Biosorption of Nickel (II) by using Waste Baker's Yeast

By

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ABSTRACT

Biological methods for removing heavy metals are in competition with chemical and physical techniques such as precipitation, ion exchange, electrochemical treatment and evaporative recovery, especially, when the concentration of the heavy metal ion is low, between 1.0 and 100 mg/L. In order to qualify for industrial applications, biosorbents have to be produced at low cost. The use of biomass from various production stages; e.g. from the pharmaceutical or the food industries, is one way to minimize the costs. This study is concerned with the binding of nickel ions onto waste biomass of *Saccharomyces cerevisiae* genus, obtained from the food industry. Since the biomass employed is a waste material, biosorption process described in this study may represent a cheap alternative to conventional methods.

Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids, offer many functional groups which can bind metal ions such as carboxylate, hydroxyl, phosphate and amino groups.

The objective of this study was to investigate the adsorption of nickel on waste baker's yeast as a function of several factors, i.e. pretreatment, pH, temperature, biomass concentrations and initial metal concentrations, in order to determine the optimum adsorption conditions of a batch process.

Pretreatment of waste yeast biomass using sodium hydroxide, formaldehyde, nitric acid and ethanol decreased the sorption of nickel (II) ions compared with live biomass. Optimum initial pH for nickel (II) ions was 5.0 at the optimum temperature of 25^{o} C. The uptake values increased with the increasing initial nickel (II) ion concentrations up to 150 mg/L. The optimum biomass concentration for this process was determined as 1.0 g/L.

The biosorption isotherms were developed at various initial pH and temperature values. The equilibrium values were expressed with the Langmuir model while nickel sorption did not fit the Freundlich plot. The Langmuir parameters q_{max} (14.30 mg/L) and b (0.0069 L/mg) have been calculated. " q_{max} " increased from 7.8 to 14.30 mg/L with the increase in pH from 3.0 to 5.0. Similar trend was observed for the " b " values; an increase from 0.0025 to 0.0069 L/mg were obtained when the pH of the solution was raised from 3.0 to 5.0. Both Langmuir model parameters were found to be the highest

values at pH 5.0 which is consistent with the results of the optimization studies as described above.

Temperature also affected the phase equilibria of nickel (II)/*S.cerevisiae* system. The highest capacity for biosorption system was obtained at 25^{o} C with the q_{max} and b values of 14.3 mg/L and 0.0069 L/mg at pH 5.0, respectively. The enthalpy change for the biosorption process have been evaluated by using the Langmuir constant "*b*", which is related to the energy of adsorption. Nickel (II) biosorption is considered to be an exothermic process since low binding occurs when the temperature increases from 25 to 45^{o} C.

The uptake of nickel (II) ions by the yeast biomass was also investigated with respect to time under optimum operating conditions. Biosorption kinetics were rapid within the first few minutes. After the initial rapid uptake, further biosorption by yeast cells continued slowly and reached an equilibrium after 2 hours at all pH values of 3.0, 4.0 and 5.0. On the other hand, the rate of adsorption was found to be the fastest at pH 5.0 with an initial rate of around 3.59 mg Ni (II) / g-min.

ÖZ

Atık sulardaki ağır metallerin arıtılmasında kullanılan fiziksel ve kimyasal tekniklere karşı, biyolojik metotlar rakip görülmektedir. Özellikle düşük ağır metal konsantrasyonlarında, örneğin; 1.0 ile 100 mg/L arasındaki derişimlerde uygulanmaktadır. Biosorbentlerin sanayide kullanılabilmesi, bunların ucuz imal edilmesini gerekli kılmaktadır. Biyomateryallerin örneğin; ilaç ve gıda sanayi sektörlerinden kolayca ve atık olarak temin edilebilmesi, biyolojik arıtma sistemlerinde kullanılması için ekonomik bir yol sağlamaktadır. Bu çalışmanın konusu, nikel iyonunun, atık olarak gıda sanayinden temin edilen bir tür maya olan *Saccharomyces cerevisiae* hücresi yüzeyine bağlanmasını içermektedir. Atık olarak temin edilen biyosorbenti, mevcut kullanımda olan ağır metal arıtma metotlarına karşı daha ucuz bir alternatif olarak düşünülmektedir.

Metal iyonlarının hücre duvarı yüzeyine bağlanmasında duvar yüzeyinde mevcut bulunan bazı polisakkaritler, proteinler ve lipidler ile bunların içinde mevcut bulunan karboksil, hidroksil, fosfat ve amino gruplarının etkin oldukları bilinmektedir.

Bu çalışma, nikelin atık ekmek mayası üzerine adsorpsiyonu, değişik çevresel faktörlerin bu mekanizmaya olan etkilerinin incelenmesi ve optimum koşulların belirlenmesini amaçlamıştır. Atık mayanın yıkanması, ortamın pH ve sıcaklığı, metal ve maya konsantrasyonları gibi faktörlerin, kesikli sistemde nikel iyonunu adsorpsiyonuna etkisi irdelenmiştir.

Sodyum hidroksit, formaldehit, nitrik asit ve etanol kullanılarak yıkanan atık mayanın nikel adsorpsiyon yüzdesini düşürdüğü gözlenmiştir. Bundan dolayı, ekmek mayası hiçbir işlemden geçirilmeden doğrudan canlı hücre olarak, biyosorpsiyon işleminde kullanılmıştır. Nikel biyosorpsiyonunda, optimum pH değeri 5.0 ve optimum sıcaklık değeri 25^{o} C olarak saptanmıştır. Nikelin maya tarafından tutunma yüzdesi sistemin başlangıç metal konsantrasyonuyla artarken, bu konsantrasyonu 150 mg/L' nin üzerine getirildiğinde, adsorpsiyon yüzdesinde önemli bir değişim gözlenmemiştir. Diğer taraftan optimum maya konsantrasyonu 1.0 g/L olarak tespit edilmiştir.

Biyosorpsiyon izotermleri değişik pH ve sıcaklık değerlerinde incelenmiştir. Nikel biyosorpsiyonu Freundlich modeline uygunluk göstermezken, Langmuir modeliyle uyum sağlamaktadır. Langmuir parametreleri olan q_{max} ve b değerleri sırasıyla 14.30 mg/L ve 0.0069 L/mg olarak hesaplanmıştır. Ortamın pH' sı 3.0' den

5.0’ e çıkarıldığında, q_{max} değeri de 7.8’ den 14.30 mg/L’a ulaşmıştır. Aynı durum *b* değeri için de gözlenmiş ve *b* değeri 0.0025’ den 0.0069’a ulaşmıştır. Her iki parametre için maksimum değer, optimum pH olan 5.0’de elde edilmiştir.

Aynı zamanda nikel / maya faz dengesini etkileyen ortam sıcaklığı, en yüksek biyosorpsiyon kapasitesi için 25^{o} C (pH 5.0) olarak saptanmıştır. Aynı sıcaklıkta, q_{max} ve *b* değerlerinin de en yüksek olduğu gözlenmiştir. Diğer taraftan *b* sabitinin adsorpsiyon enerjisine bağlı olduğu bilindiğinden, entalpi değişimi saptanmış ve sistemin ekzotermik olduğu tahmin edilmiştir. Bununla beraber, sıcaklığın artmasıyla (25’ den 45^{o} C ye) nikel biyosorpsiyonunda düşüş gözlenmiştir.

Nikel biyosorpsiyonu optimum koşullarda, üç farklı pH değerinde (3.0, 4.0 ve 5.0) zamana karşı incelenmiştir. Biyosorpsiyon kinetiğinin ilk dakikalarda çok hızlı olduğu görülmüş; nikel biyosorpsiyonu bu hızlı aşamadan sonra yavaşlamış ve 2 saat sonra dengeye ulaşmıştır. En yüksek adsorpsiyon hızı, pH 5.0 iken 3.59 mgNi(II)/ g-dk olarak belirlenmiştir.

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Chapter I

INTRODUCTION

Metal pollution is a widespread problem; in fact, in developed countries. Man's use of metals seriously began to affect the environment by the Industrial Revolution. From the environmental point of view, the metals that are of greatest concern are those which, either by the presence or by their accumulation, can have a toxic or an inhibitory effect on living things. The heavy metals escaping into the environment pose a serious health hazard because they accumulate in the tissues of humans and animals throughout the food chain. Therefore there is a need of treating and controlling the heavy metals in the environment.

The commonly used treatment processes for heavy metal removal from dilute aqueous streams are chemical reduction-oxidation, precipitation, ion exchange and adsorption. However, these high technology processes have significant disadvantages such as:

- incomplete metal removal
- need for expensive equipment and monitoring systems
- high reagent or energy requirement
- generation of toxic sludge or other waste products that require disposal

Especially, such process may be ineffective or extremely expensive when initial heavy metal concentration is in the range of 10 - 100 ppm. Also there are numerous reports in the literature documenting the capacity of pure cultures of bacteria, algae and fungi which remove heavy metal ions from solution. The mixed microbial cultures have been proposed as reasonable approach for removing heavy metals than pure cultures. The fortuitous removal of heavy metal ions by the mixed cultures used in the activated sludge process which is designed to remove BOD but not heavy metal ions, has been found to be significant but extremely variable. However, the conventional activated sludge process is considered as an unsuitable tool for the detoxification of heavy metal ions. Therefore, a new process for the efficient removal of heavy metal ions is required. An efficient process is necessary not only to detoxify metal-bearing wastewater but also

to recover precious and non-precious metal ions for recycling back to the consumers. Recently, biosorption of heavy metals has been receiving a great deal of attention for both its scientific novelty and application potential. Biosorption is defined as a property of dead or living biomass, particularly of microbial origin, to retain and concentrate metallic elements from dilute solutions (1,2,5,10,16, 20-26,35).

On the other hand, advanced technologies have been developed for remediation of heavy metal bearing wastes, where metals are amenable to removal by established biotechnological methods. However, there are some recalcitrant metals for which physico-chemical and bioremediation technology is not suitable. Therefore the choice of the microorganism for removing heavy metal ions is important because of wide differences in their capacity for sorption or in their affinity for the metal. An example of this is nickel which is difficult to bioremediate by existing technology compared with other heavy metal ions. This was probably due to the chemical properties of nickel ions leading to hindrance of bisorption. The toxic metal nickel which is an environmental pollutant is also toxic to activated sludge bacteria and concentrations can approach 0.46-3.4 ppm in mine drainage, 2-900 ppm (rinse water) in plating plants. Wastewater from paint-ink formulation and porcelain enamelling industries contain nickel concentrations of 0-40 ppm and 0.25-67 ppm Ni, respectively (19,27,33,38).

The uptake of heavy metals by the microbial species involves two mechanism. The first mechanism is the passive uptake. It is rapid and reversible accumulation step termed biosorption. The second mechanism is the active uptake. It is an irreversible and slower intracellular bioaccumulation process. The factors such as pH, temperature, initial metal and biomass concentration can affect the biosorption mechanisms. Also the pretreatment of the live waste yeast using some chemicals such as sodium hydroxide, formaldehyde, dimethyl sulphoxide, ammonium persulfate and ethanol can increase the capacity of biosorption (1,2,5,10,16,26,35,44).

These studies are focusing specially on nickel removal by using the biosorption mechanism. For biosorption, the yeast *Saccharomyces cerevisiae* is a preferred biosorbent. *S. cerevisiae* known as the baker's yeast, is an inexpensive, readily available source of biomass that can be obtain from fermentation processes. A lot of search have been focussed on biosorption of Cu (II), Cd(II), Pb(II) ,Cr(IV), Sr(II), and Fe(II) and Uranium by *S. cerevisiae* (28,30,34-36,39-45, 48). However, there is still no satisfactory precedent of employing *Saccharomyces cerevisiae* as a waste biomass in the removal of nickel. Thus, application of biowaste such as waste baker's yeast for the removal of

heavy metal ions may have some advantages because it is inexpensive, effective, environmentally safe and available sources for a long time period.

In this study, adsorption of nickel on a waste biomass (*Saccharomyces cerevisiae*) was investigated since there has been little information in the literature about this biosorption system. The effects of pretreatment, pH, temperature, initial metal concentration and biomass concentration on biosorption have been studied together with equilibrium isotherms and adsorption kinetics. Isotherms have been described by the Langmuir model and quantitative data, such as maximum biosorption capacity of *Saccharomyces cerevisiae* and affinity constants. Biosorption kinetics were also investigated in this study as a function of pH.

Chapter II

TOXIC METALS

Metals can be dispersed, both naturally and by man's activities, into any of the earth's elements: soil, water or air. However, the aquatic environment has been the most affected area of the earth. The heavy metals which have an environment effect seen in Table 2.1

Table 2.1 The most important heavy metals present in environment

Cadmium	Nickel
Chromium	Silver
Copper	Tin
Cobalt	Zinc
Lead	Mercury

2.1 Metals in the Environment

Metals are ubiquitous in nature and even those metals generally considered as pollutants are found in trace concentration in the environment (see Table 2.2). The effluent sources of metals that are discharged mainly to the rivers are :

- 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.

Table 2.2. Typical background levels of heavy metals in soil and aquatic environments

Metal	Aquatic concentration *μg/L*	Soil concentration *μg/L*
Gold (Au)	ND	0.50
Aluminium (Al)	Trace	$7.09*10^5$
Arsenic (As)	Trace	0.49
Barium (Ba)	ND	$4.34*10^3$
Cadmium (Cd)	0.06	0.60
Cobalt (Co)	0.07	79.00
Chromium (Cr)	Trace	990.00
Cesium (Cs)	Trace	59.40
Copper (CU)	0.63	296.00
Mercury (Hg)	Trace	0.29
Manganese (Mn)	10	6043.00
Nickel (Ni)	ND	397.00
Lead (Pb)	0.06	99.00
Tin (Sn)	Trace	101.00
Zinc (Zn)	19.62	496.00

ND= no data reported . trace= levels usually below detection.(Ref: 2)

For the most part, metal pollution problems arise when human activity either distrupts normal biochemical cycles or concentrates metals. Elevated metal concentrations in the environment have wide-ranging impacts on animal, plant, and microbial species.

2.2 Effects of Heavy Metals

To date humans have been exposed to a variety of metals that can cause symptoms such as hypophosphatemia, heart disease and liver damage, cancer, neurological disorders , central nervous system damage, encephalopathy and paresthesia (1,3,4). Also the plants that have been exposed to metals have been affected and it is observed of most morphological and mutational changes in plants. These include shortening of roots, leaf scorch, chlorosis, nutrient deficiency and increased vulnerability to insect attack. Similarly, microbial growth is often slowed or inhibited completely in the presence of excessive amounts of metals (1,3,10).

The heavy metals were classified according to their toxic effect (Table 2.3) which is reported by European Union under the 'framework' Dangerous Substances Directive (76/464/EEC) and include two lists, as black and grey .

