and K_1 (min^{-1}) is the first-order reaction rate equilibrium constant. The pseudo-first-order considers the rate of occupation of adsorption sites to be proportional to the number of unoccupied sites.

The pseudo-second-order equation based on adsorption equilibrium capacity assumes that the rate of occupation of adsorption sites is proportional to the square of the number of unoccupied sites and may be represented in Equation 1.2.

$$\frac{1}{q_t} = \frac{1}{K_2 q_{eq}^2} + \frac{t}{q_{eq}} \qquad \text{(Eq. 1.2)}$$

Where K_2 (g mg^{-1} min^{-1}) is the second-order reaction rate equilibrium constant.

Last but not least, exploitation of biosorption technology for heavy metals removal depends on the efficiency of regeneration of biosorbent after metal

desorption. Therefore, a non-destruction recovery using mild and cheap desorbing agents is desirable for regeneration of biomass for use in multiple cycles. Sometimes metal-selective recovery is required and this can be achieved by basic understanding of the mechanism involved particularly in metal sequestration. For metal ions which shows dependant on pH in binding to microbial cell, stripping or elution of the bound metal can be accomplished by pH adjustments. Increasing acidity generally leads to an effective removal of metal from the biomass (Gupta *et al.,* 2000). The eluent used can be dilute mineral acids (Chen *et al.,* 2005; Padmavathy, 2008), EDTA (Anand *et al.,* 2006), NaOH (Li *et al.,* 2008), NH_4OH, $KHCO_3$, $NaHCO_3$ KCN, and $CaCl_2$ (Cain *et al.,* 2008; Madhava Rao *et al.,* 2006). Screening for most effective regenerating solution is crucial. The regenerating solution should restore the biosorbent close to the original condition for effective reuse with; undiminished metal uptake and no physical changes or damage (Volesky, 2003). The desorption process would improve the economics of biosorption application.

1.3.2.3 Advantages and disadvantages of biosorption process

Until now, research in the area of biosorption suggests it as an ideal alternative for decontamination of metal containing effluents. The advantages and disadvantages of biosorption have been listed by Modak and Natarajan (1995) in Table 1.6.

Table 1.6: Advantages and disadvantages of biosorption by non-living biomass

Advantages	Disadvantages
• Growth-independent, non-living biomass is not subject to toxicity limitation of cells. No requirement of costly nutrients required for the growth of cells in feed solutions. Therefore, the problems of disposal of surplus nutrients or metabolic products are not present. • Biomass can be procured from the existing fermentation industries, which is essentially a waste after fermentation. • The process is not governed by the physiological constraint of living microbial cells. • Because of non-living biomass behave as an ion exchanger; the process is very rapid and takes place between few minutes to few hours. Metal loading on biomass is often very high, leading to very efficient metal uptake. • Because cells are non-living, processing conditions are not restricted to those conducive for the growth of cells. In other words, a wider range of operating conditions such as pH, temperature and metal concentration is possible. No aseptic conditions are required for this process. • Metal can be desorbed readily and then recovered if the value and amount of metal recovered are significant and if the biomass is plentiful, metal-loaded biomass can be incinerated, thereby eliminating further treatment.	• Early saturation can be a problem i.e. when metal interactive sites are occupied, metal desorption is necessary prior to further use, irrespective of the metal value. • The potential for biological process improvement (e.g. through genetic engineering of cells) is limited because cells are not metabolizing. Because production of the adsorptive agent occurs during pre-growth, there is no biological control over characteristic of biosorbent. This will be particularly true if waste biomass from a fermentation unit is being utilized. • There is no potential for biologically altering the metal valency state. For example less soluble forms or even for degradation of organometallic complexes.

