THE EFFECT OF pH AND BIOMASS CONCENTRATION ON CADMIUM (Cd) UPTAKE BY *Saccharomyces cerevisiae* FROM SIMULATED WASTEWATER

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ABSTRACT

The contamination of wastewaters, river sediments and soil with toxic metals, is a complex problem. The removal of these contaminations has received much attention in recent years using conventional methods such as chemical reduction, ion exchange, and electrochemical treatment**.** The alternative method is discovered which is biosorption, refers to a physico-chemical binding of metal ions to biomass. Microorganisms like bacteria, yeast and fungi as well as algae can accumulate large amounts of heavy metal ions._Biosorption is considered to be a fast physical or chemical process. The biosorption rate depends on the type of the process**.** The biosorption of cadmium ions from artificial aqueous solutions using baker's yeast biomass is investigated. The major purpose of this research is to study the effect of pH and biomass concentration on cadmium uptake by *Saccharomyces cerevisiae* from simulated waste water. The effect of pH on biosorption was studied in the pH ranges of 3.0–8.0 for Cd^{2+} while effect of concentration on biosorption in the range of 5.0 – 17.5 mg/ml of the biomass. As a result, pH value of 3.0 shows a highest percent of metal acculumated and for the biomass concentration profile, the analysis for 15mg/ ml giving the maximum uptake of cadmium from the biosorbent.

CHAPTER 1

INTRODUCTION

1.1 Background

Bioadsorption is a process that utilizes inexpensive biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminant from industrial effluents (W. Jianlong, 2001). The study of biosorption is of great importance from an environmental point of view, as it can be considered as an alternative technique for removing toxic pollutants from wastewaters. Interest has recently been focused on biomass because of its high metal-sorbing capacity, low cost and also ready abundance.

Heavy metals are discharged from various industries such as electroplating, metal finishing, textile, storage batteries, mining, ceramic and glass. As they pose serious environmental problems and are dangerous to human health, considerable attention has been given to the methods for their removal from industrial wastewaters (R. Han *et al*, 2006). One of the heavy metal, cadmium, is introduced into bodies of water from smelting, metal plating, cadmium-nickel batteries, phosphate fertilizer, mining, pigments, stabilizers, alloy industries and sewage sludge. The harmful effects of cadmium include a number of acute and chronic disorders, such as “itai-itai” disease, renal damage, emphysema, hypertension, and testicular atrophy. The drinking water guideline value recommended by World Health Organization (WHO) is 0.005 mg Cd/L. Low concentration (less than 5 mg/L) of cadmium is difficult to treat economically using chemical precipitation methodologies. The contamination of water by toxic heavy metals is a world-wide environmental problem. Discharges containing cadmium, in particular, are strictly controlled due to the highly toxic nature of this element and its tendency to accumulate in the tissues of living organisms. Ion exchange and reverse osmosis while can guarantee the metal concentration limits required by regulatory standards, have high operation and maintenance costs. These disadvantages of conventional systems together with the need for more economical and effective methods for the removal of metals from wastewater have resulted in the development of new separation technologies.

Alternative method, biosorption it is effective, simple and cheap; it is very similar to the use of ion exchange resins which can also do the clean-up job. Biosorption has the benefit of cost effectiveness as well as easing ecological concerns by, in a sense, recycling dead matter. Living and dead biomass microbial cells are able to remove heavy metal ions from aqueous solution. The need for safe and economical methods for removing heavy metals from contaminated waters and soils has resulted in the search for alternative materials that may be useful to reduce the metal content to the levels established by the legislation. Neutralization, chemical precipitation, ion exchange, adsorption, reverse osmosis, membrane filtration are conventional technologies cited in literature for removal and recovery of heavy metals from industrial effluents. However, the application of such processes is sometimes restricted because of technical or economic constraints.

As a consequence, the search for effective new technologies has directed attention to biosorption, a technically feasible and economically attractive approach using biological material as sorbents. In addition, biosorption is a methodology that is less aggressive to the environment. Uptake of heavy metal ions by microorganisms may offer an alternative method for their removal from wastewater (W. Jianlong, 2001). *Saccharomyces cerevisiae* is an inexpensive, readily available source of biomass for heavy metal removal from wastewater (Y.Goksungur, 2004).

