STUDY ON pH EFFECT OF METAL SOLUTION AND BIOSORBENT CONCENTRATION ON COPPER (Cu) REMOVAL BY *Saccharomyces cerevisiae* FROM SIMULATED WASTE WATER

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This thesis is submitted in fulfillment of the requirements for the award of the Degree of Bachelor of Chemical Engineering

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MAY 2008

“I declare that this thesis is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.”

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In the name of Allah S.W.T the Most Gracious, the Ever Merciful. Praise is to Allah, Lord of the Universe and Peace and Prayers be upon His final Prophet and Messenger Muhammad s.a.w.

To my beloved parents and family….

ACKNOWLEDGEMENT

In preparing this thesis, I was in contact with many people, academicians, and practitioners. The have contribute towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my project supervisor, Mr. Mior Ahmad Khushairi bin Mohd. Zahari for the encouragement, guidance, motivation and friendship. I am also very thankful to my panel of this project for their critics and advices. Without their continued support and interest, this thesis would not have been the same as presented here.

Not to be forgotten, all other Universiti Malaysia Pahang staff members that I have called upon for assistance since the genesis of this project and also to all my colleagues with the support, valuable opinion and sharing ideas during the progress of this project.

Finally, I would like to thank to my family especially my beloved mother, Puan Hjh. Jawiah bt. Abdullah and family members for their continuous supports from the preliminary of this project till the end of it. Thank you so much for all of you.

ABSTRACT

The production of heavy metals has increased rapidly since the industrial revolution. Heavy metals usually form compounds that can be toxic even in very low concentrations. The conventional methods of removing metals from wastewaters such as chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, membrane technologies and solvent extraction are generally expensive and have many limitations. It is also generate huge quantity of toxic chemical sludge. The search for new technologies has directed attention to biosorption. Bioadsorption is a process that utilizes inexpensive biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents. Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents. The biosorption of copper ions from simulated wastewater using baker's yeast biomass will be investigated. The major purpose of this research is to study the effect of pH and biomass concentration on copper uptake by *Saccharomyces cerevisiae* from simulated waste water. The level of copper (II) accumulation depends on the pH and initial biomass concentration. Optimum pH values for maximum copper (II) accumulation were determined in range of 3.0-8.0, while effects of concentration on biosorption were in the range 5.0-20.0 mg/ml of the biomass. As the result, there was an increase in biosorption capacity with increasing of pH. pH value of 8.0 shows the highest percent of metal accumulated which is almost 70%. For biomass concentration parameter, the analysis for 10 mg/ml of biomass concentration gives the optimum uptake of heavy metal with 60% removal from the simulated wastewater.

ABSTRAK

Penghasilan logam berat telah meningkat dengan cepat semenjak revolusi industri. Ia selalunya membentuk campuran yang boleh menjadi toksik walaupun di dalam kepekatan yang rendah. Kaedah konvensional untuk menyingkirkan logam dari air kumbahan seperti mendakan kimia, pengoksidaan atau penurunan kimia, penukaran ion, penurasan, rawatan elektrokimia, teknologi membran, dan pengekstrakan bahan pelarut selalunya lebih mahal dan aplikasinya adalah terbatas. Ia juga menghasilkan lumpur kimia bertoksik dalam kuantiti yang banyak. Pencarian teknologi baru telah tertumpu pada penjerapan biologi. Ia adalah proses yang menggunakan jisim biologi yang murah untuk mengasingkan logam berat bertoksik dan berkeupayaan untuk menyingkirkan bendasing dari kotoran industri. Rumpai, bakteria dan kulat, dan yis telah terbukti sebagai bahan penjerap berpotensi. Penjerapan biologi bagi ion kuprum daripada air kumbahan simulasi menggunakan jisim biologi yis, iaitu *Saccharomyces cerevisiae* telah diselidik. Kajian dijalankan dengan parameter nilai pH dan kepekatan jisim biologi bagi menguji kadar penjerapan logam berat menggunakan yis tersebut. Jumlah pengumpulan kuprum bergantung kepada nilai pH dan kepekatan biojisim. Nilai pH bagi pengumpulan kuprum yang maksimum dikaji dalam julat 3.0 hingga 8.0, manakala kesan kepekatan biojisim pula dalam julat 5.0 hingga 20.0 mg/ml jisim biologi. Keputusannya, kapasiti penjerapan bio meningkat dengan meningkatnya nilai pH dan bacaan pada pH 8 memberikan kadar penjerapan logam kuprum yang tertinggi iaitu hampir 70%. Untuk parameter kepekatan jisim biologi pula menunjukkan bahawa bacaan 10mg/ml menghasilkan penyingkiran ion logam kuprum yang optimum dangan peratus penyingkiran sebanyak 60%.