1.3.3 Preparation of biosorbent

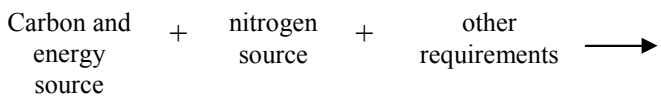
Abundant biological material could be used as biosorbent to remove heavy metals at a very low cost. When choosing the biosorbent, its origin is a major factor to be taken into account. The biomass can come from (i) industrial waste which should be obtained free of charge; (ii) organism easily available in large amounts in nature; and (iii) organism of quick growth, specially cultivated or propagated for biosorption purposes. Specificity is another factor that should be looked at before selecting the biosorbent. Some biosorbents can bind and collect a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metal.

In order to reduce the treatment cost, not only should the biomass be used directly but the biosorbent must be derived from a simple and cheap process. If by-products of fermentation process are not available, biosorbent can be produced by using relatively unsophisticated and low-cost propagation techniques. Nutrients from readily available and inexpensive sources such as carbohydrate-rich industrial effluent which often pose pollution or treatment problems such as food and dairy industry might be conveniently used (Vieira and Volesky, 2000).

1.3.4 Medium formulation and supplementation

Medium formulation is an essential stage in any biological process. The constituents of a medium must satisfy the elemental requirements for cell biomass and metabolite production and there must be an adequate supply of energy for biosynthesis and cell maintenance. Cell growth and product formation are complex processes reflecting the overall kinetics and stoichiometry of the thousands of

intracellular reactions that can be observed within a cell. The stoichiometry of growth and product formation are as shown in Equation 1.3:



$$\text{cell biomass} + \text{Products} + CO_2 + H_2O + \text{heat} \qquad \text{(Eq. 1.3)}$$

The equation should be expressed in quantitative terms, which is important in the economical design of a media if component wastage is to be minimal. Thus, minimal quantities of nutrients needed to produce a specific amount of biomass can be calculated. This requires material balances. The material balances relies on the first law of thermodynamics where all the mass entering a system must be recovered, transformed or unchanged at the output of a system, or at the end of a process. There are essentially two approaches to mass balances. The first approach is to perform a global balance, that takes into account the overall input and output of the system (i.e. microorganism), whilst disregarding the nature of the transformations taking place inside the living organism participating in the bioprocess. This approach is called 'Black Box', is most commonly used by process engineers to evaluate the performance of biotransformation. A second approach or 'Grey Box' approach, considers most reactions taking part in the metabolic machinery of an organism that participates in a particular bioprocess (Cortassa *et al.,* 2002).

The overall mass balance equation for growth of chemoorganotroph organism without product formation may be expressed as follows (Equation 1.4):

$$C_W H_X O_Y N_Z + aO_2 + bH_g O_h N_i \Rightarrow cCH_\alpha O_\beta N_\delta + dCO_2 + eH_2O \qquad \text{(E.q 1.4)}$$

Where *a, b, c, d* and *e* are the stoichiometric coefficients of the overall biomass synthesis reaction, and *α, β, δ, g, h, i, w, x, y* and *z* are the elemental formula coefficients. In such an elemental balance only the main biomass components are considered i.e. C, H, O and N as shown in Equation 1.5:

C: $w = c + d$ (E.q 1.5a)

H: $z + bi = c\delta$ (E.q 1.5b)

O: $y + 2a + hb = c\beta + 2d + e$ (E.q 1.5c)

N: $x + gb = c\alpha + 2e$ (E.q 1.5d)

A fifth equation is needed to solve the 5 unknown stoichiometric coefficients. Respiratory quotient (RQ) equation is a physiological term that tells about the type of metabolism displayed by the microorganism (Equation 1.6).

$$RQ = \frac{\text{moles of } CO_2 \text{ produced}}{\text{moles of } O_2 \text{ consumed}} = \frac{d}{a} \quad \text{(E.q 1.6)}$$

Another equation that can be used rather than the RQ equation is the yield coefficient. The biomass or product yield coefficient is defined as the amount of biomass or product synthesized per mole or gram of carbon substrate (Equation 1.7).