1.2 Problem statement

During the past decade, the world has become increasingly aware of the seriousness of one of the major consequences of development, that is, the quantity and diversity of hazardous wastes generated by its industrial activities. Such wastes are usually a by-product of industrial operations which involve heavy metals such as arsenic, cadmium, chromium, lead, mercury, etc; processes which utilize different categories of oil and petrochemicals; products such as PVC and plastics; waste products from photocopiers; chemicals; and finally, by-products such as dioxins and furans which are now recognized as extremely toxic substances, affecting all forms of life. In fact, depending upon their characteristics, nature, and concentration of contaminants, some of these wastes are extremely toxic and hazardous (Theo Colborn, 1996).

By far the greatest demand for metal sequestration comes from the need of immobilizing the metals ‘mobilized’ by and partially lost through human technological activities. It has been established beyond any doubt that dissolved particularly heavy metals escaping into the environment pose a serious health hazard. They accumulate in living tissues throughout the food chain which has humans at its top. The danger multiplies.

There is a need for controlling the heavy metal emissions into the environment as the discharge of heavy metals into aquatic ecosystems has become a matter of concern. These pollutants are introduced into the aquatic systems significantly as a result of various industrial operations. The pollutants of concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper and nickel. These toxic materials may be derived from mining operations, refining ores, sludge disposal, fly ash from incinerators, the processing of radioactive materials, metal plating, or the manufacture of electrical equipment, paints, alloys, batteries, pesticides or preservatives. Heavy metals such as zinc, lead and chromium have a number of applications in basic engineering works, paper and pulp industries, leather tanning, organ chemicals, petrochemicals fertilizers, etc. Major lead pollution is through automobiles and battery manufacturers

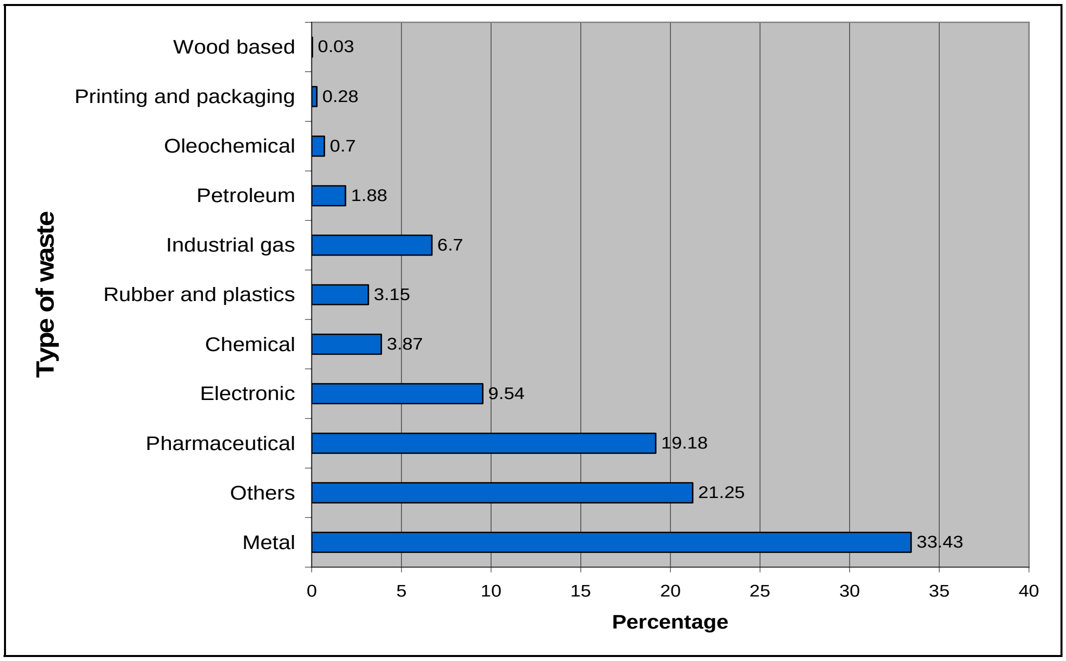

Figure 1.1 Malaysia waste sources 2004 (The Star, 2006)

