Addis Ababa University

School of Graduate Study

Department of Chemical Engineering

BIOSORPTION OF SELECTED HEAVY METALS BY BREWERY DERIVED YEAST BIOMASS

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in Partial fulfillment of the requirements of the Degree of Masters of Science in Chemical Engineering (Environmental Engineering Stream).

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Signed Declaration

I declare that the thesis for the M.Sc. degree at the University of Addis Ababa, hereby submitted by me, is my original work and has not previously been submitted for a degree at this or any other university, and that all reference materials contained therein have been duly acknowledged.

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Abstract

Waste brewery yeast has been used for biosorption of lead and nickel. The dried, ground and protonated yeast has been contacted with Lead (II) and Nickel (II) solutions so as to investigate impact of PH, Contact time, presence of co-ion, and study equilibrium and kinetics of the sorption process. PH has been found to affect lead sorption considerably in the test range of 3 to 6 where the optimum sorption capacity was found to be PH of 3 to 4. Nickel sorption remained almost unaffected in the PH range 3-7. The higher proportion of the heavy metal ion has been sorbed during 5 to 10 minutes of contacting and equilibrium has been reached within 60 minutes where the optimum sorption time is 30min.

Langmuir and Freundlich adsorption models have been used in the equilibrium study in order to fit the equilibrium data procured after 24 hours of contacting. As to the Langmuir isotherm model, maximum adsorption capacity q_{max} and affinity,b were found to be 312.5 mg/g and 0.237 for lead and 526.3 mg/g and 0.005 for nickel respectively. For Freundlich model K_f and n have been determined to be 0.946 and 0.971 for lead and 0.239 and 1.133 for nickel respectively.

Concerning the kinetics of the metal uptake process, rate of metal uptake has been determined for varying metal dose in the range of 10-200mg/l and yeast dose in the range of 0.5 – 4 g/l. the curve fitted better to freundlich model than Langmuir model. The equilibrium uptake capacity ($q_{e)}$ rate constant(k) and initial rate of uptake(h) were calculated for metal doses ranging from 10- 200 mg/l and yeast biomass dose of 0.5-4 g/l. q_e shows an increasing trend for increasing metals dose and is inversely related to yeast dose, similarly, K and h also show increasing trend for metal dose.

As to the co-ion tests conducted sorption of lead decreased from 576mg/g to 444.5mg/g when nickel concentration was increased from 50 mg/l to 700 mg/l. But to the contrary increasing dose of lead did not affect the sorption of nickel. Based on recovery tests, he recoverability of lead (80%) was significantly higher than that of nickel (38%).

Chapter One

1.1 Background

Toxic heavy metals are released into the environment from a number of industries such as mining, plating, dyeing, automobile manufacturing and metal processing. The presence of heavy metals in the environment has led to a number of environmental problems. In order to meet the water quality standards, the concentration of heavy metals in wastewater must be controlled. Moreover, metal as a kind of resource is becoming rare and rare.

The toxic characteristics of heavy meals are displayed as follows: (1) the toxicity can last for a long time in nature; (2) some heavy metals even could be transformed from relevant low toxic species into more toxic forms in a certain environment, mercury is such a case; (3) the bioaccumulation and bioaugmentation of heavy metal by food chain could damage normal physiological activity and endanger human life finally; (4) metals can only be transformed and changed in valence and species, but cannot be degraded by any methods including biotreatment; (5) the toxicity of heavy metals occurs even in low concentration of about 1.0–10 mg/l. Some strong toxic metal ions, such as Hg and Cd, are very toxic even in lower concentration of 0.001–0.1 mg/l (Volesky, 1990a; Wang, 2002a).

Due to their increasing application and the above immutable nature, the heavy metal pollution has naturally become one of the most serious environmental problems today. Conventional methods for removing metal ions from aqueous solution have been studied in detail, such as chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon etc. However, chemical precipitation and electro chemical treatment are ineffective, especially when metal ion concentration in aqueous solution is as low as 1 to 100 mg/l, they also produce large amount of sludge to be treated with great difficulties. Ion exchange, membrane technologies and activated carbon adsorption process are extremely expensive, especially when treating a large amount of water and wastewater containing heavy metal in low concentration, so they cannot be used at large scale. Alternative process is biosorption,

which utilizes various natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. These biosorbents possess metal sequestering properties and can decrease the concentration of heavy metal ions in solution from ppt to ppb level. They can effectively sequester dissolved metal ions out of dilute complex solutions with high efficiency and quickly.

Therefore biosorption is an ideal candidate for the treatment of high volume and low concentration complex wastewaters (Veglio and Beolchini, 1997; Volesky, 1990a). Heavy metal removal by biosorption has been extensively investigated during the last several decades (Volesky, 1990a). Some reviews have been published focusing on different aspects of heavy metal biosorption (Jianlong Wang , Can Chen, 2006). From these reviews, we can see that the research on biosorption is focused on the following three major fields. First, the biosorbents. It is necessary to continue to search for and select the most promising types of biomass from an extremely large pool of readily available and inexpensive biomaterials (Kratochvil and Volesky, 1998). Second, the mechanism of biosorption. The mechanism involved in the metal biosorption is only understood to a very limited extent to date. It is necessary to identify the mechanism of metal uptake by biosorbents and understand microbe–metal interactions. Third, large scale experiment. The biosorption process is basically at the stage of laboratory-scale study. It is in great difficulties and almost a failure in attempt of applying biosorption process into practice (Tsezos, 2001). Great efforts should be taken to improve biosorption process, including immobilization of biomaterials, improvement of regeneration and re-use, optimization of biosorption process. Some potential biomaterials with high metal-binding capacity have been identified in part. Among those biosorbents, there are marine algae (e.g. Sargassum natans), bacteria (e.g. Bacillus subtillis), fungi (e.g. Rhizopus arrhizus), yeast (e.g. S. cerevisae) and waste microbial biomass from fermentation and food industry. For the economical reason, researchers have paid much attention to various by-products from fermentation industry, because they are produced in large quantities. The application of these waste microbes as biosorbents for the biosorption of heavy metals and radionuclide is to kill two birds with one stone. For it uses waste to dispose waste. The enterprises can sell their waste biomass and earn some money, at the same

time, they can save the cost associated with disposing the waste biomass they produced (Kapoor and Viraraghavan, 1995). S. cerevisiae is widely used in the food and beverage industry, it is also a kind of solid waste. Although S. cerevisiae is a mediocre biosorbent, it is still a concerned biomaterial in biosorption study because its unique characteristics in comparison with other microorganisms for metal removal. 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.

Characteristics of S. cerevisiae in heavy metal biosorption are extensively researched. The yeast can be studied in various forms for different purposes. Metal-binding capacity for various heavy metals by S. cerevisiae under different conditions is another aspect of the problem. Lead and uranium, for instances, could be removed from dilute solutions more effectively in comparison with other metals. The yeast biosorption largely depends on parameters such as pH, the ratio of the initial metal ion and initial biomass concentration, culture conditions, presence of various ligands and competitive metal ions in solution and to a limited extent on temperature. An assessment of the isotherm equilibrium model, as well as kinetics has also been conducted by many researchers but a lot of work remains to come up with a model that best represent the actual process. The mechanisms of biosorption are understood only to a limited extent. Elucidation of the mechanism of metal uptake is a real challenge in the field of biosorption. (Jianlong Wang , Can Chen, 2006)

The binding mechanisms of heavy metals by biosorption could be explained by the physical and chemical interactions between cell wall ligands and adsorbates by ion exchange, complexation, coordination and microprecipitation. The diffusion of the metal from the bulk solution to active sites of biosorbents occurs predominantly by passive transport mechanisms (Veglio and Beolchini, 1997) and various functional groups such as carboxyl, hydroxyl, amino and phosphate existing on the cell wall of biosorbents can bind the heavy metals (Jianlong Wang , Can Chen, 2006). Living or dead biomass can be used to remove metals, but maintaining a living biomass during metal biosorptio is

difficult because it requires a continuous supply of nutrients and toxicity of metal for microorganism might take place.

On the other hand, the use of dead biomass can avoid these problems and the used cells can be easily regenerated. A variety of biomaterials such as bacteria, yeast, algae and fungi have been successfully used as biosorbents for the removal of heavy metals (Kapoor and Viraraghavan, 1995; Volesky, 1994). Volesky et al. [1999] defined a low cost sorbent as one that is abundant in nature, or is a by-product or waste material from industries such as breweries and dairy products. The yeast has been studied by many investigators as a biosorbent since it can be obtained without additional cost or easily cultivated in substantial amounts using simple fermentation techniques and inexpensive growth media (Jianlon, 2002; Marques et al., 1999).

This particular work is intended to investigate the sorption characteristics of brewery derived yeast biomass (S. cerevisiae) for the removal of selected heavy metals (lead and nickel) constituting effluents coming out of electroplating, metal processing and battery industries. The kinetics of the sorption process and the effect of various parameters on the sorption capacity of the yeast have been examined. And the optimum working points of the major parameters for batch operation has been determined for a standard solution. In addition, the process for recovery (elution) of the heavy metals from the yeast biomass has also been investigated to some extent.

1.2 Statement of the Problem

The presence of heavy metals in the environment has led to a number of environmental problems. Moreover, metal as a kind of resource is becoming rare and rare. Conventional physico-chemical treatment methods become generally ineffective or expensive when metals are dissolved in huge volumes at relatively low concentration. Therefore, there is a need for the development of a low cost process to remove heavy metals economically.

In this paper, the biosorption of selected heavy metals (lead and nickel) using yeast biomass (S. cerevisae) from brewery plant was investigated.

1.3 General Objective

This work had the objective of investigating the sorption and recovery of selected heavy metals by brewery derived yeast residue (Saccharomyces cerevisiae) by determining the optimum working conditions and process kinetics for batch operation based on simulated wastewater that accounts also for co-ion effect.

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1.4 Specific Objective

The specific objectives of this research work include:

- selecting the types of heavy metals to be considered in the research
- Conducting experiment to understand the effect of each parameter on the biosorption capacity of the yeast and determining the optimum working conditions and studying process kinetics using appropriate adsorption isotherms, for batch process for standard solution accounting the co-ion effect.
- Considering options for recovery of sorbed metal-ions and determining the optimum conditions.

Chapter Two

2. Literature review

2.1 Threat from the Environment

The greatest demand for metal sequestration today comes from the need to immobilize the metals released to the environment (or mobilized) by and partially lost through human technological activities. It has been established that dissolved metals (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, multiplying the danger. Thus, it is necessary to control emissions of heavy metals into the environment.

An example of one method for prioritizing the recovery of ten metals is presented in Table 2.1. This may be simplistic, but it provides a useful direction by ranking metals into three general priority categories:

(1) Environmental Risk (ER)

(2) Reserve Depletion Rate (RDR)

(3) Combination of ER and RDR.

Environmental risk assessment could be based on a number of different factors, which could also be weighted.

Table 2.1: Ranking of Risks Associated with Various Metals (Volskey, 1999)

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

Table 2.2: Industrial sources and adverse health impacts of heavy metals (African Journal of Biotechnology Vol. 6 (25), pp. 2924-2931, 28 December, 2007)

Pollutant	**Major Sources**	**Effect on Human Health**	**Permissible Level (ppm)**
Arsenic	Pesticides, fungicides, metal smelters	Bronchitis, dermatitis	0.02
Cadmium	Welding, electroplating, pesticides, fertilizer Cd Ni batteries, nuclear fission plants	Kidney damage, bronchitis, gastrointestinal disorder, bone marrow cancer	0.06
Lead	Paint pesticide, smoking, automobile emission, mining	Liver, kidney, gastrointestinal damage, mental retardation in children	0.1
Nickel	Galvanizing, paint and powder batteries processing units	Long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation.	1.0
Manganese	Welding, fuel addition and ferromanganese production.	Inhalation or contact causes damage to central nervous system.	0.26
Mercury	Pesticides, battery and paper industry.	Damage to nervous system, protoplasm poisoning	0.01
Zinc	Refineries, brass manufacture, metal plating, plumbing	Zinc fumes have corrosive effect on skin, cause damage to nervous system.	15

2.2 Conventional technologies for the removal of heavy metals

The commonly used procedures for removing metal ions from aqueous streams include chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction (Rich and Cherry, 1987). The process description of each method is presented below.