$$Y = \frac{\text{Quantity of cell dry matter/product produced}}{\text{Quantity of carbon substrate utilized}} \quad \text{(Eq. 1.7)}$$

Knowledge on the elemental composition of a named-microorganism, which should include the content of C, H, O, N, S, P, Mg and K, is required in the elemental balance equation. Trace elements i.e. Fe, Zn, Cu, Mn, Co, Mo and B may also be needed in smaller quantities (Stanbury and Whittaker, 1984) but the uptake of these

elements may be considered negligible in mass balance calculations (Cortassa *et al.,* 2002).

Most microorganisms are 70-90% water on a mass basis. The remaining dry weight is typically about 15% ash (minerals that remain upon combustion) and 85% volatile (mainly organic) material. The elemental composition of the dry matter of typical bacteria such as *Escherichia coli* is shown in Table 1.7. These values vary among strains and also depend on the physiological state of the cell i.e. growth with substrates of different degree of reduction or with different nitrogen sources (Cortassa *et al.,* 2002).

Table 1.7: Elemental composition of microbial cell

Element	Symbol	Atomic Weight	Cell Dry Weight (%)*	Element Ratio**	Formula#	Weight (%)
Carbon	C	12.01	50	4.2	5	53.1
Hydrogen	H	1.00	8	8.0	7	6.2
Oxygen	O	16.00	20	1.3	2	28.3
Nitrogen	N	14.01	14	1.0	1	12.4
Phosphorus	P	30.97	3	0.097	-	-
Sulphur	S	32.07	1	0.031	-	-
Potassium	K	39.10	1	0.026	-	-
Calcium	CA	40.08	0.5	0.012	-	-
Magnesium	Mg	24.30	0.5	0.021	-	-
Iron	Fe	55.85	0.2	0.0036	-	-
Other		-	~1.8	-	-	-

* Based on *Escherichia coli*.
** Apparent stoichiometric formula of *E. coli* based on cell dry weight.
# Useful stoichiometric ratio often used to write the components of a cell as a chemical compound formula.

Commonly used chemical formula for microorganism is $C_5H_7O_2N$. Note that this formula gives reasonably in good agreement with the values from *E. coli* for C, H, O, and N, which make up 92% of the total dry mass, but totally ignores the other elements.

Most microorganisms require an organic compound as their source of carbon. On a dry basis, a typical cell is made up of about 50% carbon (Shuler and Kargi, 2002). Numerous organic carbon compounds can be assimilated by bacteria. Amino acids, fatty acids, organic acids, sugars, nitrogen bases, aromatic compounds and countless other organic compounds have been shown to be used by bacteria. In contrast, some of the prokaryotes are autotrophs, which means that they are able to build all of their cellular structures from CO_2 (Madigan and Martinko, 2006). The carbon substrate has a dual role in biosynthesis and energy generation. The carbon requirements under aerobic conditions may be estimated from the cellular yield coefficient (Y) as described in Equation 1.7.

The method of media preparation, particularly sterilization, may affect the suitability of carbohydrates for individual fermentation processes. It is often best to sterilize sugars separately because they may react with ammonium ions and amino acids to form black nitrogen containing compounds which will partially inhibit the growth of microorganisms.

Oxygen is present in all organic components and cellular water and constitutes about 20% of the dry weight cells (Shuler and Kargi, 2002). Molecular oxygen is required as a terminal electron acceptor in the aerobic metabolism of carbon compounds.

After carbon, the next most abundant element in the cell is nitrogen. A typical bacterial cell contains about 10 to 14% (dry weight) nitrogen which can be found in proteins, nucleic acids and several other cell constituents (Shuler and Kargi, 2002). Microorganisms can utilize inorganic and organic sources of nitrogen. However, the bulk of available nitrogen is in inorganic form, either as ammonia (NH_3), N_2 or ammonium salts. Organic nitrogen may be supplied as amino acid, protein or urea.