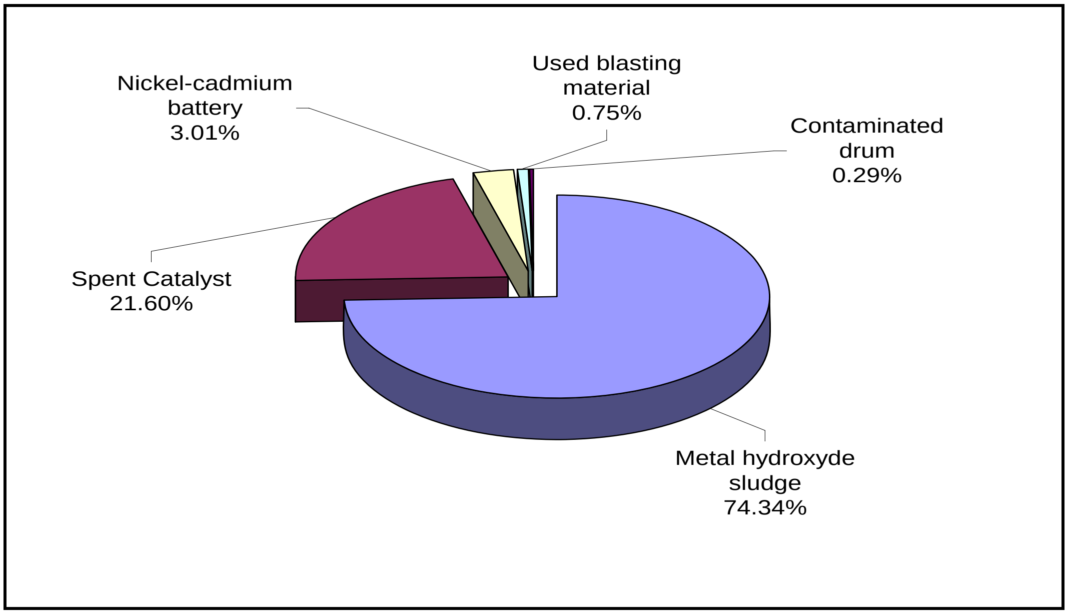

Figure 1.2 Malaysia waste under trade 2004 Exported 3,354 tonnes (The Star, 2006)

1.3 Objective

The main purpose of this project is to study the effect of pH and biomass concentration on cadmium (Cd) uptake by *Saccharomyces cerevisiae* from simulated waste water.

1.4 Scopes of Research

The scopes of the project are as follows:-

a) Study on effect of pH on cadmium uptake by *Saccharomyces cerevisiae* from simulated waste water.

b) Study on effect of initial biomass concentration on cadmium uptake by *Saccharomyces cerevisiae* from simulated waste water.

CHAPTER 2

LITERATURE REVIEW

2.1 Biosorption

2.1.1 Terminology

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. Biosorption uses the extraordinary capacity of certain types of microbial and biomass to bind with metal elements. These bio-materials are used dead just like "magic granules" which remove and concentrate heavy-metals from industrial effluents. The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents .The major advantages of biosorption over conventional treatment methods include (H.-J Rehm, 1999):

- Use of renewable biomaterials, which can reduce investment and operation cost
- Minimisation of chemical and biological sludge
- No additional nutrient requirement
- Regeneration of biosorbent
- Possibility of metal recovery
- High selectivity of biosorbents (possible to recover valuable metals)
- Cleansing of aqueous solution with low metal concentration
- High capacity by small equilibrium concentration

- Easy desorption of metals by pH swing
- Low affinity with competing cations

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

Physical Adsorption	Chemical Adsorption
The adsorbate adheres to the surface only through Van der Waals force interactions.	Molecule adheres to a surface through the formation of a chemical bond
Low temperature	High temperature
Low activation energy	High activation energy
Low enthalpy	High enthalpy
Adsorption takes place in multilayer	Adsorption takes place only in a monolayer

Table 2.1 Physical and Chemical Adsorption Differences

Figure 2.1 Biosorption Column Plant (B.Volesky, 2003)

2.1.2 Biosorbent material

Strong biosorbent behaviour of certain micro-organisms towards metallic ions is a function of the chemical make-up of the microbial cells. This type of biosorbent consists of dead and metabolically inactive cells. Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals.

Some laboratories have used easily available biomass whereas others have isolated specific strains of microorganisms and some have also processed the existing raw biomass to a certain degree to improve their biosorption properties. Recent biosorption experiments have focused attention on waste materials, which are by-products or the waste materials from large-scale industrial operations. For e.g. the waste mycelia available from fermentation processes, olive mill solid residues, activated sludge from sewage treatment plants, biosolids, aquatic macrophytes .

Another inexpensive source of biomass where it is available in copious quantities is in oceans as seaweeds, representing many different types of marine macro-algae. However most of the contributions studying the uptake of toxic metals by live marine and to a lesser extent freshwater algae focused on the toxicological aspects, metal accumulation, and pollution indicators by live, metabolically active biomass. Focus on the technological aspects of metal removal by algal biomass has been rare.