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

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

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

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

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

Phytoremediation: Phytoremediation is the use of certain plants to clean up soil, sediment, and water contaminated with metals. The disadvantages include that it takes a

long time for removal of metals and the regeneration of the plant for further biosorption is difficult.

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

- Stricter regulations with regard to the metal discharges are being enforced, particularly in industrialized countries.
- Toxicology studies confirm the dangerous impacts of heavy metals.
- Current technologies for the removal of heavy metals from industrial effluents often create secondary problems with metal-bearing sludge.

Alternative process is biosorption, which utilizes various natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. These biosorbents possess metal sequestering properties and can decrease the concentration of heavy metal ions in solution from ppt to ppb level. They can effectively sequester dissolved metal ions out of dilute complex solutions with high efficiency and quickly. Therefore biosorption is an ideal candidate for the treatment of high volume and low concentration complex wastewaters (Veglio and Beolchini, 1997; Volesky, 1990a).

2.3 Biosorption

Biosorption is the binding and concentration of heavy metals from aqueous solutions (even very dilute ones) by certain types of active, living or inactive, dead, microbial biomass (Macaskie et. al., 1992)

2.4 Biosorption Mechanisms

Various metal-binding mechanisms have been postulated to be active in biosorption, such as:

- Chemisorption 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.

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.

According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

1. Metabolism dependent and
2. Non -metabolism dependent.

According to the location where the metal removed from solution is found, biosorption can be classified as

1. Extra cellular accumulation/ precipitation
2. Cell surface sorption/ precipitation and
3. Intracellular accumulation.

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 (Kuyucak and Volesky, 1989).

In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (Ercole, et al. 1994). Further, it may be dependent on the cell's' metabolism if, in the presence of toxic metals, the microorganism produces compounds that favour 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.

Physical adsorption: In this category, physical adsorption takes place with the help of Van der Waals' forces. Kuyucak and Volesky 1989, hypothesized that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomasses of algae, fungi and yeasts takes place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells. Electrostatic interactions have been demonstrated to be responsible for copper biosorption by bacterium *Zoogloea ramigera* and alga *Chiarella vulgaris* (Aksu et al. 1992), for chromium biosorption by fungi *Ganoderma lucidum* and *Aspergillus niger* .

Ion Exchange: Cell walls of microorganisms contain polysaccharides and bivalent metal ions exchange with the counter ions of the polysaccharides. For example, the alginates of marine algae occur as salts of K^+, Na^+, Ca^{2+}, and Mg^{2+}. These ions can exchange with counter ions such as CO^{2+}, Cu^{2+}, Cd^{2+} and Zn^{2+} resulting in the biosorptive uptake of heavy metals (Kuyucak and Volesky 1988). The biosorption of copper by fungi

Ganoderma lucidium and *Aspergillus niger* was also up taken by ion exchange mechanism.

Complexation: The metal removal from solution may also take place by complex formation on the cell surface after the interaction between the metal and the active groups. Aksu et al. 1992 hypothesized that biosorption of copper by C. *vulgaris* and Z. *ramigera* takes place through both adsorption and formation of coordination bonds between metals and amino and carboxyl groups of cell wall polysaccharides. Complexation was found to be the only mechanism responsible for calcium, magnesium, cadmium, zinc, copper and mercury accumulation by *Pseudomonas syringae.* Micro-organisms may also produce organic acids (e.g., citric, oxalic, gluonic, fumaric, lactic and malic acids), which may chelate toxic metals resulting in the formation of metallo-organic molecules. These organic acids help in the solubilisation of metal compounds and their leaching from their surfaces. Metals may be biosorbed or complexed by carboxyl groups found in microbial polysaccharides and other polymers.

(a) (b)

Figure 2.1: Complexation as a mechanism of biosorption ((a) Carbonate Complex, (b) Oxalate Complex)

Precipitation: Precipitation may be either dependent on the cellular metabolism or independent of it. In the former case, the metal removal from solution is often associated with active defense system of the microorganisms. They react in the presence of a toxic metal producing compounds, which favour the precipitation process. In the case of precipitation not dependent on the cellular metabolism, it may be a consequence of the chemical interaction between the metal and the cell surface. The various biosorption mechanisms mentioned above can take place simultaneously.

In general, biosorption of toxic metals and radionuclides is based on non-enzymatic processes such as adsorption. Adsorption is due to the non-specific binding of ionic species to polysaccharides and proteins on the cell surface (Figure 2.1) or outside the cell. Bacterial cell walls and envelopes, and the walls of fungi, yeasts and algae, are efficient metal biosorbents that bind charged groups.

Biomass deriving from several industrial fermentations may provide an economical source of biosorptive materials. Many species have cell walls with high concentrations of chitin, a polymer of *N*-acetyl-glucosamine that is an effective biosorbent.

Biosorption uses biomass raw materials that are either abundant (*e.g.,* seaweeds) or wastes from other industrial operations (*e.g.,* fermentation wastes like the case in this research work). The metal-sorbing performance of certain types of biomass can be more or less selective for heavy metals, depending on the type of biomass, the mixture in the solution, the type of biomass preparation, and the chemical-physical environment.

It is important to note that the concentration of a specific metal in solution can be reduced either during the sorption uptake by manipulating the properties of the biosorbent or upon desorption during the regeneration cycle of the biosorbent.

It has been suggested that numerous chemical groups contribute to biosorption metal binding, by either whole organisms such as algae and bacteria or by molecules such as biopolymers. These include hydroxyl, carbonyl, carboxyl, sulfhydryl, thioether, sulfonate, amine, imine, amide, imidazole, phosphonate, and phosphodiester groups. The importance of any given group for biosorption of a certain metal by a certain biomass depends on such factors as the number of sites in the biosorbent material, the accessibility of the sites, the chemical state of the sites (*i.e.,* availability), and the affinity between the site and the metal (*i.e.,* binding strength). For covalent metal binding, even an occupied site is theoretically available; the extent to which the site can be used by a given metal depends on its binding strength and concentration compared to the metal already occupying the site.

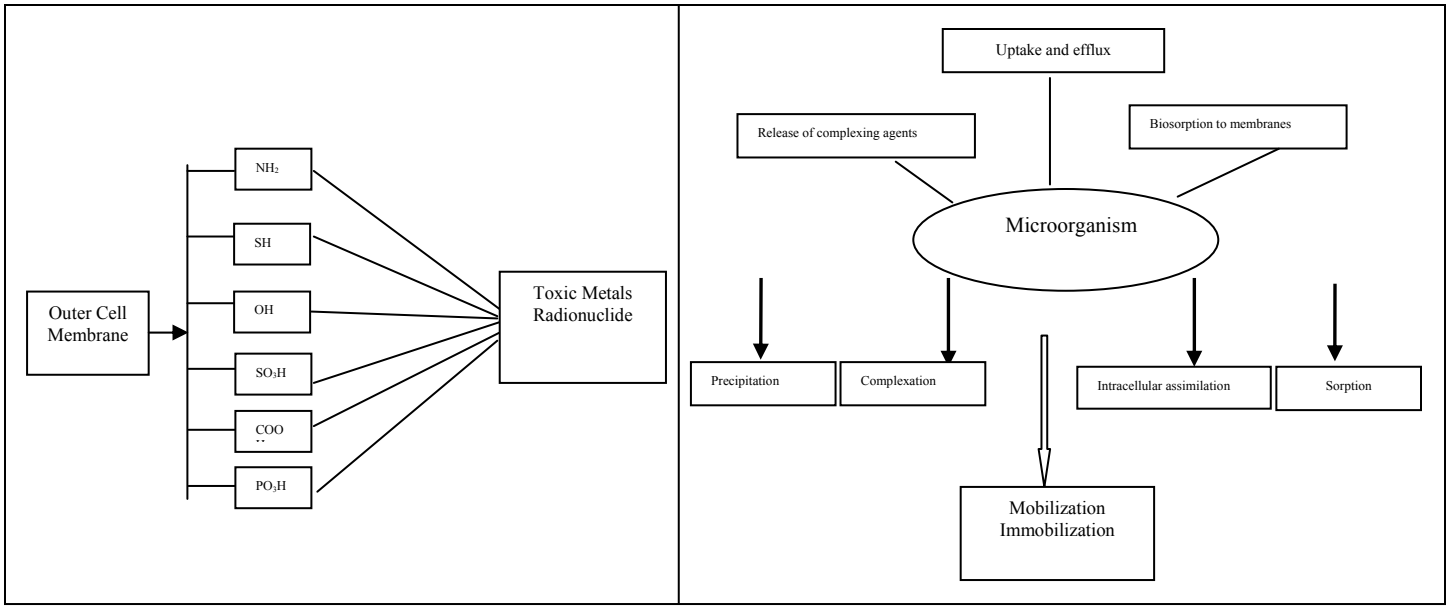

Figure 2.2: Biosorption mechanisms and active components involved in the process (Navneet Joshi, 2003)

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2.5 Biomass Types

The assessment of the metal-binding capacity of some types of biomass has gained momentum since 1985. Indeed, some biomass types are very effective in accumulating heavy metals. Availability is a major factor to be taken into account to select biomass for clean-up purposes. The economics of environmental remediation dictate that the biomass must come from nature, or even be a waste material. Seaweeds, molds, yeasts, bacteria, and crab shells, among other kinds of biomass, have been tested for metal biosorption with very encouraging results.

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 (like the case of brewery yeast which is investigated in this study)
- 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).

2.6 Organisms for Biosorption

There is a wide variety of microorganisms (Table 2.3), including bacteria, fungi, yeast, and algae, which can interact with metals and radionuclides and transform them through several mechanisms.

Table 2.3: 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, cadmium, cupper, lead, Nickel, Zinc etc...

Cost-effectiveness is the main attraction of metal biosorption. This cost-effectiveness can be maintained by using the microbial biomass directly where possible. In addition, biosorbents derived from microbial biomass through a simple process are expected to be the lowest-priced and most-economical for metal removal.

Some types of industrial fermentation waste biomass are excellent metal sorbents. It is necessary to realize that some "waste" biomass is actually a commodity, not a waste. This applies particularly to the ubiquitous brewer's yeast which is investigated in this study.

2.7 Types of Biosorption

Biosortpion can be carried out as a batch process, a continuous process, or a two-stage process with continuous metal recovery.

2.8 Desorption

Regeneration of loaded biosorbent is critical to keeping costs down and to recovering the metal(s) extracted from the liquid phase. The deposited metals are washed out (desorbed) and the biosorbent is regenerated for another cycle. The desorption process should result in:

- high-concentration metal effluent
- undiminished metal uptake upon re-use
- no physico-chemical damage to the biosorbent.

The desorption and sorbent regeneration studies might require somewhat different methodologies, beginning with screening for the most effective regenerating solution.

Because different metal ions have different affinities for the biosorbent, the uptake has some degree of metal selectivity. The selectivity of the elution-desorption operation may be different, which may serve as another means of eventually separating metals from one another if desirable.

The concentration ratio (*CR*) is used to evaluate the overall concentration effectiveness of the whole sorption-desorption process:

$$CR = \frac{Eluate\ Metal\ \ Concentration}{Feed\ Metal\ Concentration} \qquad (2.1)$$

Obviously, the higher the *CR,* the better the overall performance of the sorption process making the eventual recovery of the metal more feasible with higher eluate concentrations.

2.9 Feasibility of Biosorption

For successful application on a large scale, any operation needs to be economically viable. The feasibility of a biosorption process depends on such factors as:

- biosorbent uptake performance
- the source of the raw biomass
- biomass granulation and treatment
- the desorption and regeneration processes used.

Often, the source of the biosorbent has a major impact on the feasibility of the operation. Biosorbents (biomass) should always be obtained from the least-expensive source, such as from the effluent of a fermenter (the case in this research), seaweeds from nearby bodies of water, algae, etc. 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 (Volesky, 1997).

2.10 Advantages of Biosorption

Biosorption is highly competitive with the presently available technologies like ion exchange, electrodialysis, reverse osmosis, etc. Some of the key features of biosorption compared to conventional processes include:

- competitive performance
- heavy metal selectivity
- cost-effectiveness
- regenerative
- no sludge generation.

Biosorption is particularly economical and competitive for environmental applications in detoxifying effluents from, for example:

- metal plating and metal finishing operations
- mining and ore processing operations
- metal processing
- battery and accumulator manufacturing operations
- thermal power generation (coal-fired plants in particular)
- Nuclear power generation.