Table 2.3. Black and Grey list metals

Black list	Grey list
Cadmium	Chromium
Mercury	Copper
	Lead
	Nickel
	Zinc

Ref: 1

The heavy metals contained in black list are considered very toxic, persistent or bio-accumulative within the environment. The grey list contains those metals which are environmentally harmfull but less so than those in the black list. Therefore the limits for discharge of black list metals must be currently controlled. The standart metal concentrations in drinking water and their health effects are given in Table 2.4.

Table.2.4. The standard metal concentration in drinking water and the health effect.(Page 1 of 2)

METALS	**EFFECT**	**DRINKING WATER STANDARDS**
Cadmium	• Cause serious damage to kidneys and bones in humans • Bronchites, emphysema , aneamia • Acute effects in children	• By the Environmental Protection Agency (EPA) Maximum con.: 0.005 mg/L • By European Community (EC): 0.2 mg/L • Regulation of water quality (Turkey) 0.001 mg/L
Mercury	• Poisonous • Causes mutagenic effects • Disturbs the cholestrol	• By the Environmental Protection Agency(EPA) Maximum con.: 0.002 mg/L • By European Community (EC): 1 μ/L • Regulation of water quality (Turkey) 0.004 mg/L

Table 2.4 (Page 2 of 2)

METALS	EFFECT	DRINKING WATER STANDARDS
Chromium	• Necrosis nephrits and death in man (10 mg/ kg of body weight as hexavealent chromium) • Irritation of gastrointestinal mucosa	• By the Environmental Protection Agency(EPA) Maximum con.: (hexavealent and Trivalent) Total 0.1 mg/L • By European Community (EC): 0.5 mg/L • Regulation of water quality (Turkey) 0.1 mg/L
Copper	• Causes damage in a variety of aquatic fauna • Phytotoxic • Mucosal irritiation and corrosion • Central nervois system irritation followed by depression	• By the Environmental Protection Agency (EPA) Maximum con.: 1.0 mg/L • By European Community (EC): 3 mg/L • Regulation of water quality (Turkey) 0.01 mg/L
Lead	• Toxic to humans, aquatic fauna and livestock • High doses cause metabolic poison • Tierdness, irritability anaemia and behavioural changes of children • Hypertension and brain damage • Phytotoxic	• By the Environmental Protection Agency (EPA) Maximum con.: 0.1 mg/L • By European Community (EC): 0.5 mg/L • Regulation of water quality (Turkey) 0.1 mg/L
Nickel	• High conc. can cause DNA damage • Eczema of hands • High phytotoxicity • Damaging fauna	• By the Environmental Protection Agency (EPA) Maximum con.: 0.1 mg/L • By European Community (EC): 0.1 mg/L • Regulation of water quality (Turkey) 0.1 mg/L
Zinc	• Phytotoxic • Anaemia • Lack of muscular coordination • Abdominal pain etc..	• By the Environmental Protection Agency (EPA) Maximum con.: 5 mg/L • By European Community (EC): 5 mg/L • Regulation of water quality (Turkey) 0.1 mg/L

Ref:1,2,3.6

2.3. Nickel Ions

The metals which are of greatest enviromental concern, one of them is nickel which this study focused on nickel removal because it is difficult to bioremediate by existing technology compared with other heavy metal ions.

2.3.1 Sources and Occurrences of Nickel Ions

In the earth's crust the average concentration has been reported about 75 mg/ kg that constitutes about 0.016 % of the total mass. Its principal ores are pentlandite ((Fe Ni)$_9$ S$_8$) , millerite (NiS) and garnierite ((NiMg)$_6$Si$_4$O$_{10}$ (OH)$_8$). It occurs as the natural metal only in meteoites. It is used in the production of alloys, nickel plating for corrosion resistance and in the manufacture of batteries (e.g. nickel-cadmium bateries). The metal or its compounds are also used as catalysts, dyes, pigments and in metal process equipments that can give rise to some contamination of food. Nickel is present in crude oil in varying concentrations and the burning of petroleum products, either in combustion processes or in vehicle fuel that introduces the metal into the environment. It also enters surface water by the natural weathering and leaching processes of minerals and rocks (13).

Many nickel salts are water-soluble, therefore, contamination of water can arise ; significant problems are associated with industrial discharge of nickel containing effluents to rivers (10). Nikel concentration can approach 0.46-3.4 mg/L in mine drainage and 2-900 mg/L (rinse water) in plating plants. Wastewater from paint-ink formulation and porcelain enamelling industries contains nickel concentrations ranging from 0.40 mg/L to 67 mg/L nickel (19). Nickel can exist in the oxidation states ranging from –1 to +4, but its aqueous chemistry is dominated by the +2 (nickelous) state. This ion forms stable complexes with both organic and inorganic ligands and is also adsorbed onto particulate matter. The commonest inorganic ligands in natural water are halides, sulphate, phosphate, carbonate and carbonyls. Humic and fulvic acids from medium-strong complexes with nickel. As a result nickel is a fairly mobile metal in natural waters. Few data have been reported on nickel in air and it has been estimated to be less than 0.5 $\mu g/m^3$. However, higher levels have been reported in the past in industrial areas. A typical urban air concentration has been found to be 0.2 $\mu g/m^3$. The nickel ions in food have been reported, little is known about the chemical form of nickel

in food, although it is found to be partly complexed with phytic acid. Nickel ions have been found in wines, 100 μg/L and in beers, 50 μg/L. Moreover, it has been reported that cigarettes contain about 10-20 % of nickel. This appears to be a volatile nickel compound as nickel carbonyl . Someone who smokes 20 cigarettes a day might inhale 40-80 μg of nickel (3,13).

2.3.2 The Health Effect on Human

Nickel is regarded as an essential trace metal, but in large amounts it is toxic to humans. It is less toxic than mercury, copper, cadmium and silver, but more toxic than lead and zinc to plant. It is reported that a concentration of 0.1-9.5 mg Ni/L can reduce growth and slows down photosynthesis and toxicity to fish . The toxic effect is reduced by hardness in the water as with other metals (13).

Nikel at high doses can be carcinogenic and teratogenic to humans. Its toxicity is enhanced in the presence of other metals such as cobalt, copper, iron and zinc. As in the case of other diavelent cations, nickel can react with DNA, and at high concentrations they have been reported that can result in DNA damage (2,3). Moreover there are studies using large numbers of patients that have been investigated the role of contact dermatitis in eczema of the hands. At last between 4% and 9% of the patients were found to respond positively to nickel patch tests and especially women are seen to be more sensitive to nickel than men (2,3).

2.4. Remediation of Heavy Metals

The effluents or waters contaminated with heavy metals must be treated. The data in Table 2.5, which gives the typical composition of an untreated metal finishing effluent. The degree of treatment may range from a main process stream for a seriously polluted industrial waste to a polishing process to remove the trace concentrations which can remain after the main treatment. Thus, the type of process or combination of processes used will depend on the metal(s) involved and the ultimate concentration allowed. There are a variety of treatment process for wastewater contaminated by heavy metals.

Table 2.5. The composition of a typical untreated metal finishing effluent

Metal	Concentration (mg/l)
Chromium	5-10
Copper	3-5
Nickel	5-10
Zinc	3-5
Cyanide	1-5
Suspended solids	10-50

Ref:14

2.4.1. Physical and Chemical Remediation of Metal Ions

The most commonly used methods of treating metal ions from watewater are the physical and chemical methods which are precipitation-coagulation-flocculation, chemical precipitation, chemical oxidation-reduction, ion exchange, and adsorption (1, 8,14,15).

2.4.1.1 Precipitation-Coagulation-Flocculation and Neutralization

Precipitation is the most commonly used and the simplest physical and chemical treatment system that separates solids from liquids by gravity settling . The chemical used in this process include metal ions (as hydroxides or sulphides) which hydrolyze rapidly to form insoluble precipitates, and natural or syhntetic organic polyelectrolytes, which are rapidly adsorbed on the surface of the particulates, thereby accelerating the rate at which the particulates aggregate. These aggregates (sludge) are then removed from the water by physical means such as gravity sedimentation , flotation, or filtration through granular media (12,14,15).

The aggregation of particulate material is a two-step sequential process. In the initial step , the interparticulate forces responsible for the stability of the particulates are reduced or eliminated by the addition of suitable chemicals. Subsequently, particulate collisions occur due to transport by molecular motion or mechenical mixing. If these collisions are successful, aggregation occurs. The chemicals used to destabilize particulates are known as coagulants. Chemical handling and feeding equipment must be designed for preparation of the chemical coagulant prior to addition. The coagulant is

injected into the process stream through a mixing that should provide rapid dispersion of the coagulant in the water. This rapid mixing stage which occurs over a short time usually less than one minute and this phenomenon is called coagulation. Following destabilization , less intense mixing of the particulates must be provided to increase the rate of particulate collisions without breaking up the aggregates being formed and this phenomena is called flocculation (8).

There are many chemical processes that can be used to treat hazardous wastes and metal ions, and the process decision depends primarily on the characteristic of the waste. For example if the pH of the waste is less than 2 or more than 12.5 then it is corrosive and can effect the second treatment process efficiency. Therefore such wastes can be chemically neutralized. Acidic wastewaters are usually neutralized with slaked lime ($Ca(OH)_2$) in a continuosly stirred chemical reactor. The rate of addition of lime is controlled with a feedback control system that monitors pH and adjusts the feed rate accordingly. Alkaline wastewaters may be neutralized by adding acid directly or by bubbling in gaseous CO_2 , forming carbonic acid (H_2CO_3). The advantage of CO_2 is that it is often readily available in the exhaust gas from any combustion process at the treatment site. Simultaneous neutralization of acid and caustic waste can be accomplished in the same vessel (12,15,16).

2.4.1.2. Chemical Precipitation

The ability to adjust pH is important not only for waste neutralization, but also it facilitates other chemical processes that actually remove undesirable substances from the waste stream. For example, a common method for removing heavy metals from the waste is via chemical precipiation, which is pH dependent. By property adjusting pH, the solubility of toxic metals can be decreased, leading to formation of a precipitate that can be removed by settling and filtration. Frequently , the precipitation involves the use of lime, $Ca(OH)_2$, or caustic (NaOH) to form metal hydroxides. For example, the following reaction suggests the use of lime to form the hydroxide of a divalent metal (M^{2+}):

$$M^{2+} + Ca(OH)_2 \rightarrow M(OH)_2 + Ca^{2+} \qquad (2.1)$$

Metal hydroxides are relatively insoluble in basic solutions, and they are *amphoteric*- that is , they have some pH at which their solubility is a minimum. Since each metal has its own optimum pH (see Table 2.6) , it is tricky to control precipitation of a mix of different metals in the same waste. For a waste containing several metals , it may be necessary to use more than one stage of precipitation to allow different values of pH to control the removal of different metals. While hydroxide precipitation using lime is the most common metal removal process, even lower concentrations of metals in the effluent can be obtained by precipitating the metals as sulfides. As can be seen in the Equation 2.1, metal sulfides are considerably less soluble than metal hydoxides. A disadvantage of sulfide precipitation is the potential formation of odours and toxic hydrogen sulfide gas (8,12,18).

Table 2.6. The pH values for hydroxide precipitation

Metal	pH value
Aluminum	5.2
Iron	4.3
Chromium	6.5-7.3
Copper	7.1-7.3
Nickel	9.2-9.4
Zinc	8.3-8.5
Cadmium	9.7
Lead	6.3
Tin	1.0-4.5

2.4.2. Chemical Oxidation / Reduction

Oxidation/ reduction (redox) reactions provide another important chemical treatment alternative for heavy metals. When electrons are removed from an ion, atom, or molecule, the substance is oxidized ; when electrons are added, it is reduced. Both oxidation and reduction occur in the same reaction, hence the abbreviation redox. One of the most important redox treatment processes is the reduction of hexavalent chromium (Cr VI) to trivalent chromium (Cr III) in large electroplating operations. Sulfur dioxide is often used as the reducing agent, as shown in the following reactions:

$$SO_2 + H_2O \rightarrow H_2SO_3 \quad (2.2)$$

$$2\,CrO_3 + 3\,H_2SO_3 \rightarrow Cr_2(SO_3)_4 + 3\,H_2O \quad (2.3)$$

The trivalent chromium formed in reaction (2.3) is much less toxic and more easily precipitated than the original hexavelant chromium. The chromium in reaction (2.3) is reduced from an oxidation state of +6 to +3, while the sulfur is oxidized from +4 to +6. Another important redox treatment involves the oxidation of cyanide wastes, which are also common in the metal finishing industry. In the following reactions, cyanide is first converted to less toxic cyanate using alkaline chlorination (pH above 10) ; further chlorination oxidizes the cyanite to simple carbon dioxide and nitrogen gas (8,12-15,18).

$$NaCN + Cl_2 + 2\,NaOH \rightarrow NaCNO + 2\,NaCl + H_2O \quad (2.4)$$

$$2\,NaCNO + 3\,Cl_2 + 4\,NaOH \rightarrow 2\,CO_2 + N_2 + 6\,NaCl + 2\,H_2O \quad (2.5)$$

2.4.3. Ion Exchange

Ion exchange is primarily used for the removal of hardness ions and heavy metals. Ion exchange is a physical-chemical process by which ions are transfered from a solid to a liquid phase or vice versa. Ions held by electrostatic forces to charged functional groups on the surfaces of a solid and are exchanged for ions in solution that is charged like the solid surface being contacted. Because the exchange occurs at the surface of the solid, ion exchange is typically classified as a sorption process and can be differentiated from adsorption by the chemical and electrical potentials that control the exchange of mobile ions between the solid and solution (8).

The mechanism of all ion exchangers both natural or synthetic, have fixed ionic groups that are balanced by ions of opposite charge to maintain electroneutrality. The ions either cations or anions exchange with ions in solution. For example, mostly used cation exchange resin and the resin initially containing cation A^+ is placed in a solution containing cation B^+ due to the concentration difference between the ions A^+ and B^+ in the resin and in the solution. The equation used to describe this exchange reaction is given below:

$$B^+ + (R^-)\ A^+ \leftrightarrow A^+ + (R^-)\ B^+ \qquad (2.6)$$

Where R^- represents the negatively charged funtional groups of the resin (8).