Although abundant natural materials of cellulosic nature have been suggested as biosorbents, very less work has been actually done in that respect. The ideal microorganism should posses the following technological characteristics:-

- High affinity for the substrate
- Ability to use complex substrates
- High specific growth rate
- Low nutritional requirement, i.e., few indispensable growth factors
- Ability to develop high cell density

- Stability during multiplication
- Capacity for genetic modification
- Good tolerance to temperature and pH

In addition, it should have a balanced protein and lipid composition. It must have a low nucleic acid content, good digestibility and be non-toxic.

2.1.3 Biosorption Mechanisms

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria. Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal.

During non-metabolism dependent biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible. In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface.

Further, it may be dependent on the cell's metabolism if, in the presence of toxic metals, the microorganism produces compounds that favor the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface.

2.1.4 Factors Affecting Biosorption

The investigation of the efficacy of the metal uptake by the microbial biomass is essential for the industrial application of biosorption, as it gives information about the equilibrium of the process which is necessary for the design of the equipment. The following factors affect the biosorption process:

- Temperature seems not to influence the biosorption performances in the range of 20-35 ^{0}C
- pH seems to be the most important parameter in the biosorptive process: it affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions.
- Biomass concentration in solution seems to influence the specific uptake: for lower values of biomass concentrations there is an increase in the specific uptake.
- Biosorption is mainly used to treat wastewater where more than one type of metal ions would be present, the removal of one metal ion may be influenced by the presence of other metal ions (N. Ahalya *et al,*2001)
- Contact time, as for biosorption capacity of metal ion by *S. Cerevisiase* became higher with prolonging the contact time
- Competing ions/co-ions , the biosorption capacity of one metal ion is interfered and reduced by co-ions, including other metal ion and anions presenting in solution
- Cell age, usually the cells at lag phase or early stage of growth have a higher biosorptive capacity for metal ions than that of stationary phase (J. Wang, C. Chen, 2006)

2.2 Atomic Absorption Spectrometer (AAS)

2.2.1 Introduction

An Atomic Absorption Spectrometer is used to analyze metals by burning a solution containing the unknown metal in a gas flame, then analyzing the light emitted or absorbed by the flame with a spectrophotometer. This makes this instrument ideal for measuring trace amounts of virtually any metal. AAS is an analytical technique used to measure a wide range of elements in materials such as metals, pottery and glass. Although it is a destructive technique, the sample size needed is very small (typically about 10 milligrams - one hundredth of a gram) and its removal causes little damage. The sample is accurately weighed and then dissolved, often using strong acids. The resulting solution is sprayed into the flame of the instrument and atomized. Light of a suitable wavelength for a particular element is shone through the flame, and some of this light is absorbed by the atoms of the sample.