Many scientific studies are currently underway to provide a deeper understanding of biosorption and to support its effective application. Some pollution seems inevitable, and one might wonder what should be done to minimize it. Human populations need methods and technologies to clean waters and diminish the environmental dangers related to technological progress. Biosorption can be one such solution to clean up heavy metal contamination.

2.11 Biosorption by Saccharomyces Cerevisiae

Conventional methods for removing metal ions from aqueous solution have been studied in detail, such as chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon etc. However, as mentioned above, chemical precipitation and electrochemical treatment are ineffective, especially when metal ion concentration in aqueous solution is as low as 1 to 100 mg/L, they also produce large amount of sludge to be treated with great difficulties. Ion exchange, membrane technologies and activated carbon adsorption process are extremely expensive, especially when treating a large amount of water and wastewater containing heavy metal in low concentration, so they cannot be used at large scale. Alternative process is biosorption, which utilizes various natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. among which the yeast, S. cerevisiae is widely used in the food and beverage industry, it is also a kind of solid waste. Although S. cerevisiae is amediocre biosorbent, it is still a concerned biomaterial in biosorption study because of

its unique characteristics in comparison with other microorganisms for metal removal (J.wang et. al, 2006).

2.12 Advantages of S. cerevisiae as biosorbents in metal biosorption

Firstly, S. cerevisiae is easy to cultivate at large scale. The yeast can be easily grown using unsophisticated fermentation techniques and inexpensive growth media (Kapoor and Viraraghavan, 1995). Moreover, the yield of the biomass is also high. Secondly, the biomass of S. cerevisiae can be obtained from various food and beverage industries. S. cerevisiae as a by-product, is easier to get from fermentation industry, in comparison with other types of waste microbial biomass. Microorganisms used in enzymatic industry and pharmaceutical industry are usually involved in the secret of their products, which makes industries reluctant to supply the waste biomass. The supply of S. cerevisiae as waste residuals is basically stable. Thirdly, S. cerevisiae is generally regarded as safe. Therefore, biosorbents made from S. cerevisiae can be easily accepted by the public when applied practically. Fourthly, but not the last, S. cerevisiae, is an ideal model organism to identify the mechanism of biosorption in metal ion removal, especially to investigate the interactions of metal–microbe at molecular level. Perego1 and Howell (1997) reported that the use of yeasts as model systems is particularly attractive because of the ease of genetic manipulation and the availability of the complete genomic sequence of S. cerevisiae. In fact, S. cerevisiae, as a model system in biology, has been explored fully in molecular biology (Zhou, 2002). At the same time, S. cerevisiae can be easily manipulated genetically and morphologically, which is helpful to genetically modify the yeast more appropriate for various purposes of metal removal.

2.13 Forms of S. cerevisiae in biosorption research

S. cerevisiae in different forms has been studied for different purposes of research. For example, living cell/ dead cell (Kapoor and Viraraghavan, 1995), intact cell/ deactivated cell, immobilized cell/free cell (Veglio and Beolchini, 1997), raw material/pretreated cell by physicochemical process, wild type/mutant cell, flocculent/ non-flocculent cell

(Marques et al., 1999), engineered/ non-engineered cell, lab culture/waste industrial cell, and cells from different industries.

Veglio and Beolchini (1997) pointed out that investigation on the performance of free cells for metal uptake can provide fundamental information on the equilibrium of the biosorption process, which is useful for practical application. Meanwhile, flocculating cell has been suggested for biosorption, attempting to overcome the separation problem of free cells (Soares et al., 2002). Whether to employ living cells or non-living cells for biosorption is still at arguing stage. In the early researches on biosorption of heavy metal ions, living cells were used. However, dead cells have been found to have the same or even higher uptake capacity of metal ions, comparing with living cells. Meanwhile, dead cells can overcome some limits that living cells are used: nutrition demand, sensitivity to extreme pH value or higher metal ion concentration, etc. Therefore, biosorption studies involving dead/pretreated biomass have dominated during 1980s–90s (Malik, 2004).

2.14. Biosorption capacity of S. cerevisiae

2.14.1. Metal ion uptake

A number of references have proved that S. cerevisiae can remove toxic metals, recover precious metals and clean radionuclides from aqueous solutions to various extents. Schott and Gardner (1997) reported the recovery of light metals, such as aluminium by S. cerevisiae. Brady et al. (1994) proved that the yeast cells of S. cerevisiae treated with hot alkali were capable of accumulating a wide range of heavy metal cations (Fe^{3+}, Cu^{2+}, Cr^{3+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Ag^{+}, Ni^{2+}, and Fe^{2+}). Lead, cadmium, copper, zinc, chromium, nickel, silver and uranium, etc. have been studied much more than cobalt, molybdenum, iron, manganese, radium, selenium, lanthanide, precious metals, etc.

2.14.2. Biosorption capacity

The determination of the metal uptake rate by the biosorbent is often based on the equilibrium state of sorption system. The sorption uptake rate, q, is usually expressed in milligrams of metal sorbed per gram of the (dry) sorbent (the basis for engineering

process—mass balance calculations), or mmol/g or meq/g (when stoichiometry and/or mechanism are considered) (Kratochvil and Volesky, 1998). Metal ion uptake by S. cerevisiae has been reported in a substantial number of references. The magnitude of metal uptake by S. cerevisiae can be estimated as follows: for lead, in the order of 2–3, above 10 and less than 300 mg Pb/g dry weight biomass; for copper, in the order of 1–2, less than 20 mg Cu/g dry weight yeast; for zinc, in the order of 1–2, usually less than 30 mg Zn/g dry weight; for cadmium, in the order of 2–3, usually above 10 but less than 100 mg Cd/g dry mass; for mercury, in the order of 2; for chromium and nickel, usually in the order of 1; for seldom, more than 40 mg/g dry mass; for precious metals , such as Ag, Pt, Pd, in the order of 2, around 50 mg/g dry weight yeast. Biosorptive capacity of radionuclide uranium by S. cerevisiae is usually between 150 and 300 mg U/g dry weight biomass.

In particular, there is no standard measurement of dry weight of biomass, i.e. no standard of dry temperature and dry hours when drying biomass. Park et al. (2003) obtained the dry-cell weight by drying cells at 70 °C until the weight of the cells became constant. Liu et al. (2002a) measured the dry weight of S. cerevisiae after drying in a stove for 2h at 100–120 °C to calculate the adsorption capacities. Özer and Özer (2003) dried the yeast at 100 °C for 24h. Rapoport and Muter (1995) determined dry weight by drying the sample at 105 °C until the constant weight was achieved.
In this particular research work, drying temperature of 60^0C and drying time of 24h has been employed and the constancy of the terminal weight of dried brewery yeast biomass has been checked

2.14.3. Selectivity and competitive biosorption by S. cerevisiae

To determine which metal ions have high affinity with S. cerevisiae, it is necessary to compare the biosorptive capacity of different metal ions under the same experimental conditions. Also, it has been found that metal biosorption by S. cerevisia is selective and, in some cases, competitive.. However, only very limited information is available on the

competitive sorption of metal ions with fungal biomass (Kapoor and Viraraghavan, 1997), especially with the yeast of S. cerevisiae. In principle, S. cerevisiae has higher affinity with uranium, lead and mercury than copper, nickel or other metal ions. This particular research is partly intended to assess single metal and multi-metal competitive sorption systems for lead and nickel by brewery derived yeast biomass.

2.14.4. Comparison with other biomaterials

Bakkaloglu et al. (1998) investigated various types of waste biomass including bacteria (S. rimosus), yeast (S. cerevisiae), fungi (P. chrysogenum), activated sludge as well as marine algae (F. vesiculosus and A. nodosum) for biosorption of metals. They compared the removal efficiency for zinc, copper and nickel ions at the stage of the biosorption, sedimentation and desorption. The results showed that S. cerevisiae has a mediocre efficiency for one or multi-metal biosorption systems. By comparing the index q_{max} of Langmiur equation with seven types of waste biomass for the removal of lead ion, Kogej and Pavko (2001) indicated that lead uptake capacity by S. cerevisiae is in the middle, in comparison with the other six biomaterials they used in their study. Vianna et al. (2000) studied the biosorption capability for Cu, Cd and Zn, using three kinds of waste biomass from fermentation industries, that is, Bacillus lentus, Aspergillus oryzae and S. cerevisiae. The results showed that protonated B. lentus had the highest sorption capacity for Cu and Cd, followed by protonated biomass of A. oryzae and S. cerevisiae. S. cerevisiae was higher in comparison with other adsorbents such as aluminum oxide, activated carbon, and activated charcoal. Comparing with other fungi biomaterials, in spite of the mediocre metal biosorption capacity, S. cerevisiae is a unique biomaterial in biosorption research and practical application.

2.15. Influential factors

Biosorptive capacity is influenced by many factors, including the status of S. cerevisiae (cell age), properties of metal ions (radius of ion, valence, etc.) in aqueous solution, cultural conditions (carbon source, nutrition supply, composition of growth media, etc.),

biosorption conditions (such as pH, temperature, contact time, co-ions in solution, initial concentration of metal and biomass, availability of metal ions and micronutrition etc.).

2.15.1. Properties of metal ions in solution

Biosorption of metal ions by R. arrhizus was linearly influenced by the ionic radius, but independent of the ionic charge or electrostatic strength (Kapoor and Viraraghavan, 1997). Pearson classified metallic ions based on a "hardness scale" defined by their binding strength with F− and I− under the consideration of the thermodynamics but not the kinetics . Nierboer and Ruchardson proposed a refined classification of metals for biological systems in determining their relativeness, considering the electronegativity, charge and ionic radius of metals. Hard metals, such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, are usually nontoxic and often are essential macronutrients for microbial growth, they bind preferentially to oxygen-containing (hard) ligands, such as OH^{-}, HPO_4^{2-}, CO_3^{2-}, R–COO^{-}, and -C-O, whereas soft metals, such as Hg^{2+}, Cd^{2+} and Pb^{2+}, which often display greater toxicity, form stable bonds with nitrogen- or sulfur-containing (soft) ligands, such as CN^{-}, R–S^{-}, –SH^{-}, NH^{2-}, and imidazol. Borderline or intermediate metals are less toxic and can even be detected in certain biomolecules where they assist in mediating specific biochemical reactions, e.g., Zn^{2+}, Cu^{2+} and Co^{2+} . Based on the hard-and-soft principle of acids and bases, investigated the metal adsorption characteristics for metabolism-independent uptake of the metal ions, including Sr^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+}, Ti^{2+} by S. cerevisiae. The results showed that the complex characteristics of microbial metal uptake conformed well to the hard-and-soft principle. Biosorption of metals ions such as Sr^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+} and Pb^{2+} by freeze-dried R. arrhizus is observed to be related to covalent index (X^2_m, r), where X_m is electronegativity and r is the ionic radius (Brady and Tobin, 1995). The greater the covalent index value of metal ion is, the greater is the potential to form covalent bonds with biological ligands.

2.16 Factors affecting biosorption capacity

2.16.1 pH

For biosorption of heavy metal ions, PH is one of the most important environmental factors. The PH value of solution strongly influences not only the site dissociation of the biomass' surface, but also the solution chemistry of the heavy metals: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation, the speciation and the biosorption availability of the heavy metals (Esposito et al., 2002; Wang, 2002a: 233–248). The biosorptive capacity of metal cations increases with increasing pH of the sorption system, but not in a linear relationship. On the other hand, too high pH value can cause precipitation of metal complexes, so it should be avoided during experiments. For different biosorption system of metal ions, the optimal pH is different. It is reported that the optimal pH value is 5–9 for copper biosorption by S. cerevisiae, and 4–5 for uranium (Volesky, 1990b: 156). Mapolelo and Torto (2004) proved that the biosorption capacity of Cd^{2+}, Cr^{3+}, Cr^{6+}, Cu^{2+}, Pb^{2+} and Zn^{2+} is dependent on pH. For all metal ions they studied, the optimal pH values are all greater than 5. The optimal pH for Cd and Pb biosorption is 5.8, while for Cr (III) and Pb is 5.2. As the pH further increased, the biosorption capacity subsequently decreased. The reason may be that at low pH, the affinity with the proton at the binding site of yeast is much greater than that of the metal ion (H^+, M^{2+}), compared with that at higher pH, where M^{2+},H^+. Vianna et al. (2000) obtained a similar conclusion. They found that the biosorption capacity of metal cations strongly depends on pH value. For Cu, Cd and Zn, the biosorption capacity at pH 4.5 is far higher than that at pH 2.5 and pH 3.5. Electrostatic attraction to negatively charged functional groups may be one of the specific biosorption mechanisms. At pH 4.5, the most important group is phosphate, and the other two main active molecular groups are carboxyl and sulphate. Marques et al. (2000) studied the PH effects (i.e. initial PH value and the PH shift and control) on the removal of Cu^{2+},Cd^{2+} and Pb^{2+} from unbuffered aqueous solution by non-viable S. cerevisiae (brewery waste biomass). Özer and Özer (2003) found that optimal pH value for Pb(II) and Ni(II) ion uptake is 5.0. At lower PH, cell wall ligands were closely associated with the hydronium ions [H_3O^+] and restricted the approach of metal cations as a result of the repulsive force.