The most used applications of ion exchange are for the removal of hardness ions such as Ca^{2+} and Mg^{2+} called softening water from domestic and industrial waters and for complete demineralization of waters for industrial purposes. The softening process replaces the calcium and magnesium in the water with sodium and demineralization accomplished as a two-step. Firstly, all cations being exchanged for H^+ ions in a cation exchange and then all anions being replaced by OH^- ions in an anion exchanger. Fe (II) and Mn(II) can also be removed from water by ion exchange, altough control of oxidation states is important because Fe (III) and Mn (IV) will full the resins. Another application of ion exchange is for treatment of heavy metal ions such as chromium, copper, lead, gold, silver platinum and uranium for the electronic and pharmaceutical industries and for nuclear reactors, hospitals and laboratories (8,14,15). Today for ion exchange synthetic ion exchange resins are used for treatment process. However, some natural exchangers have been shown to be more afficient. The mostly prefered exchangers are clinoptilolite, a sodium-calcium-aluminum silicate, activated alumina and recently microorganisms that are still under investigation as ion exchangers especially for heavy metal removal.

2.4.4. Adsorption

Adsorption is another technology which has been examined and used for removing heavy metals. The adsorbent which has probably received most attention is granular activated carbon (GAC). Some metals such as chromium, lead and copper have been removed by using GAC (1,8). However carbon adsorption is an expensive treatment process . Therefore, there is a need of considerable research for the development of alternative low-cost adsorbents. Further information about adsorption processes is given in Chapter 3.

Technologies used, at present, for heavy metal removal or other hazardous wastes have some disadvantages. One of them is that they produce sludge and therefore, they transform an aquatic pollution problem to one that associated with solid waste disposal and sometimes lead to incomplete removal of heavy metals. Another important

factor is the difficulty of the application of these methods which makes the system costly. Therefore a new technology should be considered that should be cheap, effective and environmentally safe. To date a lot of researchers have mentioned about the advantages of the biosorption. Which nowadays there are some published studies about biosorption of heavy metals and many researchers focused on this process that is considered as the future's technology.

Chapter III

ADSORPTION

3.1 Definition and Application

Over the last few decades adsorption has gained importance as a purification and seperation process on an industrial scale. The ability of many porous substances to adsorb vapors in large quantities has been recognized since the 18^{th} century, but application on an industrial scale has been more recently enhanced by the advancement in the studies of adsorption fundamentals. However, to a considerable extent the mechanism of adsorption remains surrounded by queries, and therefore studies are required to establish relations between the many system variables and the performance of the adsorber. Adsorption is defined as physical and/or chemical process in which a substance is accumulated at an interface between phases. The adsorbate is the substance being removed from the liquid phase to the interface and the adsorbent is the solid phase onto which the accumulation occurs (9,11).

One of the earliest adsorption application is purification, such as the removal of H_2S obnoxius fumes from air and the removal of organic compounds from liquid water. Such materials are normally present in relatively low concentrations and are destroyed after removal. Other examples of purification are the removal of odor and color from edible oils, decolorization in the sugar industry, solute removal in tobacco manufacturing, and the removal of unwanted hydrocarbons in oil refining. For recovery purposes, adsorption has been applied to recover some biological materials , organic compounds and metals. Another recent application is the immobilization of enzymes and microbial cells for conducting biochemical reactions such as modification of edible oils and treatment of wastewater by biosorption (9,11).

3.2 The Mechanism of Adsorption

Adsorption of substances onto adsorbents takes place because there are forces that attract the absorbate to the solid surface from solution. The specific forces or

mechanism by which adsorbate is attracted to the solid solution interface can be physical or chemical (8,9,11).

3.2.1. Physical Adsorption

The electrostatic force is the basic physical principle that describes the interactions between molecules of adsorbent and adsorbate. In physisorption that is reversible, the forces between the sorbate and sorbent is weak and the system has low heat of adsorption. The physical interactions among molecules that based on the electrostatic force include dipole-dipole interactions, dispersion interactions and hydrogen bonding. When there is a net separation of positive and negative charges within the molecule, it is said to have a dipole moment. H_2O and NH_3 are example that have permanent dipoles because of the configuration of atoms and electrons within them. They are polar compounds. When two dipoles are near each other, they tend to orient their charges to lower their combined free energy by tending the negative charges of one to approach postive charges of the other. The attraction between the two molecules are the net dipole-dipole attraction. The hydrogen bonding is a special dipole-dipole interactions in which the hydrogen atom in a molecule has a partial positive charge and attracts an atom on the other molecule that has a partial negative charge. The other interaction is the dispersion interaction or the Van der Waals force. When two neutral molecules that lack permanent dipoles approach each other, a weak polarization is induced in each because of quantom mechanical interaction between them. The net effect is a weak attraction between the molecules (8,9,11).

3.2.2 Chemical Adsorption

Chemical adsorption (chemisorption) is also based on electrostatic forces. The difference between physisorption and chemisorption are; the attraction between adsorbent and adsorbate in chemisorpion that of a covalent or electrostatic chemical bond between atoms approaches with shorter bond length and higher bond energy. Adsorbates bound by chemical sorption to a surface generally cannot accumulate at more than one molecular layer or monolayer because of the specificity of the bond between adsorbate and surface. The bond may also be specific to particular site or functional groups on the surface of adsorbents. One example is the chemical bonding of

adsorbate to specific surface sites which is acid-base reactions at a functional group. The hydrated metal ions from solution with hydroxide sites on metal oxides are followed:

$$ROH\ (aq) + SOH \Leftrightarrow SOR + H_2O\ (aq)$$

R is metal ion adsorbate and S is metal oxide adsorbent. This type reaction is used for remove heavy metals by adsorption onto silica and aluminum oxide based clays and sands for water treatment (8,9,11).

3.3 Adsorption Isotherms

Adsorption isotherm specifies the equilibrium surface concentration of adsorbate on adsorbent as a function of bulk concentration of adsorbate in solution. It is called isotherm because it describes the equilibrium state of adsorbate, adsorbent and solute at a given temperature (8) . Over the years, several researchers have proposed several isotherms based on different assumptions. However, in this chapter only the Langmuir and Freundlich adsorption isotherms were discussed because these two isotherms have been used to explain the biosorption mechanism of metal species by fungi (1,10).

3.3.1 The Langmuir Adsorption Isotherms

This type of isotherm is proposed by Langmuir (1914) for homogeneous adsorption. It assumes a uniform adsorbent surface with energetically identical sorption sites. The Langmuir adsorption isotherm has found wide applicability to adsorption of metals in water treatment because of its simplicity and its ability to fit a broad range of experimental data. The Langmuir equation can be represented by the following equation:

$$q = q_{max}\, b\, C / (1+bC) \qquad (3.1)$$

Where q is the amount of adsorbed per unit mass adsorbent, q_{max} is the maximum amount of adsorbed per unit mass adsorbent or the monolayer capacity, b is

an empirical constant that reflects the affinity between adsorbent and adsorbate and C is the concentration of adsorbate in solution at equilibrium. The experimental data may be plotted to estimate q_{max} and b with rearranging Langmuir equation to:

$$1/q = 1/ q_{max} + 1/(b\ q_{max}C) \qquad (3.2)$$

so that plot of $1/q$ against $1/C$ has slope $1/b\ q_{max}$ and intercept $1/ q_{max}$.

The assumption that limited Langmuir isotherms are; firstly the energy of adsorption is independent of degree of coverage, secondly reversibly of bonding and last one is the allowance for at most only one monolayer. The q values is assumed to approach a saturating value , $q_{max,}$ if C value becomes very large (1,8,9,11).

The Langmuir model which is simply used for biosorption phenomena of one component metal ions has a theoretical basis that relies on a postulated chemical or physical interaction (or both) between solute and vacant sites on the adsorbent surface. The heat (ΔH) of adsorption should be independent of the fraction of surface covered by the adsorbed solute ($\theta = q_{eq} / q_{max}$) according to the ideal Langmuir model. q_{max} is supposed to represent a fixed number of surface sites and it should therefore be a temperature-independent constant while the temperature dependence of the equilibrium constant should follow the Arrhenius equation. The heat of adsorption related to the b constant has the form:

$$b = b_o \exp(-\Delta H / RT) \qquad (3.3)$$

Where b is constant related to the energy of adsorption in which bo is a constant containing the entropy term. ΔH is the heat of adsorption, R is the universal gas constant and T is the absolute temperature. The assumptions of identical sites with no interaction between adsorbed molecules imply that the heat of adsorption is independent of coverage and has been calculated using the Langmuir constant b. The heat of adsorption can be obtained by calculating the slope of ln b versus 1/T plot (11,22,25).

3.3.2 The Freundlich Adsorption Isotherm

The Freundlich isotherm describes equilibrium on heterogeneous surfaces and hence does not assume monolayer capacity. Mathematically , it is expressed by:

$$q = K C^{1/n} \qquad (3.4)$$

Where K is an indication of the adsorption capacity of the adsorbent and $1/n$ indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. Its values range between 0 and 1. The log-log plot q against C for this equation is linear. Here the surface concentration of adsorbate does not approach a saturation value as C increases, since there is always surface sites with higher energies of adsorption to fill. The Freundlich isotherm is very widely used to fit observed data empirically even when there is no basis for its underlying assumptions (8,9,11).

3.4 Kinetics of adsorption

The thermodynamic laws specify an equilibrium state between the adsorbent and adsorbates, the removal of compounds in water treatment is often determined by the rate of adsorption during contact with adsorbent. The adsorption rate is

$$r_a = kC\,(1 - \theta) \qquad (3.5)$$

and the desorption rate is

$$r_d = k'\theta \qquad (3.6)$$

where C is the unadsorbed solute concentration in solution, k and k' , respectively, the adsorption and desorption rate constants and θ, the fraction of surface covered by adsorbed solute. At equilibrium, the equality of these two rates leads to the Langmuir adsorption isotherm:

$$\theta = KC / (1 + KC) \qquad (3.7)$$

where the adsorption equilibrium constant is $K = k/k'$. Combining Eq. (3.5) and Eq.(3.6), the Langmuir – Hinshelwood adsorption equation modified for monolayer adsorption is obtained and the rate of adsorption is given as follows :

$$r = kC / (1 + KC) \quad (3.8)$$

In an experimental data plot of rate versus C, the rate of adsorption is proportional to the first power of the concentration of metal ion at low bulk concentrations and can be given using Eq. (3.9):

$$r = kC \quad (3.9)$$

At higher bulk metal ion concentrations, the rate of adsorption becomes independent of bulk metal ion concentration. Eq. (3.8) can describe the rate of adsorption very accurately in both of these situations. This kind of rate equation is also defined as " saturation type rate ". This rate equation can be linearized by plotting $1/r$ versus $1/C$ to determine the rate and equilibrium constants of adsorption from the slope and intercept, $1/k$ and K/k, respectively.

The process of adsorption can be categorized as a set of sequential steps. The first step is transport of solute from bulk solution phase to the boundary layer or surface film surrounding the adsorbent particle. The second step is transport of solute across the boundary layer to the exterior surface of the adsorbent particle. Molecular diffusion or eddy diffusion controls transport across the film or boundary layer. For adsorbents used in water treatment, most of the active surface area occurs in pores within adsorbent particles. The third kinetic step is diffusion of solute within these pores, from the exterior of the particle to the interior surfaces of the particle. Similarly, solute may be transported along surfaces of pore walls. The final step is the physical or chemical binding of adsorbate to the internal surface of the adsorbent. The rate of this step is controlled by chemical kinetics at the molecular level (8,11).

The kinetics of adsorption can be characterized with respect to dependence on operating parameters such as temperature and bulk concentration. The magnitude of the heat effect for the biosorption process is the most important criterion to develop a thermodynamic and kinetic relationship for metal-microorganism interaction process. However, the kinetics of the metal uptake process and the description of the thermal

properties of the biosorption remain essentially unknown and little study on the evaluation of the enthalpy change of biosorption have been given.

Chapter IV

BIOSORPTION BY FUNGAL BIOMASS

The importance of metallic ions to fungal and yeast metabolism has been known for a long time.The exact role of the metallic species in metabolic activities of the cell, however, is still being studied and in many instances it is not fully understood. Metals are known to play an important role in many key metabolic catalyzed by enzymes. The inhibitory role of metals in biochemical reactions is known but these aspects are the basis for their toxicity to the microrganisms, fungi being no exception, and to the higher organisms as well.The fungi and yeast are used in a variety of industrial fermentation processes. Such industrial fermentation processes can serve as an economical and constant supply source of biomass for biosorption of metal ions, because the biomass could be available in large quantities from established fermentation process. On the other hand, there is a possibility to use fungal biomass as biosorbent for detoxification of industrial effluent streams by removing their toxic heavy metals component. Another aspect of using fungal biomass, the sequestered metals of value could be recovered and recycled or resold. These decrease the costs of wastewater treatment process and the industrial operation (1,10,32-36,39,43,45).

4.1 Fungi

4.1.1 Characterization of Fungi

Fungi are a eukaryotic, nonphotosynthetic group of microorganisms that relying on organic substrates as their source of carbon and energy for growth and metabolic activities. Most fungi are coenocytic organisms and feature a vegetative structure known as mycelium consisting of thread-like projections called hyphae forming a multinucleate mass of cytoplasm. That enclosed within a rigid, very branched system of tubes fairly uniform in their diameter. A mycelium normally germinates from a single reproductive cell or spore which may be mobile in the water molds and phycomycetes. Although higher fungi and their spores are incapable of movement, the internal contents of mycelium show streaming movements of the cytoplasm enclosed within its

wall. In nature, the vegatative mycelium of fungi is rarely seen because it is normally embedded in soil and other opaque substrates. Some fungi (the mushrooms) form specialized spore-bearing fruiting macroscobic structures.

Their morphology (shape), physiology, reproductive mode, metabolic activities, energy storage products, chemical composition and many other features serve in their taxonomic classification. The division and classification of fungi start with four large group:

- Phycomycetes or aquatic molds are generally characterized by a mycelium with no partitions (nonseptate mycelium) and endogenous spore formation inside a sac-like structure .
- Ascomycetes form sac-like structures bearing four, eight or more ascopores originating from two meiotic followed by one or more mitotic divisions after a fusion of opposite sex nuclei.
- Basidiomycetes form four special basidiospores originating from meiotic divisions of a diploid nucleus. These are not in a sac.
- Fungi Imperfecti lack a sexual reproduction mode called asexual spores that are always exogenous and constitute a provisional taxonomic group.

A few groups in these classes have largely lost their mycelial habit of growth and became unicellular. Such fungi are collectively known as yeasts which multiply predominantly by budding and which have become very important to man. The widely used *Saccharomyces cerevisiae* which can grow anaerobically (brewer's yeast) for producing ethanol or aerobically as baker's yeast which is an ascomycetous yeast (10, 17).

4.1.1.1 The Baker's Yeast

Baker's yeast is a mass of viable yeast cells of the *Saccharomyces cerevisiae* genus. It is produced through fermentation process. A yeast which is a unicellular fungus has a nucleus contains two sets of chromosomes.