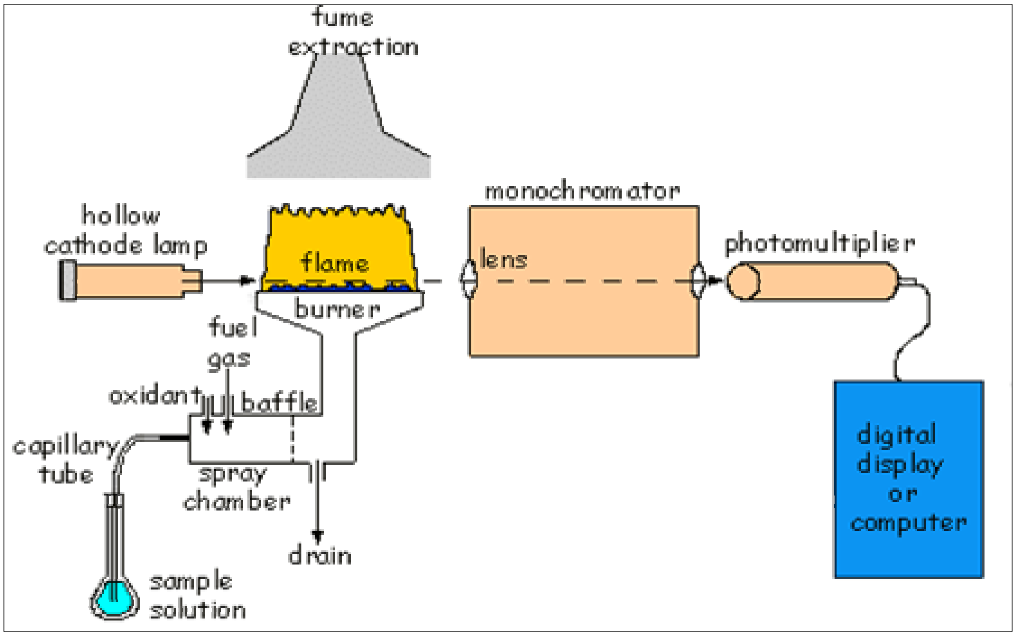

Figure 2.2 A schematic diagram of atomic absorption spectrometer

2.2.2 Operating Procedure

Atoms of different elements absorb characteristic wavelengths of light. Analyzing a sample is to see if it contains a particular element means using light from that element. For example with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample. In AAS, the sample is atomized – *i.e.* converted into ground state free atoms in the vapor state – and a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some of the radiation is absorbed by the lead atoms in the sample. The greater the number of atoms there is in the vapor, the more radiation is absorbed. The amount of light absorbed is proportional to the number of lead atoms. A calibration curve is constructed by running several samples of known lead concentration under the same conditions as the unknown. The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample. Consequently an atomic absorption spectrometer needs the following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed.

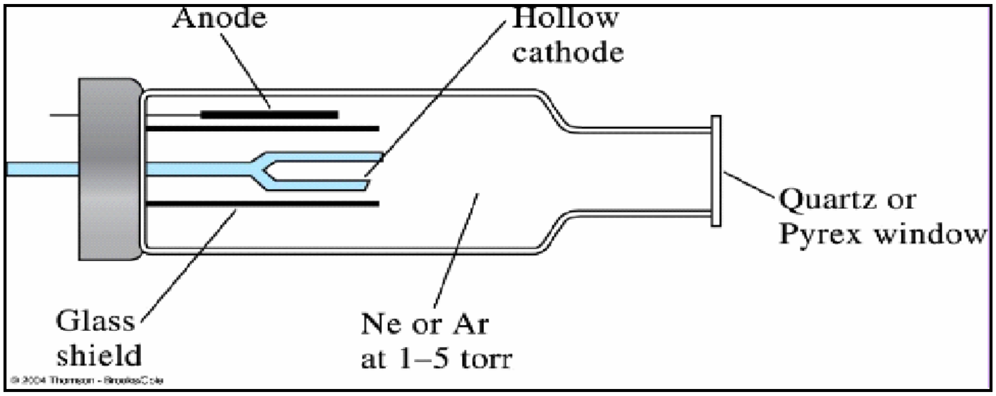

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Figure 2.3 Cathode Lamp

2.2.3 The light source

The common source of light is a ‘hollow cathode lamp’. This contains a tungsten anode and a cylindrical hollow cathode made of the element to be determined. These are sealed in a glass tube filled with an inert gas – *e.g.* neon or argon – at a pressure of between 1 Nm^{-2} and 5 Nm^{-2}. The ionization of some gas atoms occurs by applying a potential difference of about 300–400 V between the anode and the cathode. These gaseous ions bombard the cathode and eject metal atoms from the cathode in a process called sputtering. Some sputtered atoms are in excited states and emit radiation characteristic of the metal as they fall back to the ground state – *e.g.* Pb^* . Pb + *h*. The shape of the cathode concentrates the radiation into a beam which passes through a quartz window, and the shape of the lamp is such that most of the sputtered atoms are redepositing on the cathode.

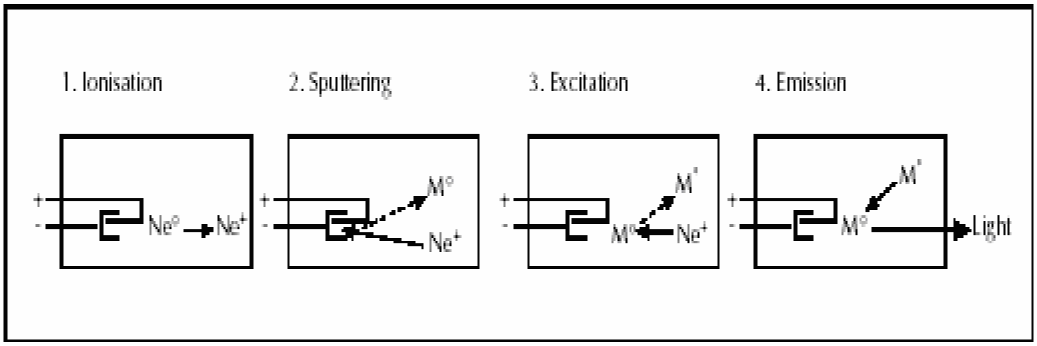

Figure 2.4 Atomization Process

A typical atomic absorption instrument holds several lamps each for a different element. The lamps are housed in a rotating turret so that the correct lamp can be quickly selected

2.2.4 The optical system and detector

A monochromator is used to select the specific wavelength of light – *ie* spectral line which is absorbed by the sample, and to exclude other wavelengths. The selection of the specific light allows the determination of the selected element in the presence of others. The light selected by the monochromator is directed onto a detector that is typically a photomultiplier tube. This produces an electrical signal proportional to the light intensity.