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LIST OF SYMBOLS

AAS	-	Atomic Absorption Spectrometer
Cu	-	Copper
C_o, C	-	Initial & Equilibrium Concentration
h	-	hour
K	-	Kelvin
L	-	Liter
mg	-	milligram
mL	-	milliliter
ppm	-	part per million
rev/min	-	revolution per minutes
^oC	-	Degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Background

Heavy metal pollution has become one of the most serious environmental problems today. Heavy metals (e.g. lead, copper, cadmium, zinc, chromium, etc.) are toxic even at low concentrations. The pollution is spreading throughout the world along with industrial progress. Copper(II) is known to be one of the heavy metals most toxic to living organisms and it is one of the more widespread heavy metal contaminants of the environment.

The commonly used procedures for removing metal ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction. The disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal has made it imperative for a cost-effective treatment method that is capable of removing heavy metals from aqueous effluents.

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (Volesky, 1986). But biomaterials such as bacteria, fungi, yeast and algae, is regarded as a cost-effective biotechnology only for the treatment of high volume and low concentration complex wastewaters containing heavy metal(s) in the order of 1 to 100 mg/L.

Among the promising biosorbents for heavy metal removal which have been researched during the past decades, *Saccharomyces cerevisiae* has received increasing attention due to the unique nature in spite of its mediocre capacity for metal uptake compared with other fungi. *S. cerevisiae* is widely used in food and beverage production, is easily cultivated using cheap media, is also a by-product in large quantity as a waste of the fermentation industry, and is easily manipulated at molecular level. The state of the art in the field of biosorption of heavy metals by *S. cerevisiae* worldwide, based on a substantial number of relevant references published recently on the background of biosorption achievements and development.

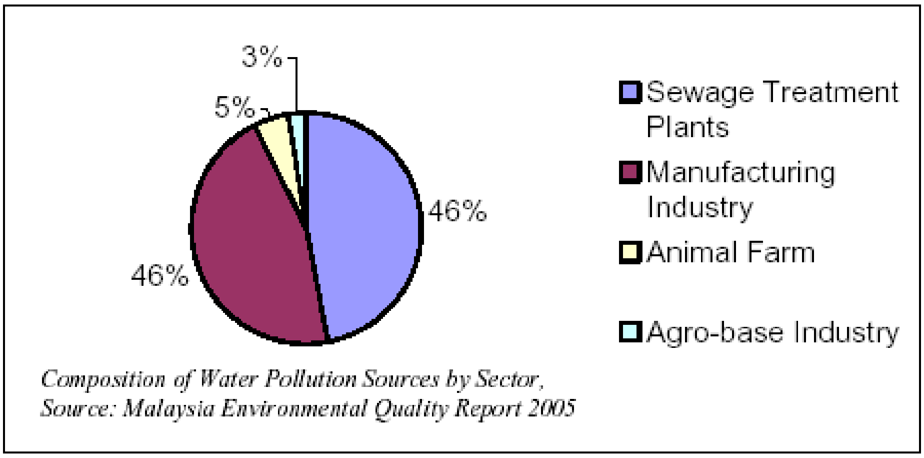
1.2 Problem statement

Environmental contamination by toxic metals is a serious problem due to their incremental accumulation in the food chain and continued persistence in the ecosystem. Heavy metal is discharged in small quantities into environment through numerous industrial activities. Heavy metals such as chromium, copper, lead, nickel in wastewater are hazardous to environment and health. The presence of copper (II) ions in water may cause toxic and harmful effects to the living organisms present and as well as to consumers.