At higher PH, e.g. 5.0, divalent positive ions are suitable to interact with negatively charged groups in biomass. On the other hand, the outer layer of the cell wall of S. cerevisiae consists of a coat protein, which can cause a charge through dissociation of ionisable side groups of the amino acids. The ionic state of ligands such as carboxyl, phosphate, imidazole and amino groups will promote reaction with the positively charged metal ions. However, metal anions, such as chromium (VI), exhibit different PH features from metal cations. Generally speaking, a low pH value is favorable for biosorption.

2.16.2. Temperature

Temperature has also an influence on the biosorption of metal ions, but to a limited extent under a certain range of temperature, which indicates that ion exchange mechanism exists in biosorption to some extent. Biosorption process is usually not operated at high temperature because it will increase the operational cost (Wang, 2002a). Brady and Duncan (1994b) found that temperature (5–40 °C) had minor effect on the accumulation level of Cu^{2+}, Co^{2+} or Cd^{2+} by free cells of S. cerevisiae in suspension. Adsorption reactions are normally exothermic, so biosorption capacity increases with decrease of temperature (Kapoor and Viraraghavan, 1997). In the range of 15– 40 °C, the maximum equilibrium biosorption capacity for Pb(II), Ni(II) and Cr(VI) ions by the inactive S. cerevisiae was reached at temperature of 25 °C. The decrease in capacity at higher temperature between 25 and 40 °C revealed that the processes of biosorption for these metal ions by S. cerevisiae are exothermic. The decrease of biosorption capacity at higher temperature may be due to the damage of active binding sites in the biomass (Özer and Özer, 2003).

2.16.3. Contact time

The biosorption process of heavy metal by S. cerevisiae usually completes rapidly. The biosorption of metals such as copper, zinc, lead and uranium by non growing cells of S. cerevisiae is a rapid process and often reaches equilibrium within several hours (Kapoor and Viraraghavan, 1997). Generally speaking, the biosorption capacity and the removal efficiency of metal ions by S. cerevisiae became higher with prolonging the contact time. However, in practice, it is necessary to optimize the contact time, considering the

efficiency of desorption and regeneration of the biomass. Ferraz et al. (2004) optimised the sorption time for Cr (III) by S. cerevisiae from a brewery company in the sorption–desorption process, the result showed that a 30 min sorption period was the best option to ensure the metal removal from solution and good recovery from biosorbent.

2.16.4. Competing ions/co-ions

Real industrial effluent usually contains various ionic components, including metal cations and anions. Some studies indicated that cations and anions additional to the ions of interest have a generally detrimental impact on metal accumulation (Suh and Kim, 2000). Usually, the biosorption capacity of one metal ion is interfered and reduced by co-ions, including other metal ions and anions presenting in solution, however the gross uptake capacity of all metals in solutions remains almost unchangeable. Competitive effect occurred in a mixed solution containing lead and chromium during the biosorption process by flocculating brewer's yeast (Ferraz and Teixeira, 1999). The yeast of S. cerevisiae seems to have more affinity, higher selectivity and biosorption capacity to Pb(II) than to Cr(III) in aqueous solutions. The decrease of metal uptake in competitive conditions was thought to be a response to increased competition between same charged species for binding sites of the yeast cells.

2.16.5. Pretreatment

Yeast cells killed by extreme chemical and physical conditions may also show very different properties for metal accumulation, compared with the original yeast (Lu and Wilkins, 1996). Now various pretreatment methods are reported to deal with the yeast cells of S. cerevisiae. Physical methods include vacuum and freeze-drying, boiling or heat, autoclaving, and mechanical disruption. Chemical methods include treatment with various organic and inorganic reagents, such as acid and caustic, methanol, formaldehyde, etc. Those methods are found to improve metal biosorption to some extent. Because of the important role of cell wall in the metal biosorption by non-viable cells,metal biosorption may be enhanced by heat or chemical sterilization or by crushing. Thus degraded cells would offer a larger available surface area and expose the

intracellular components and more surface binding sites due to the destruction of the cell membranes.

2.17. Biosorption equilibrium isotherm models and kinetics models

The assessment of a solid–liquid sorption system is usually based on two types of investigations: equilibrium batch sorption tests and dynamic continuous-flow sorption studies (Volesky and Holan, 1995). Volesky, in his book entitled "sorption and biosorption" (www. Biosorption.mcgill.ca), also offered a detailed introduction to biosorption equilibrium and kinetics, e.g. single sorbate isotherms, multi-sorbate sorption equilibrium (multi-component Langmiur models considering electrostatic binding, the effect of pH, surface complex model, Donnan model considering ionic strength, Wilson model for ion exchange etc.), biosorption batch dynamics (mass transfer model for biosorption rate), dynamic continuous flow reactor/contactor systems modelling of column performance including equilibrium column model, mass transfer model and derivation of mass transfer model to evaluate column sorption performance.

2.17.1. Equilibrium isotherm models

Equilibrium isotherm models are usually classified into the empirical equations and mechanistic models, based on the mechanism of metal ion biosorption. Mechanistic models can be used not only to represent, but also to explain and predict the experimental behavior. The empirical models for single solute systems used to describe the biosorption equilibrium are Langmuir, Freundlich and Brunauer–Emmett–Teller (BET) models. Langmuir model (L type, based on monolayer adsorption of solute) and Freundlich model (F type, developed for heterogeneous surfaces) are the most widely accepted and used in a number of references.

Researches on co-ions biosorption models are only at their preliminary stage. How to utilize mathematical model with multi-parameters to describe competitive biosorption is a future direction of biosorption research (Wang et al., 2000).

2.18. Kinetics of biosorption

Kinetics studies and, offering information on the rate of metal uptake, together with the hydrodynamic parameters, are very important for biosorption process design (Volesky and Holan, 1995). However, biosorption kinetics studies are insufficient according to the references published so far (Wang, 2002a).

2.19. Process of metal uptake

Generally speaking, the metal biosorption process by living cells is regarded as a two-step process. First, metal ions are adsorbed to the surface of cells by interactions between metal–functional groups displayed on the surface of cells, such as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide, thilo functional group, etc. The first step, also called passive biosorption, is metabolism independent and proceeds rapidly within several minutes by any one or a combination of the following metal binding mechanisms: coordination, complexation, ion exchange, physical adsorption (e.g. electrostatic) or inorganic microprecipitation. Passive biosorption is a dynamic equilibrium of reversible adsorption–desorption. Metal ions bound on the surface can be eluted by other ions, chelating agent or acid. The second step, metal ions penetrate the cell membrane and enter into the cells, also called active biosorption. Metal uptake by non-living cells is mainly in the passive mode (Volesky, 1990a,b, 1997; Veglio and Beolchini, 1997; Wang et al., 2000). By investigating the biosorption of Cr(VI) and Fe(III) on Streptococcus equisimilis, S. cerevisiae and Aspergillus niger, Goyal et al. (2003) confirmed that the metal uptake by microorganisms occurred in two stages: passive uptake which takes place immediately, and active uptake which takes place slowly. The first stage is thought to be physical adsorption or ion exchange at the cell surface, reaching the adsorption equilibrium within 30–40 min at the end of rapid physical adsorption. Ferraz et al. (2004) observed the similar phenomenon in Cr(III) uptake by S. cerevisiae over a period of 24h. Suh et al. (1998a,b) investigated the process of Pb^{2+} accumulation in the cells of S. cerevisiae by TEM (Transmission Electron Microscope) technology. The results showed that the biosorption process for Pb^{2+} was dependent upon the initial concentration of the metal ion and biomass in unbuffered aqueous solution. The time to reach an equilibrium

state was significantly shortened from 96 to 24 h as the cell dry weight increased from 0.56 to 5.18 g/l. With a cell concentration of 1g/l and initial Pb^{2+} concentration of 100 mg/l, the first step, metabolism-independent one, was a rapid process, in which Pb^{2+} binds to the cell wall within 3–5min. In the second step, Pb^{2+} penetrated through the cell membrane and entered into the cytoplasm, but this step cannot be clearly labelled as metabolism-dependent or independent.

2.20 Biosorption mechanism by the cell of S. cerevisiae

The mechanism of metal biosorption is complicated and not fully understood. The status of biomass (living or non-living), types of biomaterials, properties of metal-solution chemistry, ambient/environmental conditions such as pH, will all influence the mechanism of metal biosorption. In the last few years, some reviews have been published focusing on different aspects of biosorption mechanism, such as physical–chemical mechanism, metal detoxification, transfer mechanism and molecular biology development (Jianlong Wang , Can Chen, 2006). Two types of metal sequestering are passive mode by dead or inactive cells of S. cerevisiae and active mode by living cells. Passive mode is independent of energy, mainly through chemical functional groups of the material, comprising the cell and particularly cell wall. Active mode is metabolism-dependent and related to the metal transport and deposition. Of course, passive metal uptake may occur when the cell is metabolically active (Volesky, 1990b).

2.20.1 Extracellular accumulation/precipitation

Some prokaryotic (bacteria, Archaea) and eukaryotic (algae, fungi) microorganisms can produce or excrete exracellular polymeric substances (EPS), such as polysaccharides, glucoprotein, lipopolysaccharide, soluble peptide etc. These substances possess a substantial quantity of anion functional groups which can adsorb metal ions. References published on metal biosorption with EPS mainly focus on the bacterial organism, such as Bacillus megaterium, Acinetobacter, Pseudomonas aeruginosa, sulphate-reducing bacteria (SRB), Cyanobateria or activated sludge (Liu et al., 2001), whereas EPS study for fungi and algae is limited (Flemming and Wingender, 2001; Wang and Yang, 1996;

Pirog, 1997). The effect of EPS on Pb^{2+} removal by a polymorphic fungus Aureobasidium pullulans has been studied (Jianlong Wang , Can Chen, 2006). The results showed that Pb^{2+} only accumulated on the surface of the intact cells of A. pullulans due to the existence of EPS, whereas Pb^{2+} penetrated into the inner cellular parts of the EPS-extracted cells of A. pullulans. The longer the storage of cells, the higher the uptake capacity of Pb^{2+} by intact cells due to the increase in the amount of excreted EPS. More than 90% of the Pb^{2+} removal was due to excreted EPS, based on maximal Pb^{2+} accumulation amount. The biosorptive capacity of Pb^{2+} by the EPS-extracted cells was much less than that of the intact cells and remained constant, irrespective of the storage time. The initial rate of Pb^{2+} uptake by live cells of S. cerevisiae is lower than that of dead cells, while in the case of A. pullulans, both the capacity and the initial rate of Pb^{2+} accumulation in the live cells are higher than those in the dead cells, due to the presence of EPS for live A. pullulans. The roles of EPS on metal removal in a biosorption system are usually neglected or ignored, especially in the case of fungi and yeast. Among the limited studies on metal removal by EPS, most of them are related to the EPS extracted from intact organism cells, but not the EPS in living cells. Although conspicuous extracellular layers are mainly associated with bacterial cells, whether the yeast of S. cerevisiae excretes EPS is unclear. The strain of S. cerevisiae used in their experiment did not excrete EPS. However, flocculent strain of S. cerevisiae has been suggested to be used in metal biosorption due to higher uptake capacity than that of non-flocculent strain and other unique traits (Soares et al., 2002). The mechanism of flocculation is believed to be related to a specific protein on the surface of cell wall, i.e. lectin. Proteins on the surface of the yeast cells could be extracted by EDTA. With respect to that feature, lectin seems to be considered as a kind of EPS. Flocculation of the yeast may vary significantly, even disappear under certain conditions.