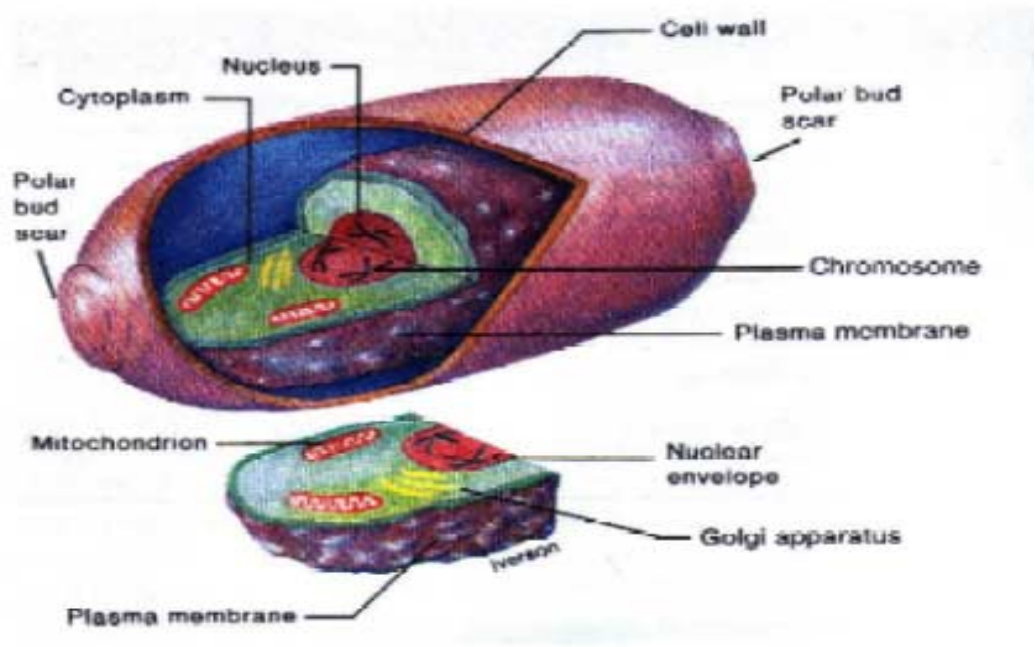

Figure. 4.1. Diagrammatic drawing of a yeast cell showing typical morphologhy

Generally yeast cells are larger than bacteria and vary considerably in size. Their appearance is spherical or egg - shaped. They have no flagella but do possess most of the other eucoryatic organelles. The yeast *Saccharomyces cerevisiae* seen in Figure 4.1.

They have a single nucleus and reproducers either asexually by budding and transverse division or sexually through spore formation. Each bud that separated can grow into a new yeast, and some group together to form colonies (see Figure 4.2). During budding there is a complete division of this double set of chromosomes, each daughter cell that is vegetatively produced again contains a complete double set of chromosomes. The cell is approximetly 5-8 μm in size (17,10).

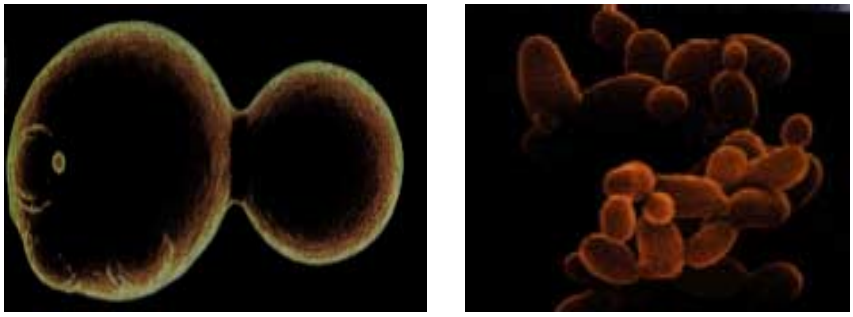

Figure.4.2. The yeast *Saccharomyces cerevisiae* reproducing colonies by budding.

4.1.1.2. The Cell Wall Structure of Yeast

The sequestering of metallic species by fungal biomass which constitutes the basis of biosorbent behaviour has mainly been traced to the cell wall. They may also be found within the cell associated with various organelles or they crystalize in the cytoplasm. However, the cell wall can be considered the primary site and the one which metallic ion from the environment encountered first and where most of the metals are found (2,4,10,16).

The cell wall of *Saccharomyces cerevisiae* is approximately 70 nm thick and contains a number of polymers including glucan (28.8 %), mannan (31%), protein (13%), lipid (8.5 %) chitin-chitosan (2%) and a small percentage of inorganic ions such as Ca^{+2}, Mg^{2+} and K^{+2} (3%) of the cell wall mass (see Figure 5.4) (10,17,30).

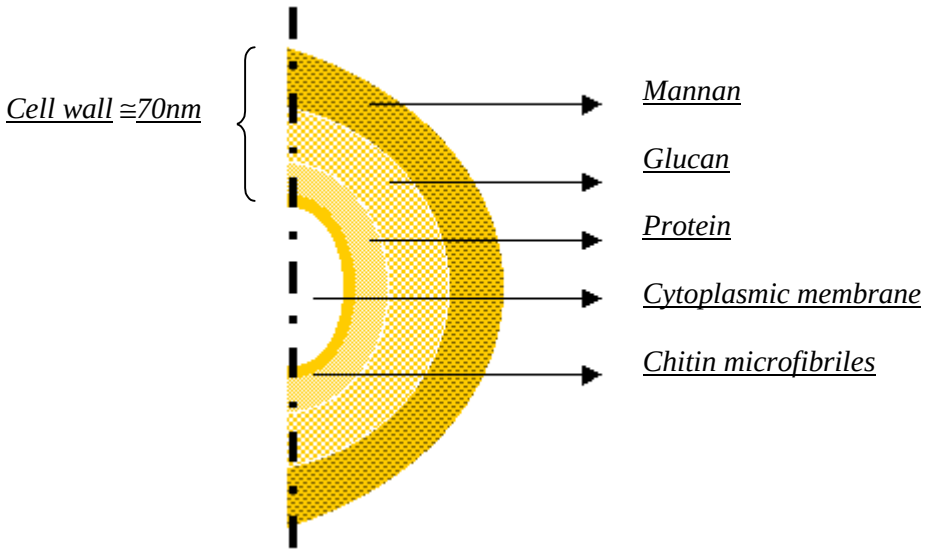

Figure. 4.3. A schematic diagram of a yeast cell wall

Glucan a polymer of β (1-3) linked glucose with β (1-6) branches. It is found primarily on the cell membrane side of the cell wall, its main function is maintaining. The outer layer of the cell wall consists of mannan polymers linked to proteins. This matrix is crosslinked by disulfide bonds and intrachain hydrogen bonding. Mannan is a polymer of mannose monomers forming a main chain linked by α (1-6) bonds and side chains with α (1-3) and α (1-3) bonded mannose residues which branch from the main chain via α (1-2) links. The mannan is found as a covelently linked protein-

polysaccharide complex of 25 to 500 kDa, of which the protein usually contributes 5 to 10 % and poly-mannose branches are crosslinked via phosphate on the mannose residues (30,17).

Protein is found throughout the cell wall of *S. cerevisiae* but it is more prominent in the outer layer. Chitin is a polymer of *N*-acetylglucosamine residues linked by β (1-4) glycosidic links and is associated with protein in the cell walls to which is linked via nonaromatic amino acid residues. Chitosan is produced by the deacetylation of chitin that is found naturally in fungal cell walls. Chitin is found as microfibrils in the inner layer of the cell wall in the glucan matrix and in bud scars.The inorganic ions generally exist in the cell membrane and in cytoplasm (30).

4.1.1.3 Essential Nutrients and Environmental Conditions for the Growth of Yeast

The cell synthesizes the many individual substances of the biological cell, including the structurall elements. One of the most important nutrients for the yeast is an assimilable carbonic organic composition that serves as a source of carbon also as a source of energy requirement for the metabolism. *S. cerevisiae* requires the following essential nutrients and growth promoters for the multiplication of its cells. For Baker's Yeast in the presence of atmosheric oxygen: (17)

- A source of assimilable organic carbon and energy
- An assimilable nitrogen composition
- The essential minerals such as PO_4^{+2} , K^+, SO_4^{-2} , Mg^{2+} and trace elements such as Fe, Cu, Zn and Mn
- The growth promoters biotin, pantothenic acid and m-inositol

4.2 The Metal Ion Uptake by Fungi

The metal ion uptake by both living and dead cells can consist of two differing modes. The first uptake mode is independent of cell metabolic activity, and is termed as biosorption or passive uptake. It involves the surface binding of metal ions to cell walls and extracellular material. The second mode of metal uptake into the cell across the cell

membrane is dependent on the cell metabolism, and is termed as intracellular uptake, active uptake or bio-accumulation. The first mode is common to metal uptake by both living and dead cells. The second mode that is depending on metabolism, occurs in living cells (see Figure 4.4). This slow phase of metal uptake can be due to a number of mechanisms, including covalent bonding, surface precipitation, redox reactions, crystallization on the cell surface or membrane transport. Sometimes this slow uptake requires metabolic energy, indicating an active transport. The metal uptake is facilitated by the production of metal-binding proteins. Therefore , metal uptake may take place by different modes, depending on whether the cells are dead or living (1,2,4,5,10,16,18).

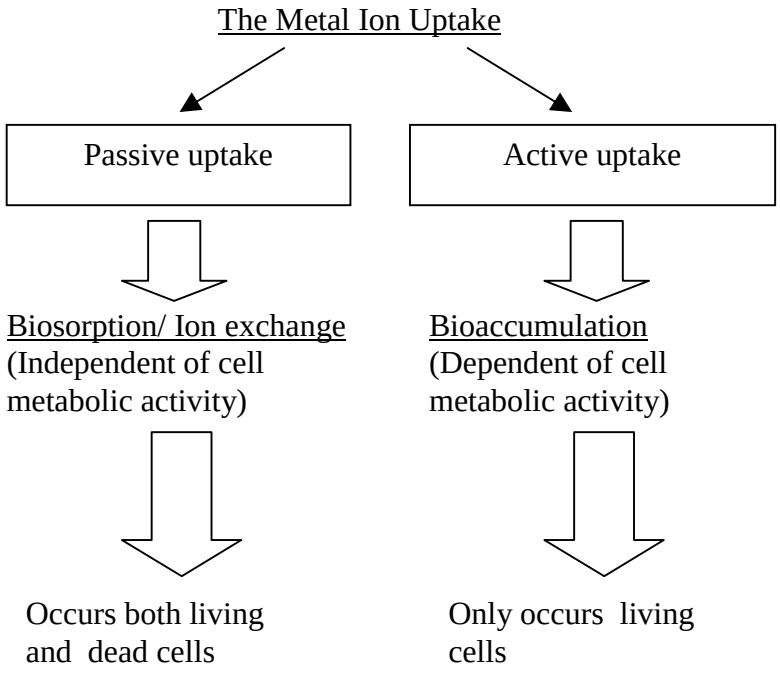

Figure.4.4 The metal ion uptake by a microorganism

4.2.1 Biosorption Mechanism

The biosorption can be demonstrated with both dead and living biomass and defined as a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions. Biomass acting just as a chemical substance, as an ion exchanger of biological origin particularly the cell wall of the microorganism was found to be responsible for this phenomenon. There are two mechanisms involved in biosorption:

- *Ion exchange* : ions such as Na, K, Mg and Ca on the cell wall surface become displaced by heavy metal ions,
- *Complexation between metal ions* ; various functional group of the cell wall such as carboxyl ; amino; thiol; hydroxy; phosphate and hydroxy-carboxyl interact in coordinated way with metal ions (1,4,5,10,16,18).

4.2.2 Factors Affecting Biosorption

Biosorption of heavy metals is affected by many experimental factors such as pH, temperature, biomass concentration and initial metal concentration. Recently, pretreatment of biomass by using some chemical substances have been reported that can increase the biosorption capacity.

4.2.2.1 pH Effect on Biosorption

Several researchers have investigated the effect of pH of heavy metals by using different kinds of microbial biomass. For example, the biosorption of Cu(II), Cd(II) and Pb(II) by S.*cerevisiae* was pH dependent and maximum biosorption was obtained in the pH range 5.0-7.0 (28,33,39,40,46). Maximum nickel biosorption by chlorella species and *R.arrhizus* were investigated at pH 7 and 4.5 (19,24,25). Biosorption of lead(II), copper(II), and nickel(II) by *R.arrhizus* and *Z.ramigera* were maximum at pH in the range 4.0-5.0 (22,37). Another search for removal of Cd(II), Pb(II) and Cu(II) by *Phanerochaete chrysosporium* was maximum at pH 6 (22,27,37,49). It is reported that at pHs lower than optimum values, protonation of the cell component adversely affected the biosorption and during ion exchange process, H^+ ions are replaced instead of metal ions on the cell wall. At higher pH values metals were precipitated because of high concentration of OH^- ions.

4.2.2.2. Temperature Effect on Biosorption

Temperature can have a significant effect on biosorption. For example , the optimum biosorption temperature for Pb(II), Cu(II) and Ni(II) by *R.arrhizus* was determined to be 25^0C for; and for Cu(II), Cd(II) and Pb(II) by *S.cerevisiae* was determined to be 25^0C (35,42,49). However , the optimum biosorption temperature of

the same metal ions by *Phanerochacte chrysosporium* and by *Sreptomyces noursei* were determined to be 30^0C (32,43). On the other hand, Fe(III) and Pb(II) biosorption by *Zoogloea ramigera* was increased with increasing temperature upto 45^0C (25). Many researchers have reported that at low temperature the binding of heavy metal ions to the microorganisms occured by a physical adsorption process and an equilibrium between the cell wall surface and the metal ions was usually rapidly and easily reversible , because of small energy requirement (22,25).

4.2.2.3. The Effect of Biomass Concentration on Biosorption

The biosorbent concentration has been shown as one of the important factor in the biosorption process. In the literature there are examples of the effect of biomass concentration on heavy metal biosorption. It has been found that the metal uptake was increased when the biomass concentration decreases (31). Such behaviours have been explained that an increase in biomass concentration leads to interference between the binding sites (31,47).

4.2.2.4. The Effect of Initial Metal Concentration on Biosorption

Another factor that affects biosorption process is the initial metal concentration. It has been reported that generally the adsorption rate increased with increasing initial metal concentration. For example, adsorption of Fe(II), Pb(II) and Cd(II) by *S. leibleini* has increased with increasing initial metal ion concentrations up to 150 mg/L , at high concentration the adsorption rates have not been changed (50). This type of reaction rate was termed as “saturation type reaction rate”. The adsorption yields ($Y= q_{1=\infty} / C_0$) decreased while the maximum adsorbed metal amounts per unit mass of dry biomass increased by increasing the initial metal ion concentration (21,24,25,27,34,35,42).