With the rapid development of various industries (including mining and smelting of metalliferous, energy and fuel production, fertilizer and pesticide industry, metallurgy, iron and steel, electroplating, electrolysis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation), wastes containing metals are directly or indirectly discharged into the environment increasingly, especially in developing countries, having brought serious environmental pollution, and threatened biolife (Bishop, 2002; Volesky, 1990a;Wang, 2002a).

Several industries, for example, dyeing, paper, petroleum, copper/brass-plating and copper–ammonium rayon, release undesired amounts of Cu (II). Discharge of heavy metals from metal processing industries is known to have adverse effects on the environment. Conventional treatment technologies for removal of heavy metals from aqueous solution are not economical and generate huge quantity of toxic chemical sludge.

Biosorption of heavy metals by metabolically inactive non-living biomass of microbial or plant origin is an innovative and alternative technology for removal of these pollutants from aqueous solution. Due to unique chemical composition biomass sequesters metal ions by forming metal complexes from solution and obviates the necessity to maintain special growth-supporting conditions. Biosorption processes are particularly suitable for the treatment of wastewater streams containing dilute heavy metal ion concentrations or when very low concentrations of heavy metals are required.



Composition of Water Pollution Sources by Sector,
Source: Malaysia Environmental Quality Report 2005

Figure 1.1 Major Sector’s Composition of Water Pollution Sources

1.3 Objective

The main purpose of this project is to study the rate of biosorption on copper removal by *Saccharomyces cerevisiae* from simulated waste water

1.4 Scopes of research

The scopes of the project are as follows:-

a) Study on pH effect of metal solution on copper removal by *Saccharomyces cerevisiae* from simulated waste water

b) Study on effect of biosorbent concentration on copper removal by *Saccharomyces cerevisiae* from simulated waste water

CHAPTER 2

LITERATURE REVIEW

2.1 Biosorption

Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions. Biomass exhibits this property, acting just as a chemical substance, 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). The major advantages of biosorption over conventional treatment methods include:

- Low cost
- High efficiency
- Minimisation of chemical and/or biological sludge
- No additional nutrient requirement
- Regeneration of biosorbent
- Possibility of metal recovery

These advantages are the primary incentives for developing full-scale biosorption processes to clean up heavy-metal pollution. 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 sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases.

Biosorption is a rapid phenomenon of passive metal uptake sequestration by non-growing biomass (Beveridge and Doyle, 1989). Results are convincing and binding capacity of certain biomass is comparable with the commercial synthetic cation exchange resins (Wase and Foster, 1997). Volesky and Holan (1995) have reviewed the exhaustive list of microbes and their metal binding capacities. Further, sorption capacity is evaluated by sorption isotherms described by Langmuir and Freundlich models. The uptake of metal by two biosorbents must be compared at the same equilibrium concentration. The adsorption is easy to understand when it refers to a single metal situation; however in a multi-ion situation, which is generally encountered in effluent, the assessment of sorption becomes complicated. Most of the work exists with single metal solution and realistic approach would be inferring results in mixed metal solution at extreme pH and variable metal concentration. Biosorption efficiency depends upon many factors, including the capacity, affinity and specificity of the biosorbents and their physical and chemical conditions effluents. Biosortpion can be carried out as a batch process, a continuous process, or a two-stage process with continuous metal recovery. Biomass should be defrosted and washed with deionized water. To ensure equal quality of the biomass during all experiments, different kinds of biomass should be mixed together to obtain a uniform mixture.