The cell's requirements for C, O, and H are typically supplied by some combination of organic material, carbon dioxide, elemental oxygen, and water (or occasionally hydrogen sulfide or methane). The other requirements can be loosely categorized as macronutrients, micronutrients, and trace elements, although the boundaries between these groups are not uniform. For microorganisms, N and P are typically considered macronutrients. These are needed in a mass ratio of about 5:1. The required C:N:P ratio is commonly accepted as 100:5:1. However, in this case, much of the carbon is used as an energy source, rather than to make cell constituents. As can be seen in Table 3.1, the C/N ratio of a typical cell itself is around 3.6 (or 4.3 in the formula $C_5H_7O_2N$) rather than 20. The term micronutrients usually includes S and Fe, and probably K, Ca, and Mg. Trace nutrients which is needed in very small amounts, usually for specific enzymes, would include other elements such as Co, Ni, Cu and Zn.

1.3.5 Application of immobilized cells in biosorption process

Use of free cells for removal and recovery of heavy metals from aqueous solution may be impractical. Cells immobilized in a matrix may offer advantages over traditional fermentation using free cells, such as ease of cell mass separation, reduced risk in contamination, ease of regeneration and reuse of biomass, minimized clogging in continuous-flow systems and operational stability where immobilized cell can withstand rough conditions during treatment process (Anisha and Prema, 2008; Kourkoutas *et al.,* 2004; Yakup Arica *et al.,* 2001).

Immobilization are the physical confinement or localization of intact cells to a certain region of space without the lost of desired biological activity (El-Mansi *et al.,* 2007). Immobilization mimics the natural process of cells ability to adhere and grow on surfaces or within natural structures. Nowadays, many researches on the use of immobilized cell in biochemical processes had been proven to increase

productivity. Immobilized cell has been used in the production of diverse valuable products such as enzyme (Anisha and Prema, 2008), ethanol (Najafpour *et al.,* 2004) and lactic acid production (Idris and Wahidin, 2006).

Immobilized cell system can be classified into four categories, which are; surface attachment of cells, entrapment within porous material, containment behind a barrier and self aggregation (Kourkoutas *et al.,* 2004). Figure 1.3 shows the classification of immobilized cell system according to the physical localization and the nature of the microenvironment. Among the immobilization system, entrapment technique is mostly reported in the literature.