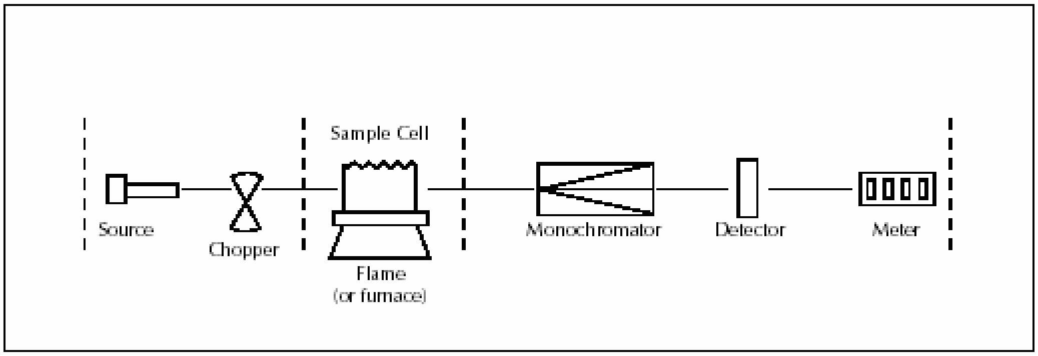

Figure 2.5 Optical System

2.3 *Saccharomyces cerevisiae*

2.3.1 Introduction

Yeast were the first microorganisms known, rarely toxic or pathogenic and can ber used in human diets. Although their protien content rarely exceeds 60%, their concentration in amino acids such as lysine (6-9%) tryptophan and threonine is satisfactory. The are larger than bacteria,facilitating separation and can be used inraw state. However, their specific growth rate is relatively slow (generation time 2 to 5 hours).

Saccharomyces cerevisiae is the budding yeast used for bread-making, where the carbon dioxide produced by growth in the dough causes the bread to rise. Essentially similar yeasts, but now given different species names, are used for production of beers, wines and other alcoholic drinks. This phase-contrast micrograph shows cells in various stages of budding. The buds are small at first, but enlarge progressively and eventually separate from the mother cell by formation of a septum (cross wall).It are perhaps the most important yeast, thanks to its use since ancient times in baking and brewing. It is believed that it was originally isolated from the skins of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-colored fruits such as plums; it exists among the waxes of the cuticle). It is the most intensively studied eukaryotic model organisms in molecular and cell biology, much like Escherichia coli as the model prokaryote. It is the microorganism behind the most common type of fermentation. *Saccharomyces cerevisiae* cells are round to ovoid, 5-10 Micrometres in diameter. It reproduces by a division process known as budding.

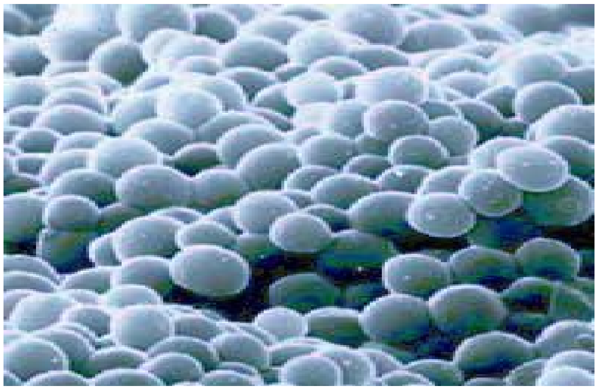

Figure 2.6 Yeast cells (Laurence Calzone *et al*, 2004)

It is useful in studying the cell cycle because it is easy to culture, but, as a eukaryote, it shares the complex internal cell structure of plants and animals. *S. cerevisiae* was the first eukaryotic genome that was completely sequenced. The yeast genome database is highly annotated and remains a very important tool for developing basic knowledge about the function and organization of eukaryotic cell genetics and physiology. The genome is composed of about 13,000,000 base pairs and 6,275 genes, although only about 5,800 of these are believed to be true functional genes. It is estimated that yeast shares about 23% of its genome with that of humans.