2.20.2. Cell surface sorption/precipitation

The cell wall tends to be the first cellular structure to come in contact with metal ions, excluding a possible existing extracelluar layer mainly related to bacterial cells. Two basic mechanisms of metal uptake by cell wall are as follows: stoichiometric interaction

between functional groups of cell wall composition, including phosphate, carboxyl, and amine as well as phosphodiester; and physicochemical inorganic deposition via adsorption or inorganic precipitation. Nowadays, complexation, ion exchange, adsorption (by electrostatic interaction or van der Waals force), inorganic microprecipitation, oxidation and/or reduction have been proposed to explain metal uptake by organism (Volesky, 1990a,b; Liu et al., 2002b).

The role of separate cell part in the removal of metals was investigated in the 1990s. The blocking of amino, carboxyl, or hydroxyl groups of isolated cell walls from the yeast S. cerevisiae reduced the uptake capacity of Cu^{2+}, indicating that these groups play a role in the binding of Cu^{2+}, and implying that both the protein and the carbohydrate fractions of the cell walls are involved in the binding of heavy metal cations (Jianlong Wang , Can Chen, 2006). Wang (2002b) also confirmed that the carboxyl and amino group in the cell wall played an important role in Cu^{2+} removal by the waste yeast cells of S. cerevisiae modified by methanol and formaldehyde. Brady et al. (1994g) obtained partially purified products (glucan, mannan, and chitin, respectively) from the isolated cell walls of the yeast S. cerevisiae by chemical enzymatic methods. It has also been confirmed that the sulphydryl groups of these amino acid residuals are major metal-binding components of the protein. Rapid release of 70% of cellular K^{+}, followed by a slower release of approximately 60% of cellular Mg^{2+}, but little loss of Ca^{2+}, was observed in Cu^{2+} accumulation by S. cerevisiae (Brady and Duncan, 1994c), indicating the existence of an ion exchange mechanism. Biosorption of monovalent ions, such as Na^{+} and K^{+} by deactivated protonated yeast of S. cerevisiae was accompanied by H^{+} release, whereas biosorption of divalent ions, such as Ca^{2+} and Mg^{2+} was sorbed not only by proton displacement, but also by additional mode, which was not accompanied by the release of H^{+}. The total maximal biosorptive capacity of divalent ions was higher than that of the monovalent ions. Is ion exchange the main mechanism in metal biosorption removal ? (Jianlong Wang , Can Chen, 2006). Brady and Tobin (1995) found that the total ions displaced ($H^{++}Mg^{2++}Ca^{2+}$) accounted for only a small portion of the metal ions taken up in the biosorption of metal ions (Sr^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}) by freeze-dried R. Arrhizus. This indicates that ion exchange is neither the sole nor the main mechanism for

metal biosorption by fungi. However, Davis et al. (2003) believed the ion exchange was the main mechanism for metal ion uptake by brown algae. In particular, they thought the term ion exchange was rather an umbrella term to describe the experimental observations, probably the precise binding mechanism range from physical binding (i.e. electrostatic or London–van der Waals forces) to chemical binding (i.e. ionic and covalent). Therefore, the definition of ion exchange should be clear before discussing the importance of its role in biosorption. Veglio and Beolchini (1997) observed that complexation was involved in metal removal. Davis et al. (2003) defined complexation or coordination as combination of cations (often called as central atom) with molecules or anions containing free electron pairs (bases, often called as the ligand (s)). A multidentate ligand contains more than one ligand atom which can be responsible for combining the metal cation(s). Avery and Tobin (1993) applied the hard-and-soft principle of acids and bases to explore the interactions between metal ions with living, non-metabolising cells of S. cerevisiae for metabolism independent biosorption. Ca^{2+}, Mg^{2+} or H^{+} displacement was observed in the biosorption process. The release of Ca^{2+} and Mg^{2+} (indication of ionic binding of the metal) and H^{+} displacement (indication of covalent binding of the metal) seemed to be clearly dependent on the metal concentration. Brady and Tobin (1995) observed that Ca^{2+} and Mg^{2+} were released for each tested ions, whereas H^{+} displacement occurred for the borderline tested ions only. They concluded that hard metal Sr^{2+} adsorption was only due to ionic binding and the borderline ions (Mn^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}) exhibited a significant degree of covalent binding.

2.20.3. Intracellular accumulation/ precipitation

When the extracellular concentration of metal ions was higher than that of intracellular, metal ions could penetrate into the cell across the cell wall and membrane of the biomass by free diffusion. Metal ions can also enter into the cell if the cell wall was disrupted by natural force (e.g. autolysis) or artificial force (mechanical force or alkali treatment etc.). The above process is independent of metabolism. However, the process of intracellular accumulation/precipitation mainly relates to the living cells of biomass, and is an energy-driven process and dependent on active metabolism. Metal ions transported across the

cell membrane, are transformed into other species or precipitated within the cell by active cells, including transportation (Jianlong Wang , Can Chen, 2006). This particular study does not involve intracellular accumulation as dead cells are employed.

Chapter Three

3. Experimental Study

3.1 Materials and Methods

3.1.1 Preparation of Biosorbent

Waste yeast biomass procured from BGI Ethiopia S.C. has been pretreated first by allowing it to settle to remove the waste beer after which the biomass was rinsed. Then the yeast biomass was separated by centrifugation and was protonated for 24 hours in 0.1 M HCL solution in order to exchange any possible attached ion with H^+ ions. Later, the yeast biomass was rinsed and vacuum filtered and was dried at 60^0C for 24 hours. The dried yeast biomass was ground, sieved and the mass with particle size in the range of (125 μm - 225 μm) was stocked for biosorption tests.

3.1.2 Metal Solutions and Reagents

The chemicals used for the study were Analytical grade Pb (II) NO_3, Ni (II) $NO_3.6H_2O$, for metal ion sorption study and Nitric acid, Hydrochloric acid and Sodium hydroxide for PH adjustment. Fresh dilutions and metal solutions were used in each sorption study.

3.1.3 Methods of adsorption study

Batch adsorption experiments were carried out by shaking the flasks at 200 rpm for a period of contact time using an automatic shaker with temperature and time control with the parameters of concern set to the required condition. Following systematic process, the absorption uptake capacity of Ni (II) and Pb (II) in batch system was studied in the present work. The data obtained in batch mode studies was used to calculate the equilibrium metal adsorptive capacity. It was calculated for each sample of Ni (II) and Pb (II) by using the following expression:

$$q_e = \frac{v(C_o - C_e)}{m} \quad (3.1)$$

where q_e is the amount of heavy metal ions adsorbed onto per unit weight of beer yeast in mg/g, v the volume of solution treated in L, C_0 the initial metal ion concentration in mg/ l, C_e the equilibrium metal ion concentration in mg/l, and m is the dry weight of the biomass in g.

3.2 Studies of Factors Influencing the Biosorption Process

3.2.1 Effect of PH

Experiments were conducted to study the effect of solution pH on lead (II) and nickel (II) ion adsorption by contacting 0.1 g of sorbent with 100 ml of 1000 mg/l of lead (II) and nickel (II) ion solution. The mixture was agitated using a rotary shaker at 200 rpm and 25^0C for 60 min. The pH of the solution was adjusted by the addition of 0.1M NaOH or 0.1M HCl as needed, and studies were conducted at pH 3, 4, 5, 6 and 7. The biosorbent was then removed from the solution by centrifugation. The residual lead (II) and nickel (II) ion concentration in solution was analyzed in Atomic Absorption Spectrophotometer as described below. All experiments were conducted two times.

3.2.2 Effect of Contact Time

Batch biosorption tests were done at different contact time at the initial concentration of 1000 mg/l of Ni (II) and Pb (II) , and waste beer yeast dose concentration is 1g/l in 100mL solution. The samples were shaken in the rotary shaker at 200 rpm with the temperature controlled at 25^0C. The Samples were then taken at different time intervals (5, 10, 20, 30, 60 and 90 minutes) and were centrifuged. The concentration of metal ions in the supernatant solutions was analyzed using flame atomic absorption spectrometry (SpectrAA plus 20). Each determination was performed twice.

3.2.3 Effect of Initial Metal Concentration

Kinetic studies were conducted by varying the initial lead (II) and nickel (II) ion concentration and the yeast doses. 0.1 g of protonated yeast was suspended in 100 ml of lead (II) and nickel (II) solution (10, 100 and 200 mg/l) and the pH was adjusted to a value of 5.2 and 6.75 for lead and nickel with 0.1M NaOH and 0.1M HCL . The mixture was continuously stirred at 200 rpm and temperature was kept at 25^0C.and samples were withdrawn at predetermined time intervals (5, 10, 20, 30, 60 and 90 minutes) and finally the solutions were centrifuged and the supernatant solutions were analyzed for residual lead and nickel ions by Atomic Absorption Spectrophotometer.

3.2.4 Effect of Yeast Concentration

Kinetic studies on the effect of protonated yeast doses on lead (II) and nickel (II) ion sorption were carried out at an initial concentration of 1000 mg/l at pH 5.2 and 6.75 for lead and nickel respectively, and at an agitation speed of 200 rpm and temperature of 25^0 C. A series of kinetic experiments were conducted for four different protonated yeast doses varying between 0.5-4g/l. Samples were withdrawn at pre-determined time intervals (5, 10, 20, 30, 60, 90 minutes), Centrifuged and analyzed for residual lead (II) and nickel(II) ion concentration using flame atomic absorption spectrophotometer.

3.2.5 Equilibrium Study

Equilibrium sorption experiments were carried out at a pH of 5.2 and 6.75 for lead (II) and nickel (II) ion respectively by contacting 0.1 g of sorbent with 100 ml of lead (II) and nickel (II) ion solution at the concentrations varying over the range 5-1000 mg/l. The pH was adjusted using 0.1M NaOH and 0.1M HCl. The biosorbent was separated from the solutions after 24h. The residual metal ion in the solutions was estimated. All experiments were conducted in duplicates at a temperature of 25^0C. The maximum variation in metal sorption data between duplicate experiments was 5%.

3.2.6 Adsorption Isotherms

Adsorption isotherms show the distribution of solute between the liquid and solid phases equilibrium conditions. Many different isotherm models have been proposed for the adsorption of solutes in a liquid solution onto a solid surface. Langmuir model is probably the most popular isotherm models due to its simplicity and its good agreement with experimental data. The Langmuir model, the saturated monolayer isotherm, can be described by the linear form:

$$\frac{1}{q_e} = \left(\frac{1}{C_e} * \frac{1}{b q_{max}}\right) + \frac{1}{q_{max}} \qquad (3.2)$$

where c_e is the equilibrium metals concentration in aqueous phase (mg/l); q_{max} is the q_e for a complete monolayer (mg/g), a constant related to sorption capacity (the maximum amount of metal ion per unit weight of adsorbent); and b is a constant related to the affinity of the binding sites and energy of adsorption(l/mg). By plotting $1/q_e$ versus $1/c_e$, q_{max} and b can be determined.

Keeping the sorbent concentration constant at 1 g/l. The linearized plot of Langmuir (plot of $1/q_e$ and $1/C_e$) isotherm was given for both metals in single ion and multi-ion systems. The Langmuir constant q_{max}, defined as the amount of adsorbate per unit weight of adsorbent to form a complete monolayer on the sorbent surface and b which reflects quantitatively the affinity between the adsorbent and adsorbate were calculated for both metals in single ion and multi ion systems.

The equilibrium established between the adsorbed metal ions (q_e) and that remained free in the solution (C_e) was also represented by the Freunlich adsorption isotherm, by the linear equation as follows.

$$\ln q_e = \ln K_f + \frac{1}{n} * \ln C_e \qquad (3.3)$$

Where K_f and n are the Freundlich constants related to the adsorption capacity and adsorption intensity of the sorbent, respectively.