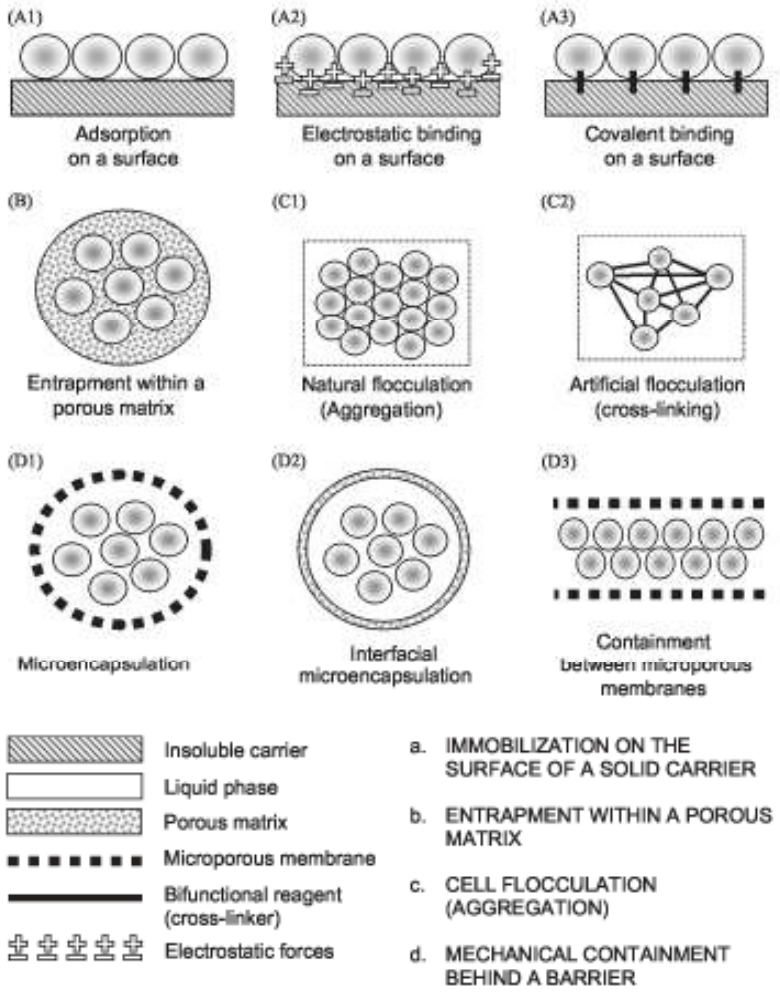


Figure 1.3: Techniques of cell immobilization (Kourkoutas *et al.,* 2004)

Cell entrapment can be achieved through in situ immobilization in the presence of porous matrix (i.e. gel entrapment), or by allowing cells to penetrate and move into preformed porous matrix until their mobility is obstructed by the presence of other cells. Both entrapment methods are based on the inclusion of cells within a rigid network to prevent the cells from diffusing into the surrounding medium while allowing mass transfer of nutrient and metabolites. Cell immobilized by entrapment can reach high densities in the matrix. However, dense cell packing may lead to mass transport limitation (El-Mansi *et al.,* 2007; Kourkoutas *et al.,* 2004).

A wide variety of material had been use as entrapment matrix based on the formation of gelling matrix i.e. polysaccharides such as alginates, chitosan, κ-carragenan, agar or other polymeric matrixes like gelatin, collagen and polyvinyl alcohol. Gel entrapment has the disadvantage of limited mechanical stability. However, the gel structure can be made stronger by reaction with other molecules such as polyvinyl alcohol, polyethyleneimine, gluteraldehyde cross-linking, silica or by partially drying the gel. Among the natural polymer used for immobilization purposes, alginate is most discussed in the literature. The mild condition for immobilization and its simplicity are some of the reasons why calcium alginate was chosen as the immobilization matrix (Bayragmoğlu and Yakup Arica, 2009; Idris and Wahidin, 2006). Examples of cell immobilization by entrapment into alginate matrix are in the production of lactic acid from whey by *Bifidobacterium longum* (Shahbazi *et al.,* 2005) and production of α-galactosidase by *Streptomyces griseoloalbus* (Anisha and Prema, 2008).

Alginate or alginic acid is found in all brown algae where it constitutes 10 – 40 % of dry weight, occurs in both the cell wall matrix and in the mucilage or intracellular material. It is also known to be produced by two bacterial genera *Pseudomonas* and *Azotobacter* (Rehm, 2006). Alginate are linear unbranched copolymer containing β-1,4 D-mannuronic (M) and its C5- epimer α-1,4 L-guluronic acid (G) residues with mannuronic acid occuring mostly intracellularly and in young cell wall. The monomers are arranged in a pattern of blocks of continuous

mannuronate residues (M-blocks), guluronate residues (G-blocks), or alternating residues (MG-blocks) (Figure 1.4).