"*Saccharomyces*" derives from Greek, and means "sugar mold". "*Cerevisiae*" comes from Latin, and means "of beer".

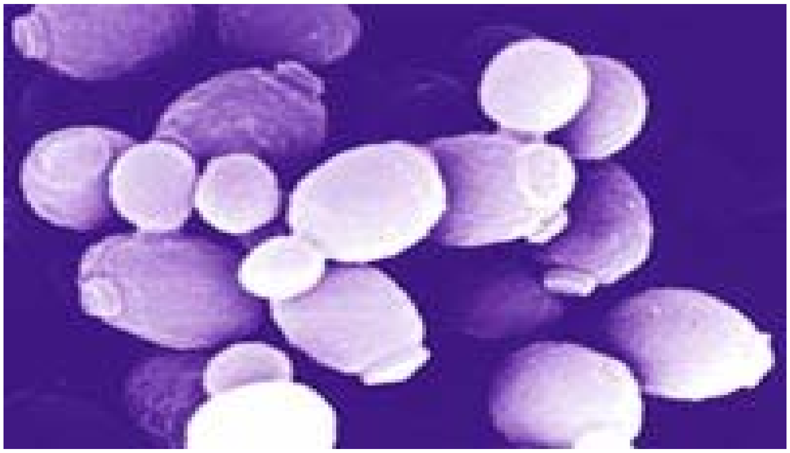

Figure2.7 *Saccharomyces cerevisiae*

2.3.2 Lifecycle

Yeast has a sexual life cycle like other higher organisms such as plants and animals. Haploid cells harbor one set of chromosomes (n = 16) and are either of mating type a or mating type a. Such haploid cells cannot sporulate. When mixed, a-cells can mate with a-cells, forming diploid (2n = 32) zygotes containing a double set of chromosomes. Both haploid and diploid cells can multiply asexually by budding. Under certain starvation conditions, diploid yeast cells can undergo sporulation, resulting in the formation of asci containing four spores. These spores contain the haploid (n = 16) number of chromosomes and can germinate giving rise to two a-, and two a-cell cultures. Lager brewing yeast strains are genetically more complicated, being species hybrids carrying the tetrapoid (4n = 64) number of chromosomes. Sporulation and subsequent inter-crossing of the spore clones, may form new combinations of genes, resulting in yeast strains with altered characteristics, some of which may be attractive to the brewer. There are two forms in which yeast cells can survive and grow, haploid and diploid. The haploid cells undergo a simple lifecycle of mitosis and growth, and under conditions of high stress will generally simply die. The diploid cells (the preferential 'form' of yeast) similarly undergo a simple lifecycle of mitosis and growth, but under conditions of stress can undergo sporulation, entering meiosis and producing a variety of haploid spores, which can go on to mate (conjugate), reforming the diploid.

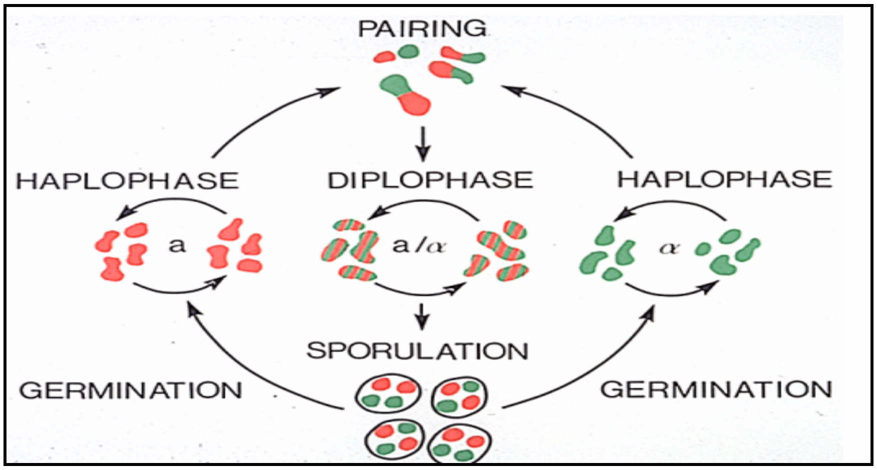

Figure 2.8 Yeast Cycle

2.4 Heavy Metal

2.4.1 Terminology

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources, or intake via the food chain.

2.4.2 The Toxicity

Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater. Heavy metals may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings.