3.2.7 The competitive adsorption of lead and Nickel (Co-ion effect)

In this group of experiments, competitive adsorption of Ni (II) and Pb(II) ion from their binary solutions was investigated by following a similar procedure as described above. These studies were performed at a initial pH of 5.2 and 6.75 for lead (II) and Nickel (II) respectively at 25^0C. The experiments of competitive adsorption of Ni (II) and Pb(II) included two parts: (i) the competitive adsorption of Ni (II) and Pb (II) with the total metal concentration fixed at (1000mg/l); (ii) in a series of two metal ions solution, the initial concentration of Ni (II) was fixed to 1000 mg/l, whereas, the concentration of Pb(II) was varied from 50 to 700 mg/l. In another binary system, the initial concentration of Pb (II) was constant in1000mg/l, and the concentration of Ni (II) were varied from 50 to700mg/l.

3.2.8 Experiment on Biosorbent Recovery.

The experiment on biosorbent recovery was conducted by eluting the biosorbed metal ions using 2M HNO_3 both for Lead (II) and Nickel (II) ions both in the single ion and multi ion systems where the biosorption was initially conducted at conditions expressed in the above sections (nickel recovery test was conducted on yeast residue centrifuged after sorption test at PH from 3 to 6 , both lead and nickel recovery test was performed on yeast biomass residue from multi-ion sorption test at Pb(II) 1000mg/l and Ni(II) 200mg/l and Pb(II) 1000mg/l and Ni(II) 500mg/l initial metal doses . The Elution (recovery) tests were done for contact time of 60 minutes and metal ion concentrations and metal ion recovery was calculated.

$$\text{Recovery} = (C_{fel} V_{el} / m) / q * 100 (\%) \quad (3.4)$$

Where q is the metal uptake (mg/g); m the biosorbent dry weight (g); C_{fel} the Cr(III) final concentration in eluant (mg/L); V_{el} the eluant volume (L).

Chapter Four

4. Results and Discussion

4.1 Effect of PH

Among the major factors affecting biosorption capacity of saccharomyces cerevisiae, pH is considered to be the most important. According to the results of the experimentation conducted as shown in 3.2.1, pH has been found to affect sorption of lead significantly. When the pH was increased from 3 to 6 the sorption capacity has decreased from an average of 905mg/g at pH 3 to 302.5 mg/g at pH 6. On the other hand when we consider impact of pH on sorption of nickel in the range of 3 to 7, no significant variation was observed in the sorption capacity (Figure 4.1(b)). The experiments were not conducted at a pH greater than 7 in order to prevent precipitation of the heavy metals. The results obtained in this thesis work seem to fairly deviate from the findings of other workers (V. Padmavathy et.al., 2002 ,Runping Han, 2004). This can possibly be attributed to the alteration in the net ionic state of ligands such as carboxyl, Phosphate, imidazole, and amino groups where at lower pH (3-4) the concentration of H^+ ions is higher making the ligands have higher affinity for the increased sorption of Pb.

Unlike the case of Pb pH has no significant influence on the ionic state of the active sites and ligands involved in the sorption of nickel. In general, the optimum pH for Pb sorption was found to be 3-4 while nickel sorption is almost constant in the pH range of 3-7 as shown in Figure 4.1.

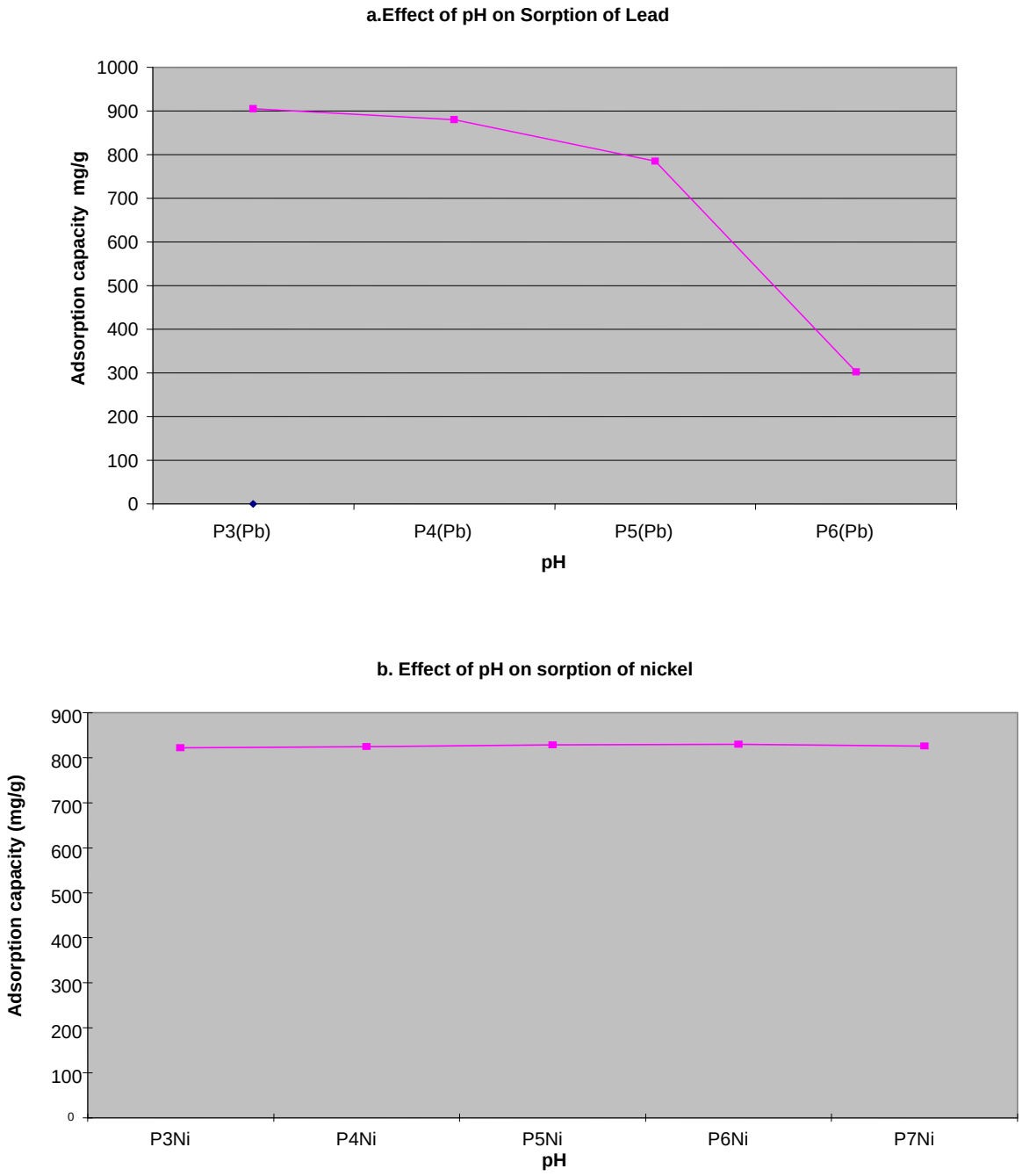

Figure 4.1: Effect of pH on Sorption of (a) Lead and (b) Nickel (average of duplicate data).

4.2 Effect of Contact Time

As the sorption tests were conducted based on the non-living dead deactivated cells, only surface sorption is responsible for the process. The slow intracellular accumulation or metabolism was not accounted in this thesis work. Hence the rate of sorption or removal of heavy metals from the solution was a very quick process. Both in the case of Pb and Ni. Even though prolonged adsorption brought a slight increase in the removal of the heavy metals from the solution, quasi-equilibrium was reached within 30 minutes and minor changes were observed until 90 minutes.

4.3 Equilibrium Study and Adsorption Isotherms

The equilibrium study has been conducted based on the commonly used monolayer and multi layer adsorption isotherm models of Langmuir and Freundlich. According to the plots of $1/q_e$ versus $1/C_e$ for Langmuir isotherms and LnK_f versus LnC_e for Fleundlich, the experimental data was found to fit better to Freundlich model. The sorption affinity (n) of Nickel was found to be 1.1339 and that for lead was found to be 0.971. Unlike the findings of other workers, the maximum adsorption capacity and sorption affinity for Nickel is greater than that for Lead. And this is linked to the greater affinity of the active sites for nickel.

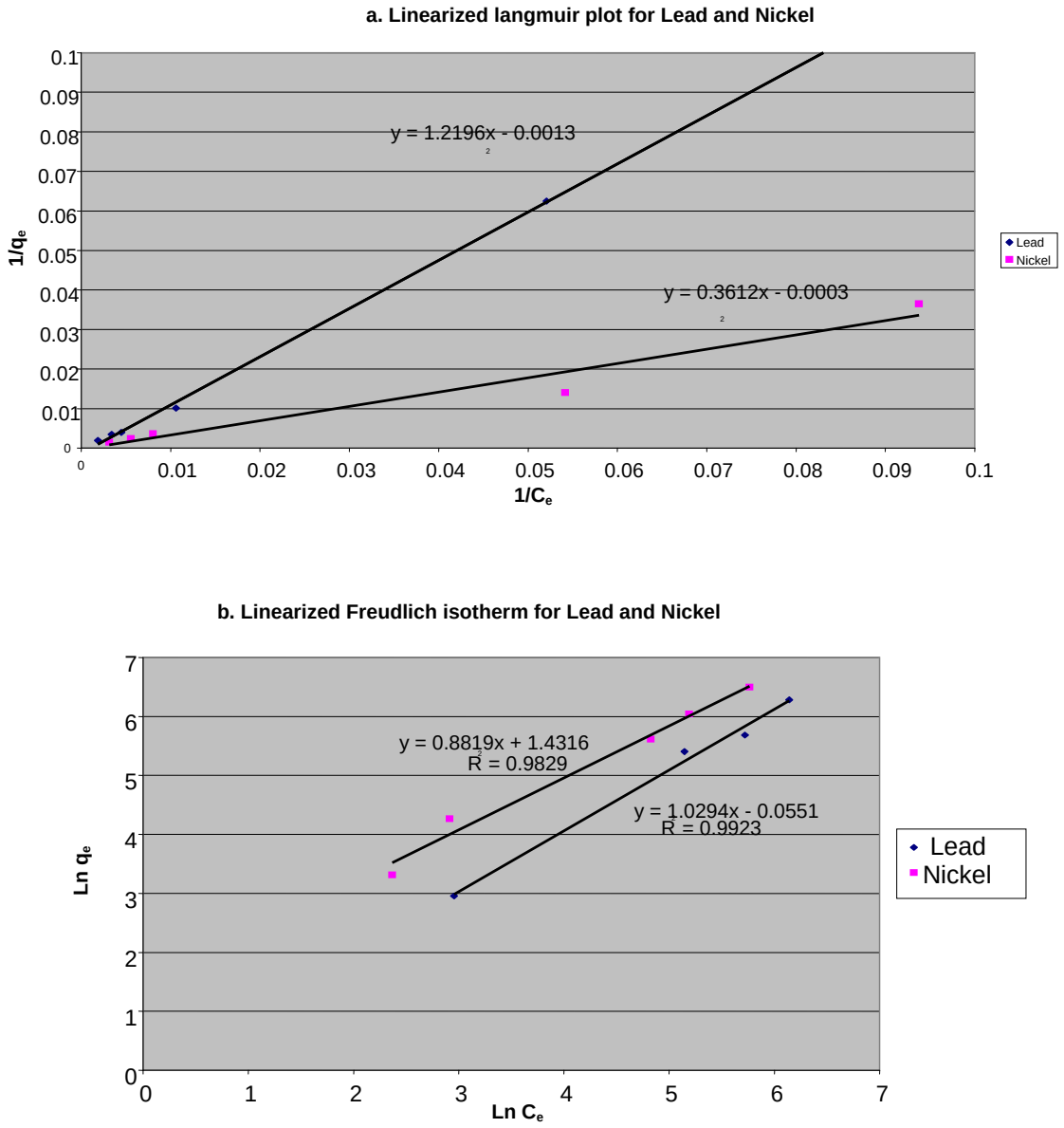

Figure 4.2. (a) Langmuir and (b) Freundlich isotherms for sorption of Lead and Nickel (average duplicate data)

Table 4.1: Parameters for Langmuir and Freundlich adsorption models

	Parameters of the Langmuir Model			Parameters of the Freundlich model		
Metal	Q_{max} (mg/g)	b (l/mg)	R^2	K_f	n	R^2
Nickel	526.3	0.005	0.944	0.239	1.133	0.983
Lead	312.5	0.237	0.924	0.946	0.971	0.992