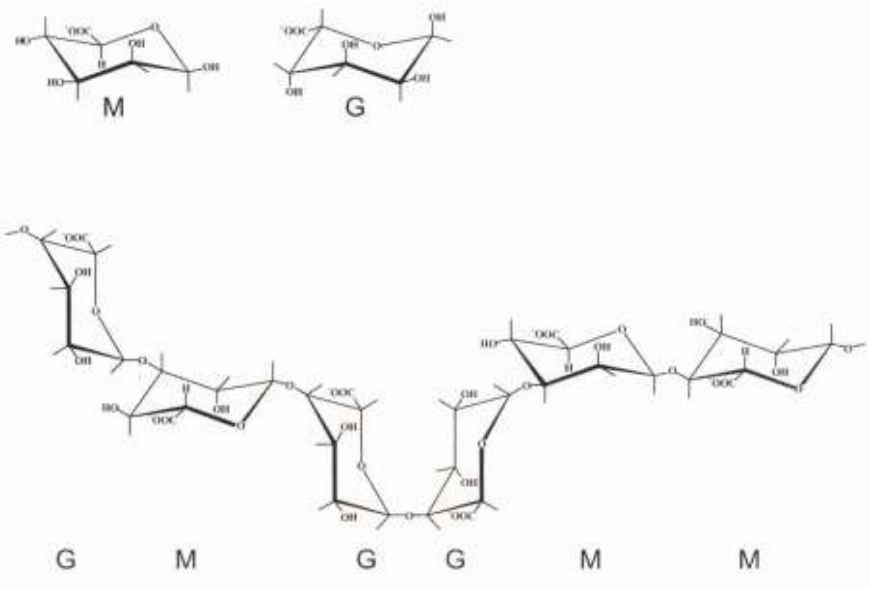


Figure 1.4: The chemical structure of alginate. M, β-D-mannuronic acid; G, α-L-guluronic acid (Rehm, 2006)

Alginate is used for a variety of industrial purposes i.e. as a stabilizing, thickening and gelling agent in food production or to immobilize cells in pharmaceutical and biotechnology industries. It was preferred over other materials due to various advantages such as biodegradability, hydrophilicity and consists of carboxylic groups that contribute to metal binding properties. Application of alginate entrapment in the field of metal removal study has also been reported. By immobilizing microbial cells in the alginate matrix, the microbial cell performance and adsorptive capacity of biosorbent system for heavy metal removal can be enhanced. Table 1.8 lists some recent application of cell entrapment in alginate for removing metal ions in aqueous solution.

Table 1.8: Recent application of cell entrapment in the metal removal studies

Organism	Metal ion	Reference
Bacteria		
Oscillatoria sp.	Cd(II)	Katırcıoğlu *et al.,* 2008
Fungi		
Phanerochaete chrysosporium	Zn^{2+}	Lai *et al.,* 2008
Pycnoporus sanguineus	Cd(II)	Mashitah *et al.,* 2008
Trametes versicolor	Cd(II)	Yakup Arica *et al.,* 2001
Algae		
Scenedesmus quadricauda	Cu(II), Zn(II) and Ni(II)	Bayragmoğlu and Yakup Arica, 2009
Ascophyllum nodosum	Cu(II), Cd(II) and Zn(II)	Carvalho *et al.,* 1994

In the presence of multivalent cations, α-L-guluronic acid residues from two or more alginate chains form a three dimensional network that can be described by the "egg box model" as shown in Figure 1.5 (Grant *et al.,* 1973). The egg box model shows clearly that the binding abilities of divalent ions depend on G residues of alginate. The more the content of G residues the stronger the ability of alginate to bind to divalent ion. Alginates with a high proportion of poly α-1,4 L-guluronate blocks therefore develop stiff porous gels that maintain their integrity for long periods of time.

The binding ability towards different divalent ions also tends to be different depending on the hydratation volume (ionic radius) (Rehm, 2006). Zheng *et al.* (1992) indicated that the Sr^{2+} ions chelated more closely by the $–COO^-$ and –OH groups of the alginate cavities rather than Cd^{2+} ions. This is because the ionic radius of Sr^{2+} (1.18Å) is larger than that of Cd^{2+} (0.99Å). Therefore by replacing calcium with higher ionic radius of divalent cation (Ba^{2+} > Sr^{2+} > Ca^{2+} >> Mg^{2+}) or multivalent cations i.e. Al^{3+} or Ti^{3+} can increase the mechanical strength of the gel formed. Other stabilization methods involve the use of polyelectrolytes such as