4.2.2.5 The Effect of Pretreatment on Biosorption

Living cells have been pretreated using physical and chemical methods to increase the metal biosorption capacity. Physical pretreatment methods have included heat treatment, autoclaving, freeze drying and boiling. Chemical pretreatment methods

such as contacting especially fungal cells with acids, alkaline and organic chemicals that increase the biosorption capacity have been reported in the literature (1,26,27,41,42.44). One of them is pretreatment of *Aspergillus niger* using sodium hydroxide, formaldehyde, dimethyl sulphoxide and detergent. This has increased in biosorption of lead, cadmium, copper (26). The pretreated *R. arrhizus* with formaldehyde have increased biosorption of lead and nickel (27). Moreover, *S.cerevisiae* with hot alkali treatment by using NaOH have been observed as increases in biosorption of lead, copper and cadmium (41,42,44).

Chapter V

EXPERIMENTAL

5.1 Materials

5.1.1 Microorganism

The industrial strain of *Saccharomyces cerevisiae,* collected from the waste of Pakmaya Baker's Yeast Industry (İzmir, Turkey) was used as the biosorbent in this study. The waste biomass was stored at 4 ^{o}C after biomass suspensions were prepared in concentrations of 10 g dry cell per litre deionized water. pH of this stock yeast solution was around 5.5. The suspension was directly diluted into the flasks containing metal solutions to be used for biosorption experiments as live biomass.

5.1.2 Chemicals

Nickel (II) solutions were prepared by diluting 1000 ppm stock solutions of nickel (II) obtained by dissolving Ni $(NO_3)_2 .6H_2O$ in deionized water. The live biomass was pretreated with sodium hydroxide, formaldehyde, nitric acid and ethanol. The pH of biosorption medium was adjusted by using 0.1 M HCl and 0.1 M NaOH. The chemicals used in this study is given in Table 5.1.

Table 5.1 The chemicals and their properties.

Chemical	**Purity and properties**	**Producer**
Ethanol (C_2H_5OH)	% 95	Carlo-Erba
Formaldeyhde	% 37, d=1.09 g/cm^3	Merck
Hydorchloric acid (HCl)	% 37, d= 1.19 g/cm^3	Aldrich
Nitric acid (HNO_3)	% 65, d= 1.40 g/cm^3	Merck
Sodium hydroxide (NaOH)	% 98.99, pellet	Aldrich
Nickel (II) Nitrate (Ni ($NO_3)_2$. $6H_2O$)	% 99.98, d= 2.05 g/cm^3	Aldrich
Nickel (ICP)	ICP std solution, 1000 ppm	Merck
Deionized water (WaterPro PS, model 90007 – 05)	18.1 megaohm	Labconco

5.2 Methods

5.2.1 Pretreatment of Waste Yeast

The live *S.cerevisiae* was treated with some chemicals in order to prepare its surface for biosorption. Thirty gr dry weight of biomass was pretreated in five different ways as descibed below (26):

A. Live biomass without pretreatment.
B. Boiled for 15 min in 500 ml of 0.5 N sodium hydroxide solution.
C. Boiled for 15 min in 500 ml of 10 % (v/v) formaldehyde solution.
D. Boiled for 15 min in 500 ml of 10 % (v/v) nitric acid solution.
E. Heated for 15 min in 200 ml of ethanol under reflux condition.

After each pretreatment , the biomass was washed with excess amount of deionized water and then dried at 60 ^{o}C for 12 h. Sodium hydroxide pretreated biomass was washed with deionized water until the pH of the wash solution was in near neutral (6.8-7.2) range since high pH values (≥ 9) are responsible for nickel hydroxide precipitation.

5.2.2 Effect of pH on Biosorption

The experiments were carried out in well - stirred solutions with a volume of 100 ml. 100 mg/L nickel ion concentration and 1.0 g/L biomass concentration were used for each run. A series of biomass suspensions from pH 3 to 6 were adjusted with 0.1 M HCl and 0.1 M NaOH. For adjusting the pH's of biosorption medium to 3, 4, 5 and 6; 422 µl of 0.1 M HCl, 90 µl of 0.1 M HCl, 5 µl of 0.1 M HCl and 156 µl of 0.1 M NaOH were used, respectively. Adsorption experiments for each metal solution and pH were carried out in an orbital shaker at 150 rpm and 25^{o} C for 24 hours. Samples were then assayed and metal uptake by biomass for each run were calculated at different pH values.

5.2.3 Effect of Temperature on Biosorption

150 mg/L nickel solutions were prepared separately in total volume of 90 ml at pH 5.0. 10 ml concentrated biomass (1.0 g/L) was then added to the metal solutions. Samples were placed in an orbital shaker (150 rev min^{-1}) at different temperatures (15, 25, 35 and 45^{o} C). Under the specified conditions the same concentration of nickel without biomass were placed in order to check the loss in metal solution. After 24 hours (sufficient to reach equilibrium), 1.5 ml samples were taken, centrifuged and supernatants were analysed for nickel concentration. The uptake of nickel was determined from the difference between the initial and equilibrium concentrations.

5.2.4 Effect of Biomass Concentration on Biosorption

Biomass concentrations ranging from 0.5 to 5.0 g/L were used. The initial nickel concentration was 100 mg/L in 100 ml total solution volume and pH was adjusted to 5.0 with trace amount of 0.1 M HCl. After shaking the solutions at 150 rpm in an orbital shaker for 24 hours, the equilibrium was reached and samples were taken, centrifuged and analysed for final nickel concentration.

5.2.5 Effect of Initial Metal Concentration on Biosorption

90 ml of metal solutions containing 0, 50, 100, 150, 200 and 250 mg/L nickel(II) ion were mixed with 10 ml of 1.0 g/L biomass, and pH of the solution was adjusted to pH 5.0 with trace amount of 0.1 M HCl. The solutions were shaken in an orbital shaker at 25^{o} C, 150 rev min^{-1} for 24 hours. After reaching the equilibrium, 1.5 ml samples were taken, centrifuged and residual nickel concentrations were determined. From the difference between the initial and residual nickel concentration, amount of nickel adsorbed by biomass for each run were determined.

5.2.6 Determination of Biosorption Isotherms

The equilibrium adsorption isotherms were conducted in batch mode for different pH (3.0, 4.0, 5.0, 6.0) and temperature (15, 25, 35, 45^{o}C) ranges. The range of concentration of prepared nickel(II) solutions varied from 0 to 250 mg/L in biosorption

medium. All reactions were carried out in stirred solutions (total volume about 100 ml) with a typical volumetric ratio between biomass/bulk liquid of 1.0 g/L in an orbital shaker over 24 hours. Samples (1.5 ml) were taken, centrifuged in a Hettich, model EBA 12/12R centrifuge and analysed. The initial and equilibrium concentration of nickel was determined in each run. The initial and residual nickel(II) ion concentrations (C_{eq}) in solution were determined by using the ICP – AES equipment and the adsorbed metal amount per unit mass of waste yeast (q_{eq}) was calculated from the mass balance. Equilibrium adsorption isotherms were prepared by plotting the amount of nickel sorbed per mass of dry cells as a function of residual concentration of nickel at equilibrium. The mean values of triplicates of each concentration were plotted and fitted to both Langmuir and Freundlich isotherm equations.

5.2.7 Determination of Biosorption Kinetics

Adsorption kinetic experiments were performed in batch mode in well stirred vessels (100 ml). A nickel solution of known concentration was added to an appropriate amount of the yeast, pretreated before. The initial concentration of nickel (II) in the liquid phase was 100 mg/L and adsorption kinetic experiments were conducted for four different pH ranges (3.0, 4.0, 5.0 and 6.0) using 1.0 g/L biomass concentration. The solutions were agitated continuously in an orbital shaker at 25^{o}C and 150 rpm to ensure homogenous exposure to the metals. The decrease in the nickel concentration in solution due to adsorption was analysed by pipetting small amounts of solution (1.5 ml) and centrifuged. Samples were taken at short intervals (at the very beginning approximately every 60 seconds, then every 5 minutes) starting from the initial time for 60 minutes. After 2 hours equilibrium was reached, but in order to be sure more samples were taken and analysed over the 24 hours period. Each experiment was repeated three times, and mean values were presented (Chapter VI).

5.3 Analysis of Metal Ions

The concentration of unadsorbed nickel ions in the sample supernatant was determined by using an Inductively Coupled Plasma-Atomic Emission Spectrometer (Varian, ICP-AES, Axial Liberty). 1.5 ml samples were taken from the biosorption

medium and were centrifuged in a Hettich, model EBA 12/12R centrifuge at 10,000 rpm, 4^{o} C for 3 minutes. 1ml alliquot from each sample supernatant was pipetted into 10 ml volumetric flasks containing 18.1 megaohm deionized water and HNO_3. The acidity of the solution in each volumetric flask was adjusted to 5 % (v/v) HNO_3.

The standard nickel solution concentrations were prepared from 1000 ppm Ni standard solution in 5 % (v/v) HNO_3. The concentration range of the standard solutions covered the Ni (II) concentration in the samples after each biosorption experiment.

The blank solution was prepared in 100 ml volumetric flasks using 18.1 megaohm deionized water with HNO_3 concentration of 5 % (v/v).

The instrument operating conditions of ICP – AES are described in Table 5.2.

Table 5.2 ICP – AES (Axial Liberty) operating conditions

Argon gas flow	15 L/min
Argon auxiliary flow	1.50 L/min
PMT voltage	650 V
Sample uptake	30 sec
Rinse time	10 sec
Line for nickel element	231.604 nm

All the equipment (glass and plastic ware) were soaked in 1:5 nitric acid solution for 24 hours, rinsed thoroughly with deionized water and dried in an oven at 70-80^{o} C after each use (51,52).

Chapter VI

RESULTS AND DISCUSSION

6.1 Factors Affecting the Biosorption of Nickel by Waste Baker's Yeast

It is known that some parameters such as pH, temperature and bulk concentration may affect the biosorption capacity. Therefore, biosorption data of nickel(II) by waste yeast are presented in this section under different pH, temperature, biomass and metal concentration values at equilibrium to obtain the optimum conditions for this system.

6.1.1 Effect of Pretreatment

Live or dead cells of biomass can be used as an adsorbent material for the removal of toxic metal ions from aqueous solutions. The efficiency of dead cells in biosorbing metal ions may be greater, equivalent to, or less than that of living cells and may depend on factors such as the microorganism under consideration, pretreatment method used, and type of metal ion being studied (26). The biosorption capacities of live or dead biomass may vary great deal. Therefore, in this study live cells were used and the waste baker's yeast was pretreated in four different ways (see ChapterV) to determine the effect of pretreatment methods on the biosorption capacity of nickel since there is no information about this subject in the literature for *Saccharomyces cerevisae.*

Figure 6.1 shows the effect of pretreatment of *S. cerevisae* done by using sodium hydroxide, formaldehyde, nitric acid and ethanol on biosorption of nickel in comparison with live cells. Pretreatment of live biomass using ethanol and formaldehyde yielded with the 5.70 and 4.80 mg Ni(II)/g biomass uptake values, respectively. These values were found to be lower than that observed for live biomass, but higher than other pretreatment results. Pretreatments using NaOH decreased the biosorption of nickel to a value of 2.55 mg Ni(II)/g biomass. The lowest biosorption capacity of 0.54 mg Ni(II)/g biomass was found for the cells pretreated with HNO_3. Pretreatment of live biomass using four different chemicals did not improve the nickel biosorption in comparison with live biomass.

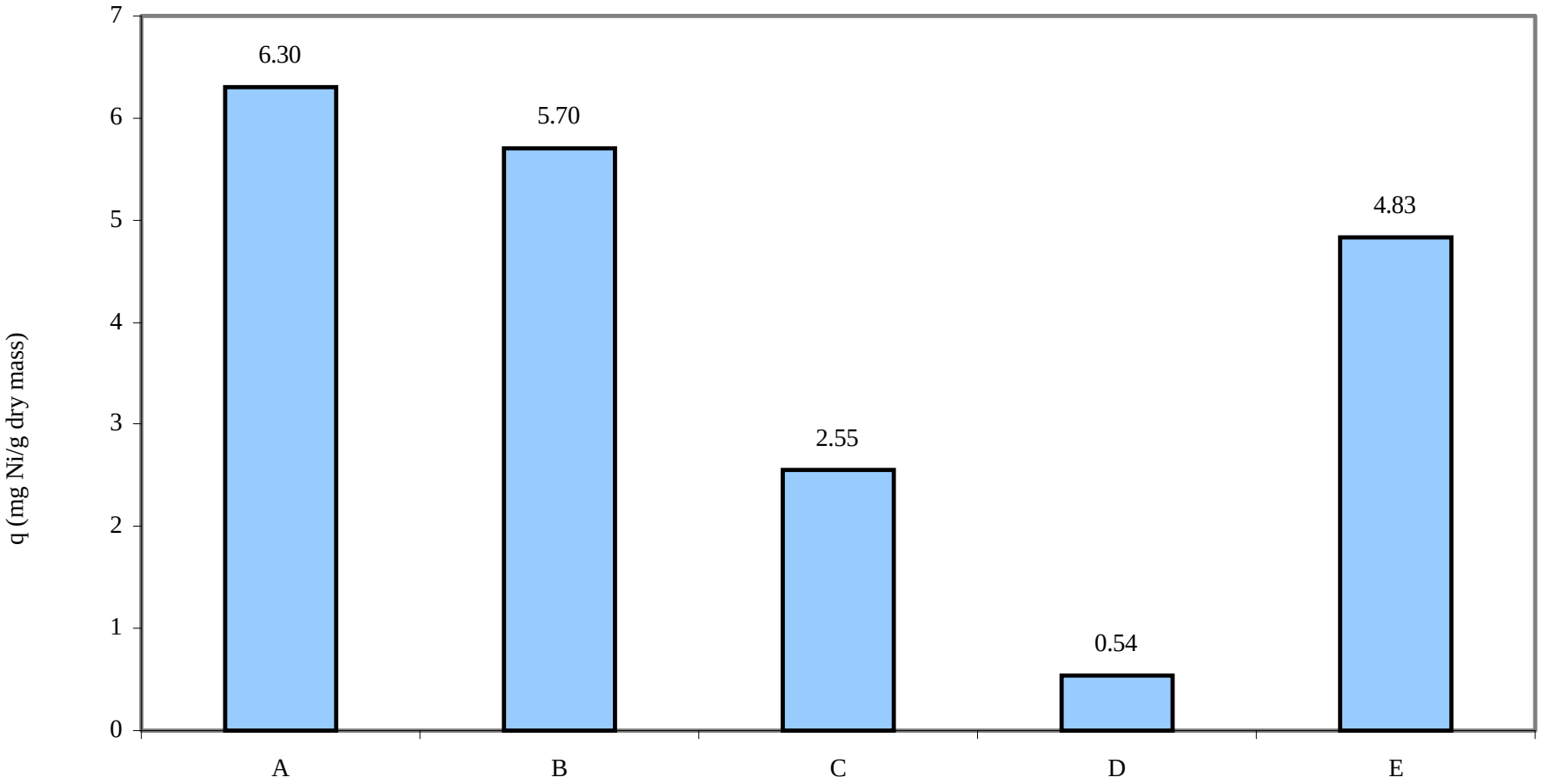

Figure 6.1 The effect of pretreatment on biosorption (pH=5.0; temperature 25^{o}C; biomass concentration=1.0 g/L; Ci = 100 mg/L agitation rate= 150 revmin^{-1}; A= Live biomass without pretreatment ; pretreatment by using: B= ethanol, C= NaOH, D= nitric acid E= formaldehyde)

Live biomass was observed to possess highest nickel biosorption (6.30 mg Ni(II)/g biomass) capacity; HNO_3 pretreatment completely inhibited nickel biosorption. This observation was found to be similar to the findings of Kapoor and Viraraghavad, obtained for biosorption of nickel by *Aspergillus niger*. But the pretreatment with same methods as we used, increased the biosorption of lead, cadmium, and copper in case of the *A. niger* research (26). The accumulation of lead (II) in *S. cerevisiae* cells decreased because the number of binding sites were decreased by autoclaving (33).