4.4 Kinetic Study

4.4.1 Effect of initial concentration of heavy metal

In this research work, the concentration of nickel (II) ion and lead (II) ion was varied between 10 and 200 mg/l at a given sorbent dosage. As discussed in the literature review section of this document various kinetic expressions, namely pseudo-first order and pseudo-second order equations were used to fit the experimental data. The pseudo-first order rate expression of Lagrangian is generally described by the following equation.

$$\frac{dq_t}{dt} = k_1 (q_e - q_t) \tag{4.1}$$

where q_e and q_t are the amounts of lead(II) and nickel(II) ions, (mg/g) adsorbed on the sorbent at equilibrium, and at time t, respectively and k_1 is the rate constant. Integrating and applying the boundary conditions where at $t = 0$, $qt = 0$ and at $t = t$, $qt = qe$ Equation (4.1) takes the form:

$$\log (q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \tag{4.2}$$

The results of the experimentation on effects of metal dose did not fit to the pseudo-first order kinetic model that the sorption data was again analyzed in terms of a pseudo-second order mechanism, described by

$$\frac{dq_t}{dt} = K_2 (q_e - q_t)^2 \tag{4.3}$$

Where, k_2 is the rate constant of pseudo-second order biosorption (g/mgmin). Integrating and applying boundary conditions $t = 0$ and $qt = 0$ to $t = t$ and $qt = qe$, Equation (4.3) becomes

$$\frac{t}{q_t} = \frac{1}{h} + \frac{1}{q_e} t \tag{4.4}$$

If second order kinetics is applicable, the plot of *t/qt* against *t* of Equation (4.4) should give a linear relationship from which the constants *qe, h* and k_2 can be determined. The values of the constants *qe, h* and k_2 for pseudo-second order reaction have also been given in Table 4.2. As can be seen from Table 4.2, the correlation coefficient for the second order kinetics at all Lead(II) and Nickel(II) ion concentrations was greater than 0.99. On comparing the pseudo-first order and pseudo-second order adsorption rate constants at different Lead (II) and nickel (II) ion concentrations, it is seen that the second order model, provides the best correlation of the data. The values of *qe* increased from 8.31 to 104.7(mg/g), as the initial concentrations of Lead (II) ion varied form 10 to 200 mg/l. where as the value of q_e in the case of nickel increased from 8.17 to 169.49(mg/g) as the initial concentration varied from 10 to 200 mg/l. The values of initial sorption rate h increased from 7.84 mg/g min for lead (II) and 8.25 mg/gmin for nickel (II) at a concentration of 10mg/l to value of 1000 mg/g min for Lead (II) and 909.09 mg/g min for Nickel at a concentration of 200mg/l. the rate constant K again generally shows an increasing trend as concentration of lead(II) and nickel(II) rises from 10mg/l to 200mg/l. The equilibrium uptake capacity increases proportionally with initial concentration of heavy metals. But the values calculated from this particular study for equilibrium uptake capacity are significantly higher that that reported in literature.

Table 4.2: Parameters for Pseudo-second order kinetic model for varying metal dose

Metal type	Metal dose (mg/l)	(qe)Ion uptake capacity at equilibrium (mg/g)	(h) Initial sorption rate (mg/g min)	(k) rate constant (g/mg min)	(r^2) correlation coefficient
Lead	10	8.31	7.84	8.808	0.999
	100	99	526.32	18.622	0.999
	200	104.17	1000	10.851	0.999
Nickel	10	8.17	8.25	8.091	0.999
	100	89.28	588.23	13.551	0.999
	200	169.49	909.09	31.6	0.999

4.4.2 Effect of sorbent dose

The effect of deactivated protonated yeast dose on the sorption kinetics of lead(II) and nickel(II) ion was studied at a pH of 5.8 for lead and 6.75 for nickel and 1000 mg/l initial metal concentration. The sorbent dose was varied between 0.5 and 4 g/l. For all the sorbent doses, the amount of Lead(II) and nickel(II) ion sorbed at 1000 mg/l concentration increased rapidly in the beginning and slowly at the end. While at higher biomass concentrations (4 g/l) the equilibrium reached within 20-30 min, at lower biomass concentrations (0.5 and 1 g/l) equilibrium was not observed even until 60 min. Probably at high sorbent dosage the available ions are inadequate to cover all the available sites on the sorbent. Thus, the time of contact required to reach saturation varied with the biomass dose. The plot also shows that within a short time a large fraction of the total amount of Lead (II) and nickel (II) ion was removed but the uptake capacity of Lead (II) and nickel (II) ion per unit amount of sorbent (mg/g) decreased with increase in biomass concentration. From a plot of t/q_t against t for different sorbed dosages the rate constant, k, the equilibrium sorption, qe and the initial sorption rate, h, were calculated (Eq. (4.4)). The values of k, q_e and h for different sorbent doses are given in Table 4.3. There is a decrease in q_e with increase in sorbent dosage the ion uptake capacity at equilibrium (q_e) decreased from 106.38 mg/g to 2.506 mg/g as the sorbent dose increased

from 0.5 g/l to 4g/l for lead (II) and from 158.78 mg/g to 21.41 mg/g for nickel(II). Where as the values for rate constant (k) and initial sorption rate (h) seem not to show any trend. This may be attributed to the nature of the data obtained after measurement in atomic absorption spectrophotometer.

Table 4.3: Parameters for Pseudo-second order Kinetic model for varying yeast biomass dose

Metal type	Yeast dose (g/l)	(qe)Ion uptake capacity at equilibrium (mg/g)	(h) Initial sorption rate (mg/g min)	(k) rate constant (g/mg min)	(r^2) correlation coefficient
Lead	0.5	19.801	121.951	3.215	0.999
	1	106.38	10.331	1095.412	0.999
	2	5.02	3.124	8.067	0.999
	4	2.506	12.722	0.494	0.99
Nickel	0.5	158.78	476.19	52.943	0.999
	1	84.75	2000	3.591	0.999
	2	41.49	909.1	1.894	0.999
	4	21.41	400	1.146	0.999

4.5 The competitive adsorption of lead and Nickel

4.5.1 Impact of existence of nickel ion on sorption of lead ion.

The effect on biosorption of Lead ion was studied in the presence of nickel ion. As can be seen from the result shown in Figure 4.3, with increasing Ni (II) concentration, the biosorption quantity of Pb(II) decreased. The biosorption quantity of lead ion decreased from 576mg/g to 444.5 mg/g when the concentration of Nickel ion increased from 50ppm to 700ppm. Thus it can be seen that the existence of Nickel has great effect on the biosorption of Lead by brewery yeast biomass. The nickel ion existing in the solution, up on adsorbed by beer yeast and compete to the lead ions, and occupy some activated sites on beer yeast; decreasing the adsorption capacity of lead. So in the presence of Ni-ion, sorption of lead is seriously affected and the effect is augmented with increasing concentration of lead.

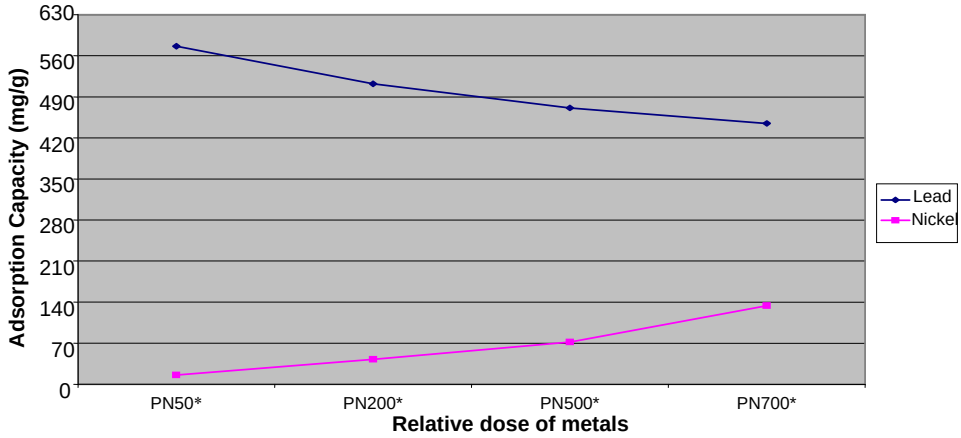

Figure 4.3. Impact of existence of nickel ion on sorption of lead ion. ("*" represents co-ion system with dose of P (Lead 1000mg/l) and N Nickel with 50, 200, 500 and 700 mg/l as shown on labels.

4.5.2 Impact of existence of lead ion on sorption of nickel

In this Study , the effect of presence of lead ion on biosorption of nickel ion was also studied . The result is shown in Figure 4.4. With increasing Pb (II) concentration, the biosorption quantity of Ni (II) remained almost unaffected. The biosorption quantity of nickel ion was almost 760mg/g when the concentration of lead ion was increased from 50ppm to 700ppm. Thus it can be seen that the existence of lead has almost no effect on the biosorption of nickel by brewer yeast biomass. This situation may be attributed to the difference in the ligands and active sites involved in the sorption of lead and nickel ions though there are common terminals of sorption for both ions on the yeast biomass.

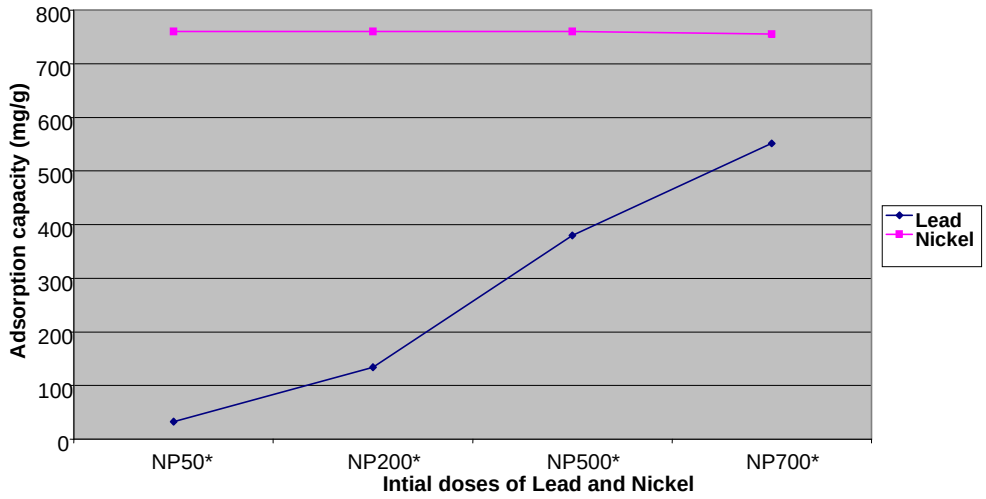

Figure 4.4. Impact of existence of Lead ion on sorption of Nickel ion. ("*" represents co-ion system with dose of N (nickel 1000mg/l) and P lead with 50, 200, 500 and 700 mg/l as shown on lebels.

4.6 Experiment on Biosorbent Recovery

The options for recovery of the sorbed heavy metal has been conducted based on the yeast biomass recovered after sorption tests have been performed on single ion and multi ion systems where initially the sorption has been conducted at different PH conditions for single ion systems and at the multi –ion test conditions indicated above. The tests are very preliminary and crude that they can only reveal the great possibility of recovery and require further intensive research in order to clearly understand the process and determine the optimum working conditions and furnish viable data for design of recovery systems. For Nickel ion recovery test conducted by adding the residual heavy metal laden yeast biomass into 3M HNO_3 and contacting time of 60min. Higher Nickel recovery (5.83%) has been obtained for the sample where the sorption has been conducted at a pH 3 and even more higher recovery was achieved from the elution test based on the multi-ion sorption system where nickel recovery was found to be 38.1% from initial sorption capacity of 157.5 mg/g in the presence of lead ion and nickel recovery of 18.7% was achieved when the initial

sorption capacity was 428mg/g. On the other hand Lead sorption was found to be 80.3 % for initial sorption capacity of 512 mg/g. In general Lead recovery was more efficient than that of nickel. And it can be seen from Table 4.4 that Nickel recovery is higher when initial doses and corresponding adsorption capacity are lesser.

Table 4.4: Percentage recovery of nickel (II) and Lead (II) from sorbents recovered from different biosorption test conditions.