polyethyleneimine (PEI) and polypropyleneimine (PPI), gluteraldehyde, colloidal silica, propylene glycol ester of alginic acid with PEI and potassium poly(vinyl alcohol) sulphate and trimethylammonium glycol chitosan iodide. Entrapment of cells within the alginate polymer is through ionotropic gelation mechanism (El-Mansi *et al.,* 2007). The cell polymer-cell mixture can be formed in different sizes and shapes. However, the most common forms are small beads about 1 – 5 mm in diameter. Other additional advantages of alginate beads are low density, mechanical stability and can withstand experimental pH range of 3 – 8 (Yakup Arica *et al.,* 2001).

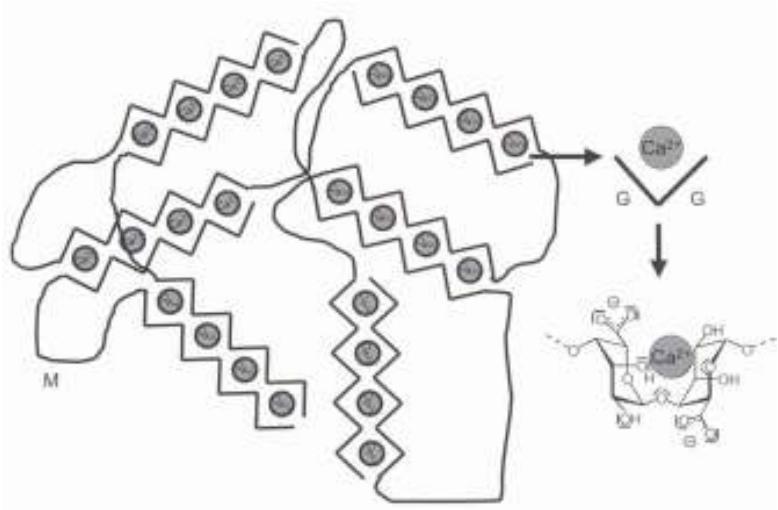


Figure 1.5: Alginate gel formation and cross-linking with Ca^{2+} ions. Gray oxygen atoms of guluronic acid (G) are interacting with Ca^{2+} ions. M, β-D-mannuronic acid (Rehm, 2006)

1.4 Objective of Thesis

The aim of this study is to develop a two stage biological treatment processes for the removal of Cu(II) and Ni(II) from the electric and electronic industry wastewater.

1.5 Scope of Thesis

Initially, electrical and electronic industry was identified where sampling and characterization of its wastewater was carried out in order to screen its heavy metal content. Bacteria previously isolated from textile wastewater i.e. *Acinetobacter haemolyticus, Acinetobacter calcoaceticus, Clavibacter agropyri* and *Cellulosimicrobium cellullans* were used throughout this research as the bacterial strain were already exposed to metal ions present in the textile wastewater and therefore capable of tolerating the metal ions. The bacterial strains were subjected for Cu(II) and Ni(II) toxicity test in liquid pineapple waste using repli-plate technique The most tolerant strain was chosen for biosorption study. Cultivation and formulation of cheap growth medium such as liquid pineapple waste and brown sugar for large scale cultivation of bacteria was also looked into. Biological waste material, rubber wood shavings (RWS) served as first stage metal removal followed by second stage removal using dead cell of *A. haemolyticus* immobilized in gel matrix of alginate (SAIB). The effect of operational parameters were investigated i.e. the effect of pH, contact time, biomass dosage, pretreatment of biomass and initial metal concentration. Equilibrium isotherms were used to evaluate the adsorption data. Eventually, the best operational parameters were used to treat wastewater from the electric and electronic industry. The mechanism of Cu(II) and Ni(II) biosorption by *A. haemolyticus* were elucidated using FTIR and FESEM-EDAX.