Huang and Huang suggested that increase in metal biosorption after pretreating the biomass could be due to removal of surface impurities and exposure of available binding sites for metal biosorption (26). But, here in this study pretreatment decreased biosorption of nickel in comparison with live cells which might indicate that it may be advantageous to use live cells. This may be due to the fact that microorganisms can take up nickel intracellularly. It is possible that better nickel removals by live biomass could have been due to intracellular nickel uptake or the presence of chelating ligands that may be present on the cell surface in trace amounts, even after washing the biomass thoroughly before the biosorption experiments. It needs to be pointed out that reduction in nickel biosorption by ethanol and formaldehyde in comparison with live cells was in the range of 10-25 % only, while the lowest results were obtained with NaOH and HNO_3 in comparison with live cells were 60 % and 90%, respectively. Thus, it was demonstrated in this study that pretreatment of *S. cerevisiae* did not have an effect on biosorption capacity for nickel ions. Therefore, live cells of *S. cerevisiae* without applying any pretreatment were used for biosorption experiments throughout this study.

6.1.2 Effect of pH on Biosorption

pH is one of the major factor affecting biosorption of metal ions since cation competition may occur with hydrogen ions. Hence, in this study biosorption was studied with respect to the different pH values using constant nickel and biomass concentrations at 25^{o} C. Figure 6.2 shows the effects of pH on biosorption capacity at equilibrium.

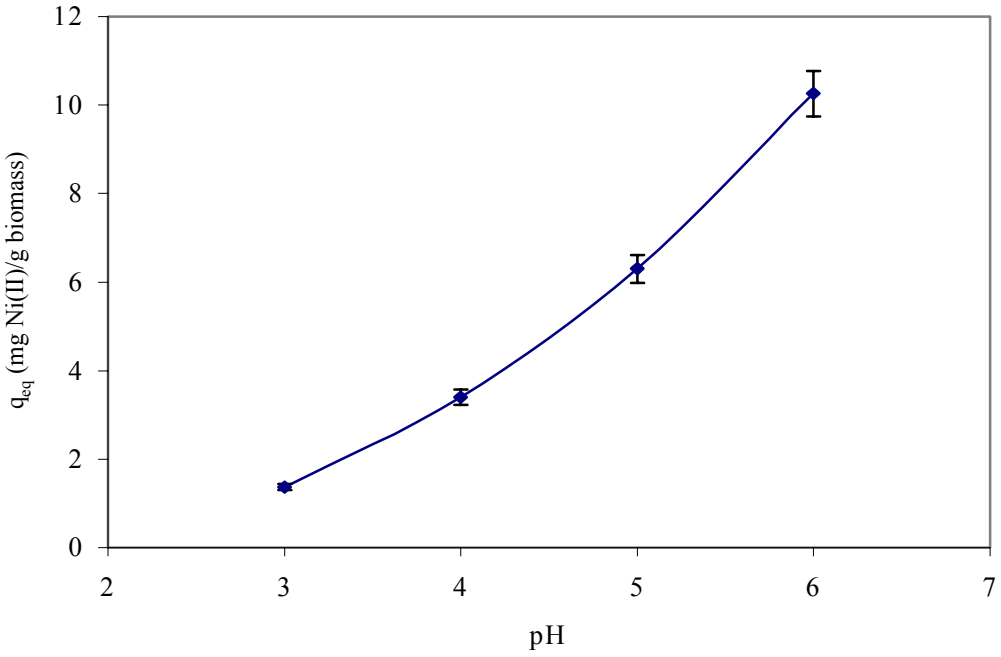

Figure 6.2 Effect of pH on nickel biosorption capacity by *S. cerevisiae* at a constant initial metal ion concentration of 100 mg litre^{-1}(temperature = 25^{o}C; biomass concentration= 1.0 g litre^{-1} ; agitation rate = 150 rev min^{-1})

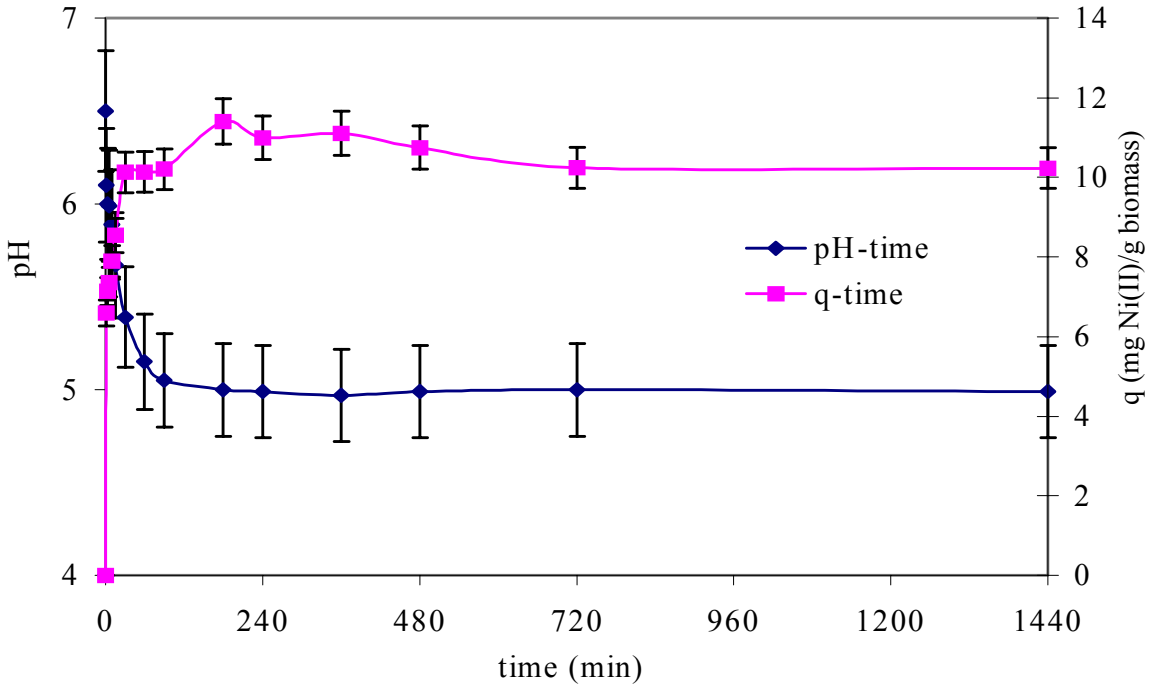

Figure 6.3 The pH change during biosorption started with pH 6.5 and the precipitation of Ni (II) ions (Ci = 100 mg/L; temperature = 25^{o}C; biomass concentration = 1.0 g litre^{-1} ; agitation rate = 150 rev min^{-1})

As can be seen from Figure 6.2, the maximum biosorption of nickel on biomass was observed at around pH 6.0. During the time course of biosorption, when pH of the solution was checked it was observed that the pH was not constant when the initial pH was 6.0. The nickel ions precipitated at the bottom of the flasks because of the high OH^- ions in the adsorption medium. Figure 6.3 shows the change in pH during biosorption at pH close to 6.0. Here, it can be seen that the initial pH was 6.0 and changed during the time course of biosorption. The pH gradually decreased and after 90 minutes the pH change was not recorded upto 24 hours. pH was maintained at 4.98, while nickel uptake on biomass increased and penetration by cells occured at the same period of time. When the similar observation was made for the initial pH values of 3.0, 4.0 and 5.0, there was no change in pH during the time course of biosorption.

In Figure 6.2, it can be seen that there is a decrease in nickel ion adsorption per unit weight of biomass when pH was decreased from 6.0 to 3.0. The maximum nickel ion uptake by waste baker's yeast was obtained as 1.39 and 3.66 mg Ni(II)/g biomass at pH 3.0 and 4.0, respectively. At pH 5.0, nickel uptake was maximum, 6.30 mg Ni(II)/g biomass, which can be selected as the optimum pH value for Ni(II) uptake even pH seems to yield with the maximum uptake. Since this result was thought not to be the real adsorption value because of the precipitation; pH 5.0 was selected as the optimum pH for biosorption.

The nickel ion uptake found to be decreased with decreasing the pH. The medium pH affects the solubility of metals and the ionisation state of the functional groups (carboxylate, phosphate and amino groups) of the fungal cell wall. The carboxylate and phosphate groups carrying negative charges on the fungal cell wall components are the potent scavengers of cations (32). At acidic pH (≈3.0), protonation of the cell wall component adversely affected the biosorption capacity of the fungal biomass, but this effect became negligible with increasing the pH of the medium. With an increase in pH, the negative charge density on the cell surface increases due to the deprotonation of the metal binding sites and thus increases biosorption.

The pH effect on biosorption was reported by many researchers and heavy metal biosorption by *S. cerevisiae* was found to be efficient in the pH range between 4.0 and 5.5. For example, the uptake of lead (II), cadmium (II), copper (II) and zinc (II) by *S. cerevisiae* have been found maximum at the pH value 4.5 and 5.5. Below these pH values biosorption has not been effective (33-35,39-46,48,49). However, the uranium uptake by using *S. cerevisiae* has reached the maximum value at pH 4.0 (46). In

another report, the biosorption for strontium (II), Mn (II), and TI (II) by *S. cerevisiae*, havee been found high at pH of 5.5 (40).

6.1.3 Effect of Initial Metal Concentration on Biosorption Capacity

The effect of initial metal concentrations of nickel (II) ions from 50 mg litre $^{-1}$ to 250 mg litre^{-1} was studied and results were presented in Figure 6.4. The biosorption of nickel(II) ions by waste yeast increased with increasing the initial metal ion concentration upto 150 mg/L. At higher concentrations the adsorption of nickel(II) ions did not change and reached to a saturation value. The maximum uptake of nickel(II) ions reached to 7.82 mg Ni(II) g^{-1} biomass at 150 mg/L initial metal concentration.

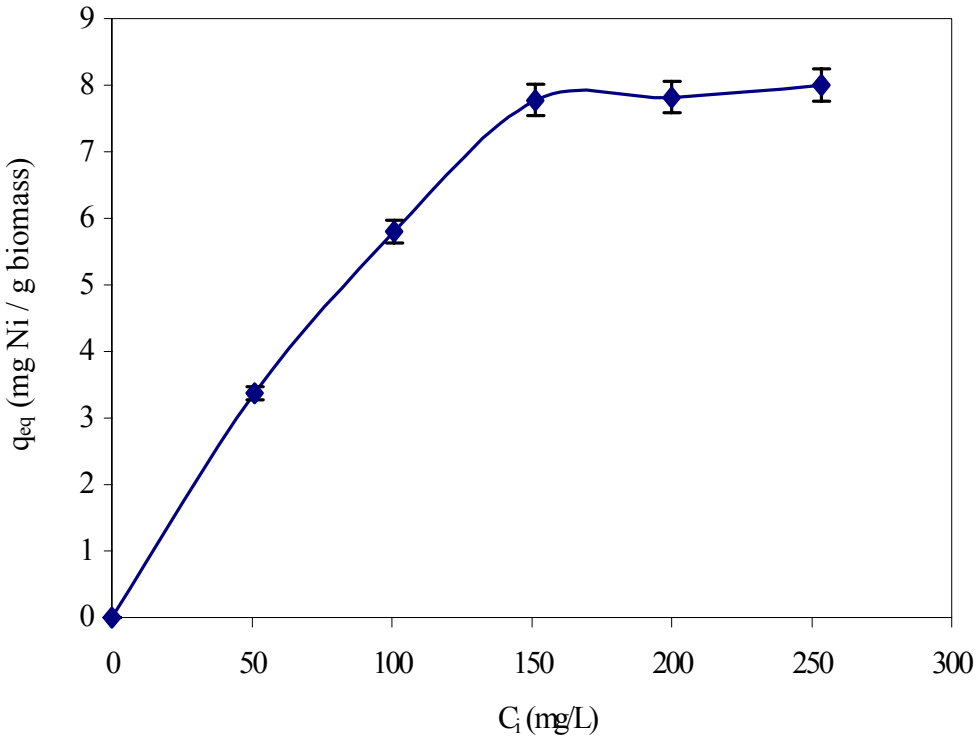

Figure 6.4 The effect of initial metal concentration on biosorption capacity (pH=5.00; temperature = 25^{o}C; biomass concentration = 1.0 g litre^{-1} ; agitation rate = 150 rev min^{-1})

This type of reaction can be termed as " saturation type reaction" as some other researchers have also showed similar results for different metal ion uptake by

Z.ramigera, R. arrhizus, S. cerevisiae and *S. leibleinii* (24,25,34,35,42). The effect was further investigated with biosorption isotherms (see Section 6.2).

6.1.4 Effect of Biomass Concentration on Biosorption capacity

The biosorbent concentration has been shown to be one of the important factor in biosorption processes. In this study, the waste yeast concentration from 0.1 to 5.0g/L was used to determine the effect of biomass concentration on nickel biosorption capacity and Figure 6.5 shows the results for the different biomass concentration on the capacity of biosorption.