Till now, research in the area of biosorption suggests it an ideal alternative for decontamination of metal containing effluents. Advantages and disadvantages of biosorption by non-living biomass are as follows (Modak and Natarajan, 1995).

2.1.1 Advantages

- Growth-independent, non-living biomass is not subject to toxicity limitation of cells. No requirement of costly nutrient required for the growth of cell in feed solutions. Therefore, the problems of disposal of surplus nutrients or metabolic products are not present.
- Biomass can 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 anion 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 condition 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.

2.1.2 Disadvantages

- Early saturation can be problem i.e. when metal interactive sites are occupied, metal desorption is necessary prior to further use, irrespective of 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. These will particularly true if waste biomass from 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.

Biosorption does offer a competitive wastewater treatment alternative, the basis of which needs to be well understood in order to prevent application failures.

2.1.3 Sources of Biomass for Biosorption

Some biosorbents can bind and collect a wide range of heavy metals with no specific priority, whereas others are specific for certain types of metals. When choosing the biomass for metal biosorption experiments, its origin is a major factor to be considered. Biomass can come from:

- Industrial wastes which should be obtained free of charge
- Organisms that can be obtained easily in large amounts in nature (e.g., bacteria, yeast, algae)
- Fast-growing organisms that are specifically cultivated or propagated for biosorption purposes (crab shells, seaweeds).

Some examples of sources of biomass include:

- Seaweeds
- Microorganisms (bacteria, fungi, yeast, molds)
- Activated sludge
- Fermentation waste

- Other specially propagated biomasses

Biosorbents must be hard enough to withstand the application pressures, porous and/or transparent to metal ion sorbate species, and have high and fast sorption uptake even after repeated regeneration cycles. The need to transport raw biomass may also present some logistical problems. Microbial biomass has a high water content and is prone to decay, so drying may be required if it cannot be processed and/or granulated directly on location in the wet state. There is a wide variety of microorganisms (Table 2.1), including bacteria, fungi, yeast, and algae that can interact with metals and radionuclide and transform them through several mechanisms.

Table 2.1 Examples of toxic heavy metals accumulating microorganisms

Organism	Element
Citrobacter sp.	Lead, Cadmium
Thiobacillus ferrooxidans	Silver
Bacillus cereus	Cadmium
Bacillus subtilis	Chromium
Pseudomonas aeruginosa	Uranium
Micrococcus luteus	Strontium
Rhisopus arrhizus	Mercury
Aspergillus niger	Thorium
Saccharomyces cerevisiae	**Uranium**

Often, the source of the biosorbent has a major impact on the feasibility of the operation. The spent biosorbents can be regenerated at very low cost using water, so the material can be reused many times. Hence, considering the overall unit operations involved in biosorption, we can conclude that the process is generally economically viable.

2.1.4 Biosorption mechanisms

There are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms may be classified according to the dependence on the cell's metabolism and according to the location where the metal removed from solution is found. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into; *1)* Metabolism dependent and *2)* Non -metabolism dependent.

Dependent on the cell's metabolism is when transport of the metal across the cell membrane yields intracellular accumulation. 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. The mechanism of biosorption is complex, mainly ion exchange, chelation, adsorption by physical forces, entrapment in inters and intrafibrilliar capillaries and spaces of the structural polysaccharide network as a result of the concentration gradient and diffusion through cell walls and membranes (Kuyucak and Volesky, 1988). Various metal-binding mechanisms have been postulated to be active in biosorption, such as:

- Chemisorptions by ion exchange, complexation, coordination and/or chelation.
- Physical Adsorption
- Microprecipitation
- Oxidation/Reduction.

Due to the complexity of the biomaterials used, it is possible that at least some of these mechanisms are acting simultaneously to varying degrees, depending on the biosorbent and the solution environment.

Ion exchange is a reversible chemical reaction wherein an ion in a solution is exchanged for a similarly charged ion attached to an immobile solid particle. Ion