Metal type	**Initial sorption test condition**	**Biosorption capacity (mg/g)**	**Final concentration of metal after recovery (mg/l or ppm)**	**Percentage recovery (%)**
Nickel	PH = 3	824.5	60	5.83
	PH = 4	828.5	10	0.9
	PH = 5	830	10	1
	PH = 6	826	40	3.2
Nickel and Lead co-ion test	Co-ion Test with initial concentration of lead = 1000mg/l and nickel = 200mg/l	157.5 for Nickel(II)	60	38.1
		512 for Lead (II)	993	Unacceptable As precipitated metal ion has excessively increased the recovery
	Co-ion Test with initial concentration of lead = 1000mg/l and nickel = 500mg/l	428 for Nickel (II)	80	18.7
		471 for Lead (II)	514	**80.3**

Chapter Five

5. Conclusion and Recommendation

5.1 Conclusion

Biosorption being a very cost effective and eco-friendly option for the removal of heavy metals from waste streams, this particular research work analyzed the sorption capacity and vital factors affecting the sorption capacity of the two selected heavy metals Lead and Nickel

Expertimental results show that pH significantly affects sorption of lead . On the other hand when we consider impact of pH on sorption of nickel in the range of 3 to 7, no significant variation was observed in the sorption capacity. Unlike the case of Pb, PH has no significant influence on the ionic state of the active sites and ligands involved in the sorption of nickel. In general, the optimum PH for Pb sorption was found to be 3-4 while nickel sorption is almost constant in the PH range of 3-7.

The rate of sorption or removal of heavy metals from the solution was a very fast process. A significant percentage of the metal ions Lead (II) and Nickel(II) has been removed during the first five to ten minutes of the contacting and equilibrium has been reached in almost 60 minutes.

The equilibrium data can be represented by both Langmuir and Freundlich isotherm models but with higher correlation coefficient for Freundlich model. Concerning the maximum sorption capacity and sorption affinity of the heavy metals, unlike the findings of other researchers the brewery yeast involved in the study has been found to have higher maximum sorption capacity Q_{max} for nickel than for lead. And this is linked to the greater affinity of the active sites for nickel. The equilibrium uptake capacity increases proportionally with initial concentration of heavy metals. But the values calculated from this particular study for equilibrium uptake capacity are significantly higher than that reported in literature.

In the study of the kinetics of sorption for both heavy metals sorption capactiy versus time data did not fit to first order kinetic model rather it fitted better to the pseudo-second order model and the values of the constants q_e, h and k_2 for pseudo-second order reaction have also been evaluated. The correlation coefficient for the second order kinetics for all the tests conducted for varying metal dose and yeast dose Lead(II) and Nickel(II) ion was greater than 0.99. The values of q_e tends to increase as metal dose was increases from 10mg/l to 200mg/l for both metal ions. The values of initial sorption rate h has also got an increasing trend. The rate constant (k) again generally shows an increasing trend as concentration of lead(II) and nickel(II) ions rises from 10mg/l to 200mg/l.

There is a decrease in equilibrium uptake capacity q_e with increase in sorbent dosage as the uptake capacity is measured per unit of sorbent. But the total sorption capacity evidently rises. Where as, the values for rate constant (k) and initial sorption rate (h) seem not to show explicable trend. This may be attributed to the nature of the data obtained after measurement in atomic absorption spectrophotometer.

The co-ion effect analysis has well proven the interference between the two metal ions, where the sorption of lead is seriously affected by the presence or increasing dose of nickel in the metal dose range considered in the study. To the contrary increasing dose of lead did not draw any impact on the sorption of nickel which possibly implies that nickel has predominantly got more exclusive ligands of sorption though it shares common sites with Lead.

As to the recovery or elution tests conducted to observe degree of recoverability, recovery of lead has been considerably bigger than that of nickel which is possibly due to the higher ion exchange capacity and higher covalent index of Lead . The study generally depicts the very wide possibility of recovery which is mainly dependent upon the conditions at which the sorption was conducted initially but further intensive research is required in order to clearly understand the process and determine the optimum working conditions and furnish viable data for the design of recovery systems.

5.2 Recommendation

Such biosorption tests have been intensively conducted on various metals and conventional and non conventional sorbents. Saccharomyces Cerevisae considered in this research has shown higher adsorption capacity as compared to what is reported in literature for both metal ions Lead (II) and Nickel (II). Further research in this stride is recommended in order to confirm this fact in the test conditions not included in this study and or perform some refinement and further study to design pilot scale and ultimately industrial scale biosorption unit (column) based on brewery derived waste yeast biomass.

The findings of this study seem to show promising possibilities of lead and nickel and other heavy metal removal and recovery that further study should be conducted. The bisorption tests should better be under taken by measuring the amount of heavy metal removed both from the residual solution and by eluting that adsorbed on the biomass.

In addition to conventional sorbents like Saccharomyces Cerevisae considered in this study, the sorption capacity of other locally available and cultivable biosorbents and non conventional biosorption agents should be studied and options for scale up should be devised.

Apart from purely experimental study, some modeling works on sorption capacity, sorption mechanism and design optimization can greatly save resource and lead to workable biosorption technology.

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Annex 1: Experimental Data

Table A1(a) Impact of PH on adsorption capacity, Lead		
Test Code	**Initial Dose of H.metal(mg/l)**	**Average Adsorption capacity(mg/l)**
P3(Pb)	1000	905
P4(Pb)	1000	880
P5(Pb)	1000	785
P6(Pb)	1000	302.5

Table A1(b) Impact of PH on adsorption capacity, Nickel		
Test Code	**Initial Dose of H.metal(mg/l)**	**Average Adsorption capacity(mg/l)**
P3(Ni)	1000	822
P4(Ni)	1000	824.5
P5(Ni)	1000	828.5
P6(Ni)	1000	830
P7(Ni)	1000	826

Table A2(a) Equilibrium uptake capacity for varying metal dose, Lead		
Test Type	**Initial dose of metal (mg/l)**	**Average Adsorption capacity at equilibrium (mg/g)**
E1(Pb)	40	20
E2(Pb)	100	94.5
E3(Pb)	400	225
E4(Pb)	600	295
E5(Pb)	1000	535

Table A2(b) Equilibrium uptake capacity for varying metal dose, Nickel		
Test type	**Initial metal dose (mg/l)**	**Average Adsorption capacity at equilibrium (mg/g)**
E1(Ni)	40	28
E2(Ni)	100	74
E3(Ni)	400	275
E4(Ni)	600	420
E5(Ni)	1000	670

Table A3(a) Test for Co-ion effect, Impact of existence of nickel on sorption of Lead				
Test Code	**Initial Metal dose (Pb)**	**Initial Metal Dose (Ni)**	**Average sorption capacity Pb**	**Average sorption capacity Ni**
PN50	1000	50	576	34.25
PN200	1000	200	512	157.5
PN500	1000	500	471	398
PN700	1000	700	444.5	566

Table A3(b) Test for Co-ion effect, Impact of existence of Lead on sorption of Nickel				
Test Code	**Initial Metal dose (Pb)**	**Initial Metal Dose (Ni)**	**Average Adsorption Capacity Pb (mg/g)**	**Average Adsorption capacity Ni (mg/g)**
NP50	50	1000	32.5	760
NP200	200	1000	134.15	760
NP500	500	1000	379.55	760
NP700	700	1000	551.5	755

Table A4 (a) Impact of metal dose on sorption of Lead and sorption kinetics			
Test Code	**Initial Dose of H.metal(mg/l)**	**Residual Dose of H.metal(mg/l)**	**Adsorption capacity (mg/g)**
M1T1-5(Pb)	10	-	-
M1T1-10(Pb)	10	-	-
M1T1-20(Pb)	10	-	-
M1T1-30(Pb)	10	3.8	6.2
M1T1-60(Pb)	10	0.3	9.7
M1T1-90(Pb)	10	0.3	9.7
M2T1-5(Pb)	100	10	90
M2T1-10(Pb)	100	1	99
M2T1-20(Pb)	100	1	99
M2T1-30(Pb)	100	1	99
M2T1-60(Pb)	100	1	99
M2T1-90(Pb)	100	1	99
M3T1-5(Pb)	200	90.8	109.2
M3T1-10(Pb)	200	96.8	103.2

M3T1-20(Pb)	200	95	105
M3T1-30(Pb)	200	7.5	192.5
M3T1-60(Pb)	200	0.8	199.2
M3T1-90(Pb)	200	0.8	199.2

Table A4 (b) Impact of metal dose on sorption of Nickel and sorption kinetics

Test Code	Initial Dose of H.metal(mg/l)	Residual Dose of H.metal(mg/l)	Adsorption capacity (mg/g)
M1T1-5(Ni)	10	-	-
M1T1-10(Ni)	10	3	7
M1T1-20(Ni)	10	2	8
M1T1-30(Ni)	10	2	8
M1T1-60(Ni)	10	2	8
M1T1-90(Ni)	10	-	-
M2T1-5(Ni)	100	11.4	88.6
M2T1-10(Ni)	100	10.3	89.7
M2T1-20(Ni)	100	11	89
M2T1-30(Ni)	100	11	89
M2T1-60(Ni)	100	11	89
M2T1-90(Ni)	100	10.4	89.6
M3T1-5(Ni)	200	33	167
M3T1-10(Ni)	200	33	167
M3T1-20(Ni)	200	33	167
M3T1-30(Ni)	200	30	170
M3T1-60(Ni)	200	-	-
M3T1-90(Ni)	200	-	-

Table A5(a) Impact of yeast biomass dose on sorption of Lead and sorption kinetics

Test Code	Initial Dose of H.metal(mg/l)	Residual Dose of H.metal(mg/l)	Adsorption capacity (mg/g)
Y1T1-5(Pb)	100	0.1	19.98
Y1T1-10(Pb)	100	-	20
Y1T1-20(Pb)	100	-	20
Y1T1-30(Pb)	100	0.1	19.98
Y1T1-60(Pb)	100	0.3	19.94
Y1T1-90(Pb)	100	1	19.8
Y2T1-5(Pb)	1000	409	59.1
Y2T1-10(Pb)	1000	469	53.1
Y2T1-20(Pb)	1000	496	50.4
Y2T1-30(Pb)	1000	14	98.6
Y2T1-60(Pb)	1000	0.4	99.96
Y2T1-90(Pb)	1000	0.4	99.96
Y3T1-5(Pb)	100	2	4.9
Y3T1-10(Pb)	100	8.5	4.575
Y3T1-20(Pb)	100	17	4.15
Y3T1-30(Pb)	100	0.9	4.955

Y3T1-60(Pb)	100	16.5	4.175
Y3T1-90(Pb)	100	-	-
Y4T1-5(Pb)	100	2	2.45
Y4T1-10(Pb)	100	1.4	2.465
Y4T1-20(Pb)	100	1	2.475
Y4T1-30(Pb)	100	0.9	2.476
Y4T1-60(Pb)	100	-	-
Y4T1-90(Pb)	100	-	-

Table A5(b) Impact of yeast biomass dose on sorption of Nickel and sorption kinetics			
Test Code	**Initial Dose of H.metal(mg/l)**	**Residual Dose of H.metal(mg/l)**	**Adsorption**
Y1T1-5(Ni)	1000	170	166
Y1T1-10(Ni)	1000	170	166
Y1T1-20(Ni)	1000	170	166
Y1T1-30(Ni)	1000	200	160
Y1T1-60(Ni)	1000	-	-
Y1T1-90(Ni)	1000	-	-
Y2T1-5(Ni)	1000	154	84.6
Y2T1-10(Ni)	1000	158.4	84.16
Y2T1-20(Ni)	1000	154.4	84.56
Y2T1-30(Ni)	1000	154	84.6
Y2T1-60(Ni)	1000	154	84.6
Y2T1-90(Ni)	1000	154	84.6
Y3T1-5(Ni)	1000	160	42
Y3T1-10(Ni)	1000	170	41.5
Y3T1-20(Ni)	1000	170	41.5
Y3T1-30(Ni)	1000	170	41.5
Y3T1-60(Ni)	1000	-	-
Y3T1-90(Ni)	1000	-	-
Y4T1-5(Ni)	1000	142.2	21.445
Y4T1-10(Ni)	1000	145.8	21.355
Y4T1-20(Ni)	1000	136.8	21.58
Y4T1-30(Ni)	1000	-	-
Y4T1-60(Ni)	1000	143	21.425
Y4T1-90(Ni)	1000	-	-

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