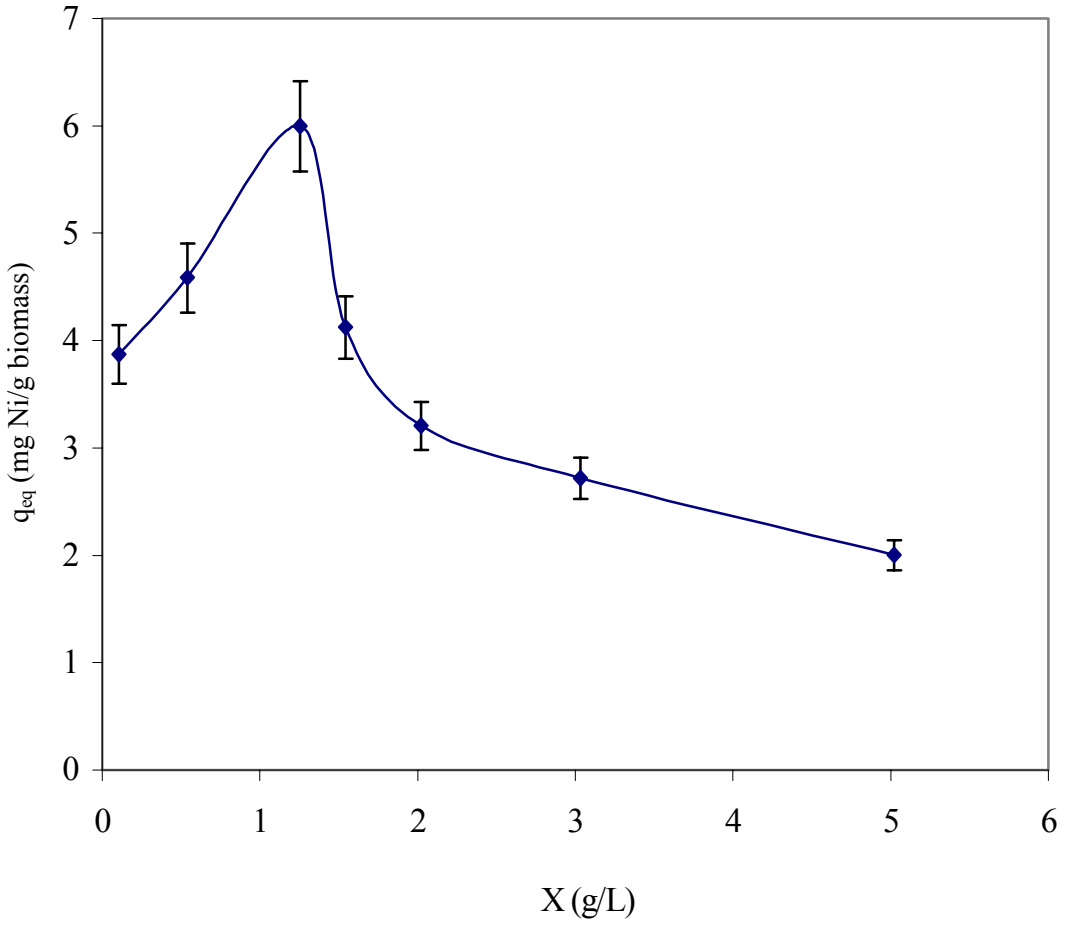

Figure 6.5 The effect of biomass concentration on biosorption capacity (pH=5.00; temperature = 25°C; C_i = 100 mg/L ; agitation rate = 150 rev min^{-1})

The biosorption of nickel(II) ions decreased with increasing the waste yeast concentration (Figure 6.5), eventhough an increase in biosorbent concentration generally increases the uptake of the substances. Such a behaviour has been explained by some researchers and hypothesized that an increase in biomass concentrations leads to interference between the binding sites (31,47). The nickel (II) adsorption by waste yeast decreased with decreasing biomass concentration owing to decreasing surface area of the cell wall that decreased the binding sites. In the present study, optimum waste yeast concentration was found to be as 1.0 g/L.

6.1.5 The Effect of Temperature on Biosorption Capacity

The effect of temperature on nickel biosorption was shown in Figure 6.6. As can be seen in this figure, the maximum biosorption of nickel(II) ions by waste yeast was obtained at 25^{o} C. The adsorptive capacity of the yeast biomass for nickel(II) ions decreased with increasing temperatures above 25^{o} C . Below 25^{o} C, biosorption capacity decreased. The biosorption capacity was found to be the highest at 25^{o} C with the value approximately 8.0 mg Ni(II)/g biomass. The biosorption capacity values were found to be nearly the same for the temperature ranges 15, 35 and 45^{o} C and around 2.30 mg Ni(II)/g biomass was obtained.

Adsorption is an exothermic process, therefore, the adsorptivity is expected to decrease with increasing temperature. Here, a maximum adsorption value was obtained at 25^{o} C. *S.cerevisiae* yeast is known to be very active at this temperature. Therefore, temperature effect experiments were conducted using different biomass concentrations at different temperature values. The results were shown in Figure 6.7 and experimental data were given in Table B.2 (see App.B).

As can be seen from Figure 6.7, biosorption capacities changed with respect to temperature at different biomass concentrations. The lowest biosorption value was observed when experiments were conducted at 35 and 45^{o} C. The uptake of nickel were not changed considerably and found to be approximately 2.0 mg Ni(II)/g biomass by changing the biomass concentrations from 0.5 to 5.0 g/L. Uptake of nickel by changing the biomass concentration at 15 and 25^{o} C resulted with an increase. In the case of the experiments conducted at 15^{o} C, maximum biosorption was around 1.5 g/L biomass concentration with the value of 3.0 mg Ni(II)/g biomass.

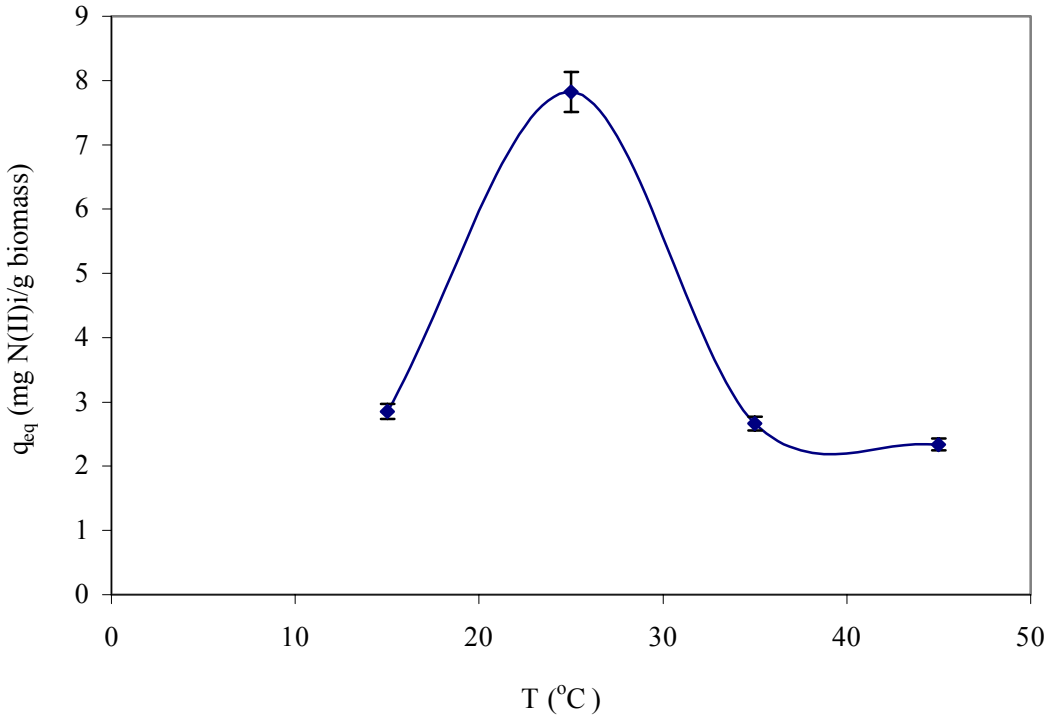

Figure 6.6 The effect of temperature on biosorption capacity (pH=5,00; biomass concentration =1.0 g/L; C_i = 150 mg/L; agitation rate =150 rev min^{-1})

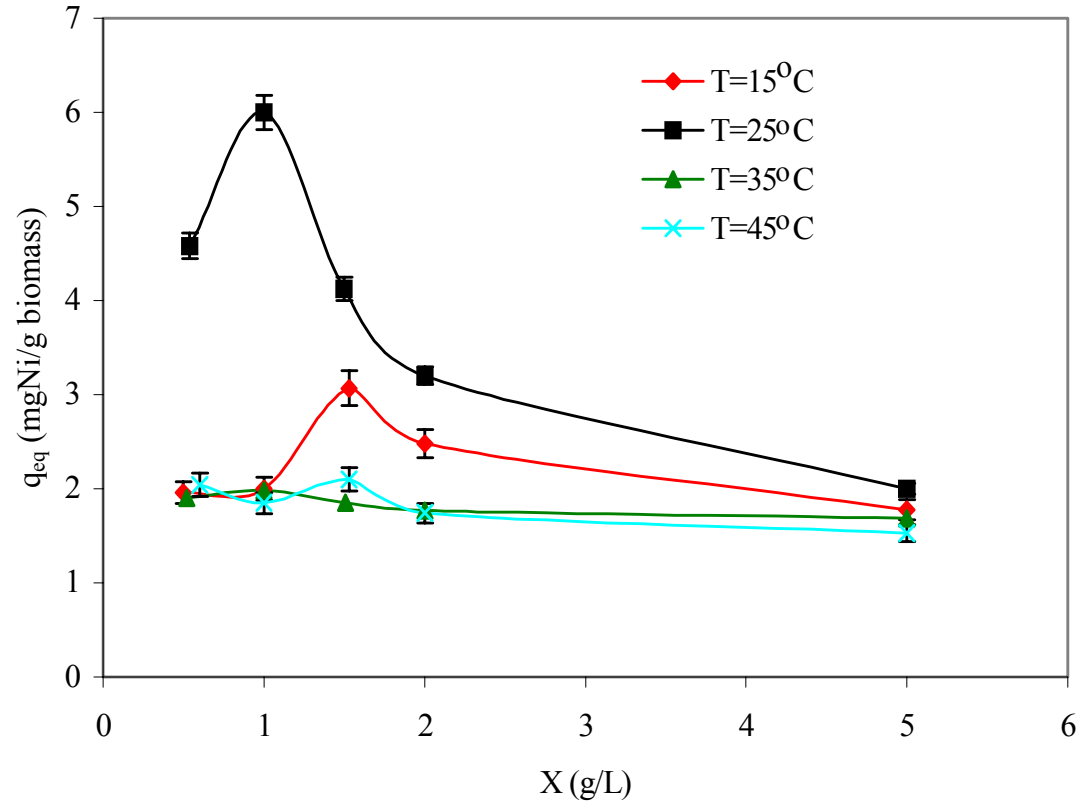

Figure 6.7 Biosorption of nickel(II) ions for different temperature and biomass concentration (pH=5,00; C_i = 100 mg/L; agitation rate=150 rev min^{-1})

The maximum uptake of nickel ion per biomass was occurred at 25^{o} C as also indicated in Figure 6.6, with the uptake value of 6.30 mg Ni(II)/g biomass. Here, it can be seen that as the biomass concentration increases, the biosorption capacity decreases at all the temperature values. It has been reported that at low temperature values the binding of heavy metal ions to the microorganisms occured by a physical adsorption and an equilibrium between the cell wall surface. The metal ions were usually rapidly bound and easily dissociated because of small energy requirement (22,25). Accumulation processes that depend on cellular metabolism, such as active uptake, would be those that are the most likely to be inhibited by low temperatures, whereas high temperatures could affected the integrity of the cell membranes and hinder compartmentalization of metal ions, also leading to reduced uptake levels (45).

6.2 Equilibrium Isotherms for Biosorption of Nickel(II) Ions by Live Cells of *S.cerevisiae*

The nickel(II) biosorption experiments were performed in batch mode in stirred solutions as a function of pH and temperature since these are the main process variables affecting the equilibrium of metal - microorganism systems as seen in Section 6.1. The equilibrium relationship between the adsorbed metal amount per unit mass of *S.cerevisiae* (q_{eq}) and the residual nickel(II) ion concentration (C_{eq}) in solution phase were expressed by adsorption isotherms. The initial nickel ion concentrations were changed from 50 to 250 mg/L while the yeast concentration in each sample was held constant at 1.0 g/L. The applicability of the Langmuir and Freundlich adsorption isotherms for the metal - microorganism system was tested under these specified conditions by only changing the pH and temperature.

6.2.1 Biosorption Isotherms for Nickel(II)/Baker's Yeast System at Different pH Values

The biosorption equilibrium isotherms were generated for different pH values. The temperature of 25^{o} C and biomass concentration of 1.0 g/L were held constant since these were the optimum values found from the previous biosorption experiments.

Figure 6.8 and Table 6.1 show the biosorption isotherms for nickel(II) – baker's yeast system at the pH values of 3.0, 4.0 and 5.0. The isotherm data were tried to fit the

Langmuir and Freundlich adsorption isotherms. In Figure 6.8 solid lines show the best fit Langmuir isotherms using the parameters reported in Table 6.1. The parameters were estimated for each pH values from the linearized equations of Langmuir and Freundlich which were given in Chapter III. The calculated parameters were given in Table 6.1 and 6.2 and the linearized Langmuir and Freundlich isotherm curves can be seen in Figure C.1-14 (see App.C).

Table 6.1 The biosorption parameters obtained from the Langmuir adsorption isotherms for nickel (II) ions at different pH values.

pH	q_{max} (mg/g)	b (L/mg)	R^2 (regression coefficient)
3.00	7.82	2.5×10^{-3}	0.985
4.00	14.00	4.6×10^{-3}	0.986
5.00	14.30	6.9×10^{-3}	0.989

Table 6.2 The biosorption parameters obtained from the Freundlich adsorption isotherms for nickel (II) ions at different pH values.

pH	K	$1/n$	R^2 (regression coefficient)
3.00	0.015	0.9701	0.894
4.00	0.300	0.5791	0.854
5.00	0.470	0.5392	0.910

The data did not fit the Freundlich adsorption isotherms as seen in Table 6.2. It can be seen that all isotherms follow the Langmuir relationship and fitted with high correlation coefficients as seen in Table 6.1. A linear approximation can be made for the nickel (II) concentrations below 30 mg/L solution. The ratio between equilibrium concentrations in the biomass and liquid bulk increased with an increase in pH, particularly at pH 5.0, which is consistent with the trend shown in Figure 6.2.