Heavy metals are major toxicants found in industrial wastewater and may adversely affect the biological treatment of wastewater. The source of heavy metals in wastewater treatment plants are mainly industrial discharges and urban storm water runoffs. Heavy metals toxicity in mainly due to soluble metals. Toxicity is controlled by various factors such as pH, type and concentration of complexing agents in wastewater, antagonistic effects by toxicant mixtures, oxidation state of the metal and redox potential [*G. Bitton, 2005*]

Industrial exposure accounts for a common route of exposure for adults. Ingestion is the most common route of exposure in children. Children may develop toxic levels from the normal hand-to-mouth activity of small children who come in contact with contaminated soil or by actually eating objects that are not food (dirt or paint chips). Less common routes of exposure are during a radiological procedure, from inappropriate dosing or monitoring during intravenous nutrition, from a broken thermometer, or from a suicide or homicide attempt.

2.4.3 Cadmium Properties

Figure 2.9 Cadmium Metal Solid

Cadmium is a soft, malleable, ductile, bluish-white bivalent metal which can be easily cut with a knife. It is similar in many respects to zinc but lends itself to more complex compounds. Cadmium has no constructive purpose in the human body. This element and solutions of its compounds are extremely toxic even in low concentrations, and will bioaccumulate in organisms and ecosystems.

One possible reason for its toxicity is that it interferes with the action of zinc-containing enzymes. Inhaling cadmium laden dust quickly leads to respiratory tract and kidney problems which can be fatal (often from renal failure). Ingestion of any significant amount of cadmium causes immediate poisoning and damage to the liver and the kidneys. Compounds containing cadmium are also carcinogenic. Cadmium is considered as an etiological agent for essential hypertension and increase in systolic and diastolic blood pressure has been reported in cadmium workers. A number of epidemiologic studies suggest a relationship between occupational exposure to cadmium and lung and prostrate cancers.

Other health effects that can be caused by cadmium are:-

- Diarrhea, stomach pains and severe vomiting
- Bone fracture
- Reproductive failure and possibly even infertility
- Damage to the central nervous system
- Psychological disorders
- Possibly DNA damage or cancer development

Cadmium also may derive its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and humans. Cadmium is biopersistent and, once absorbed by an organism, remains resident for many years (over decades for humans) although it is eventually excreted. Another important source of cadmium emission is the production of artificial phosphate fertilizers. Part of the cadmium ends up in the soil after the fertilizer is applied on farmland and the rest of the cadmium ends up in surface waters when waste from fertilizer productions is dumped by production companies. Cadmium can be transported over great distances when it is absorbed by sludge. This cadmium-rich sludge can pollute surface waters as well as soils.

Cadmium strongly adsorbs to organic matter in soils. When cadmium is present in soils it can be extremely dangerous, as the uptake through food will increase. Soils that are acidified enhance the cadmium uptake by plants. This is a potential danger to the animals that are dependent upon the plants for survival. Earthworms and other essential soil organisms are extremely susceptive to cadmium poisoning. They can die at very low concentrations and this has consequences for the soil structure. When cadmium concentrations in soils are high they can influence soil processes of microorganisms and threat the whole soil ecosystem as equally to the green environment. In aquatic ecosystems cadmium can bio accumulate in mussels, oysters, shrimps, lobsters and fish. The susceptibility to cadmium can vary greatly between aquatic organisms.

2.4.4 Act Relevant to Heavy Metal Discharges

Metal	Unit	Standard A	Standard B
Sianide	mg/L	0.05	0.10
Cadmium	**mg/L**	**0.01**	**0.02**
Plumbum	mg/L	0.10	0.50
Arsenium	mg/L	0.05	0.10
Nickel	mg/L	0.20	0.10
Zinc	mg/L	2.00	2.00

Table 2.2 Permissible Concentrations for Effluents (Malaysian Environmental Quality Act)

Metal	Contaminated Water Discharged Guide Value (mg/L)	Drinking Water Limiting Value (mg/L)	Drinking Water Maximum Concentration (mg/L)	Drinking Water Guide Value (mg/L)
Cadmium (Cd)	**0.005 total**	**0.005**	**0.005**	**0.005**

Table 2.3 Permissible Concentrations for Direct Discharges in Receiving Streams; Limiting Value of Drinking Water (H.-J Rehm, 1999)

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

The following factors affect the biosorption process:-

a) pH seems to be the most important parameter in the biosorptive process: it affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions

b) Biomass concentration in solution seems to influence the specific uptake for lower values of biomass concentrations there is an increase in the specific uptake. Hence this factor needs to be taken into consideration in any application of microbial biomass as biosorbent.