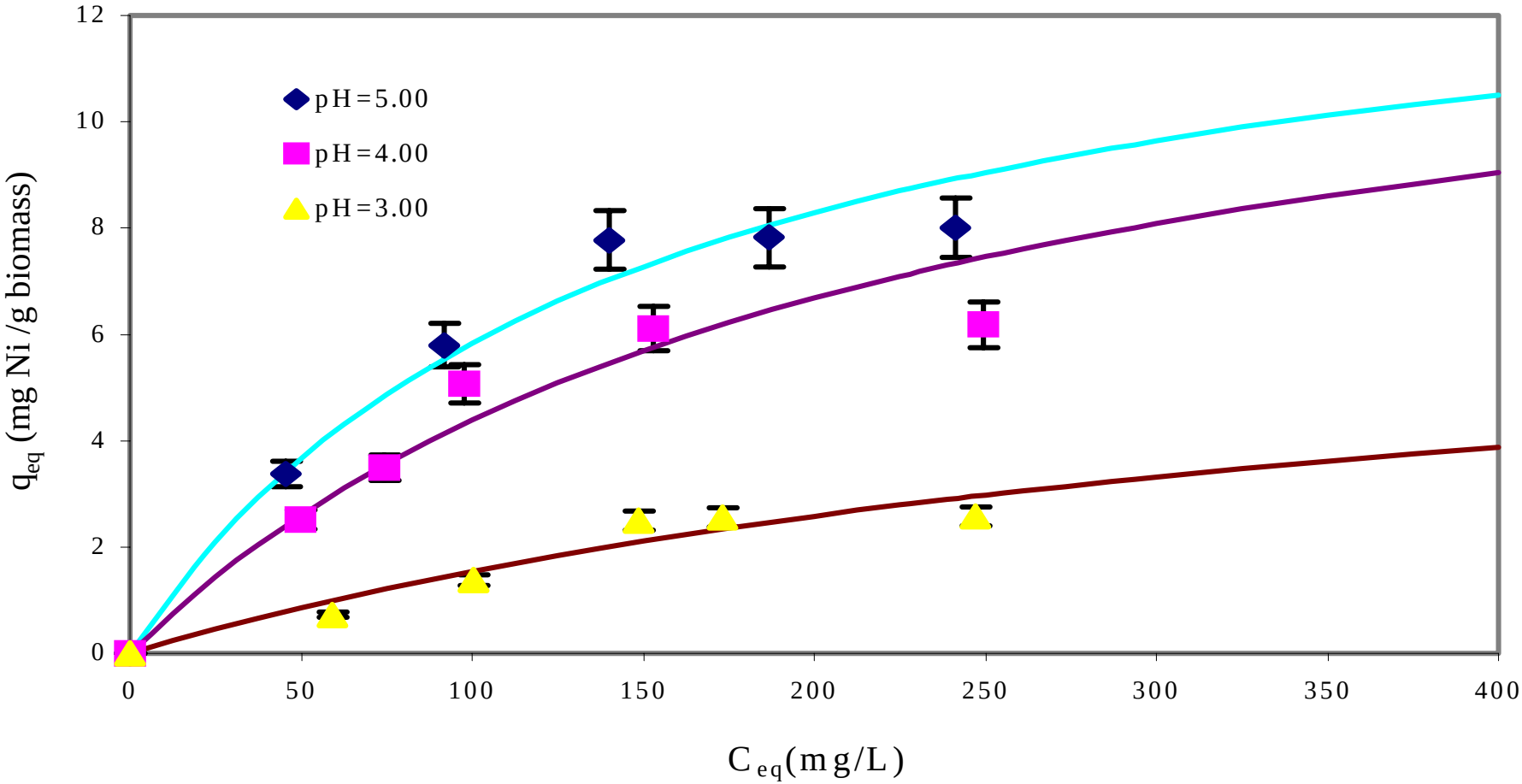

Figure 6.8 Biosorption isotherms for nickel(II)/ baker's yeast system at different pH values (biomass concentration= 1 g/L; temperature 25^{o}C ; agitation rate = 150 rev min^{-1}) Lines corresponds to the Langmuir isotherms using values reported in Table 6.1.

Table 6.1 shows the biosorption parameters q_{max} and b as a function of pH for the nickel(II) / baker's yeast adsorption system. As can be seen from Table 6.1, as pH increases both the q_{max} and b values increase. These parameters are strongly affected by pH. This increase can also be seen from Figures 6.9 and 6.10.

Figure 6.8 shows the change of the highest possible sorbate uptake q_{max} for different pH values and Figure 6.10 shows the change of the b values for different pH values. As shown by Table 6.1, the highest coefficient b value was obtained at pH 5.00 as 0.0069 L/mg which is related to the affinity between the biosorbent and sorbate. A large value of b shows the strong bonding. The highest possible sorbate uptake, q_{max} were determined as 7.80, 14.00 and 14.30 mg/L at pH 3.00, 4.00 and 5.00, respectively. On the other hand, q_{eq} values were determined as 2.55, 6.11 and 7.82 mg Ni(II)/g biomass at pH 3.0, 4.0 and 5.0, respectively, was smaller than q_{max}. That might indicate that the biosorption of nickel ions on *S. cerevisiae* could be expressed by monolayer type of adsorption in which the surface of the yeast was not fully covered by nickel ions.

6.2.2 Biosorption Isotherms for Nickel(II) / Baker's Yeast System at Different Temperature Values

The experimental values of biosorption equilibrium and the calculated Langmuir adsorption isotherms for the adsorption of nickel ions were given in Figure 6.11. The Langmuir model parameters were given in Table 6.3 which were estimated from the linearized Langmuir isotherm curves at different temperature values were given in Figures C.1-14 (see App.B).

As shown in Figure 6.11, the experimental adsorption equilibrium data for nickel(II) ions were well fitted to the Langmuir model for different temperature values. The highest coefficient b and q_{max} values were obtained at 25^{o} C. The large value of b constant as 0.0069 L/mg shows the strong bonding. The q_{eq} value at optimum pH and temperature was determined as 7.82 mg Ni(II)/g biomass which was smaller than q_{max} (14.30 mg/g). This might also be an indication for the monolayer type adsorption of nickel ions on *S. cerevisiae.*

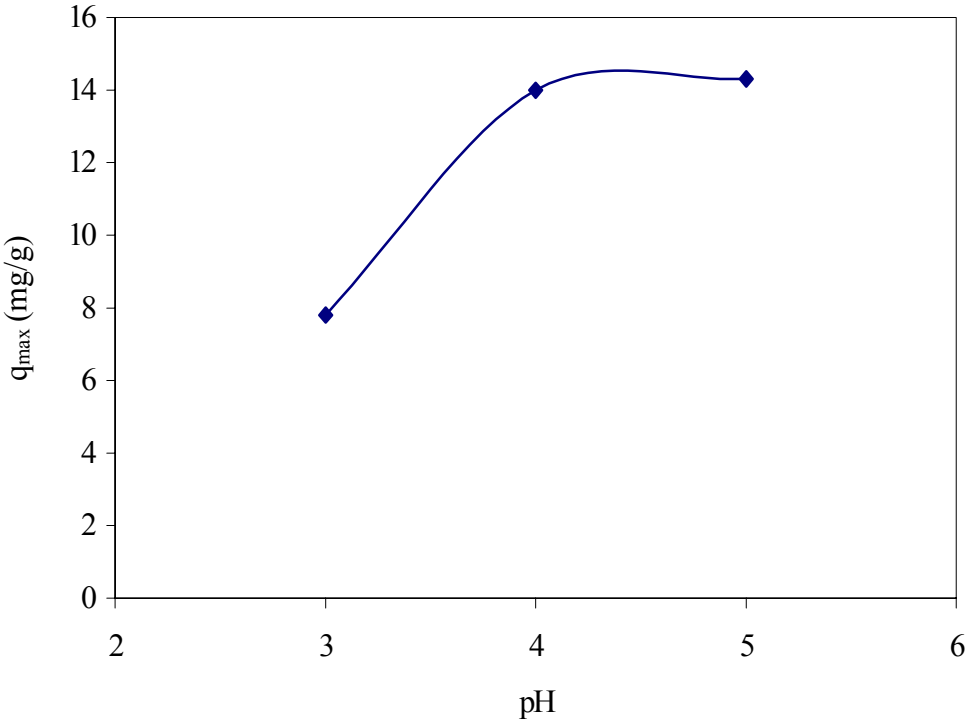

Figure 6.9 The Langmuir parameters of q_{max} at different pH values

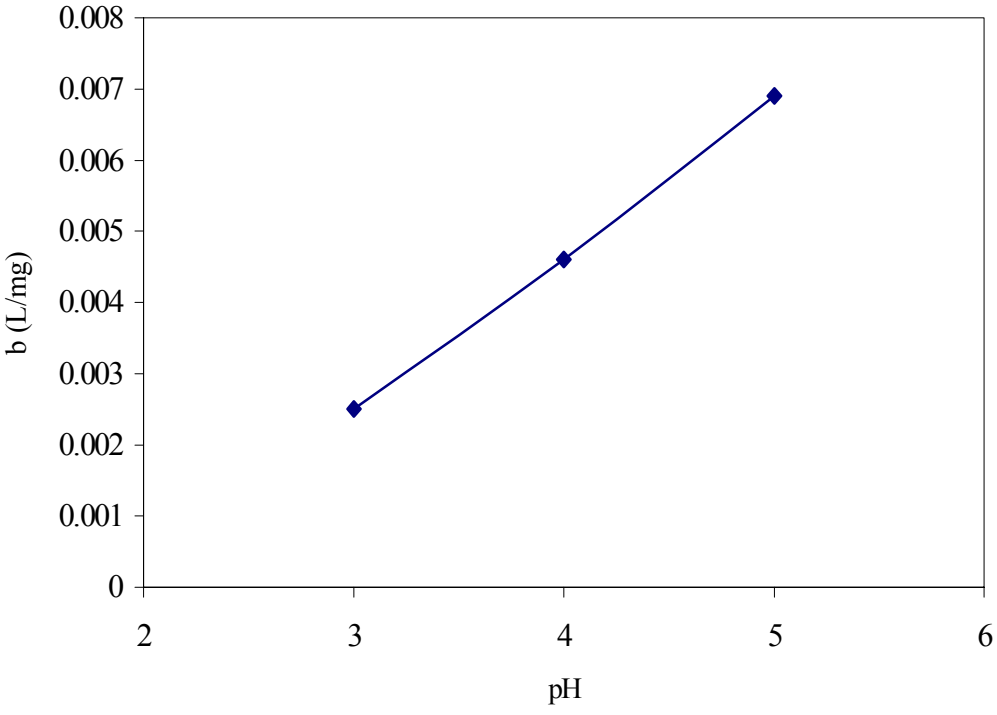

Figure 6.10 The Langmuir parameters of *b* at different pH values

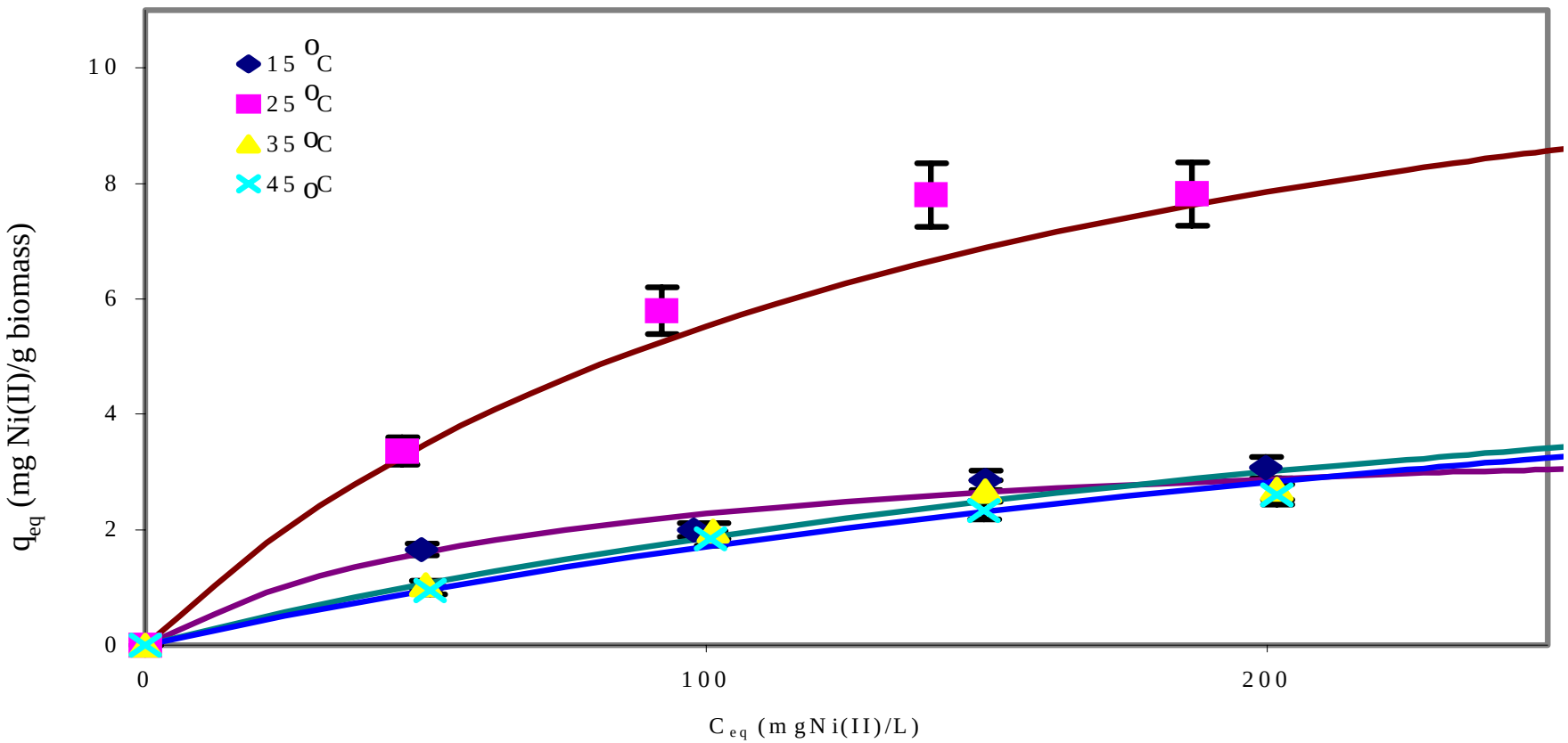

Figure 6.11 Biosorption isotherms at different temperatures (biomass concentration 1.0 g/L; pH: 5.00 ; agitation rate : 150 rev min^{-1}) Lines correspond to the Langmuir isotherms using values reported in Table 6.3

The experimental equilibrium data of nickel(II) biosorption on *S.cerevisiae* for different metal concentrations and temperatures were given in Table 6.4A (see App.A). Figure 6.12 and 6.13 show the change of the Langmuir parameters by varying the temperature of the nickel(II) biosorption on waste yeast.

Table 6.3 The adsorption constants obtained from the Langmuir adsorption isotherms for nickel (II) ions at different temperatures

Temperature (C^o)	q_{max} (mg/g)	b (L/mg)	R^2 (regression coefficient)
15	3.907	0.0014	0.957
25	14.30	0.0069	0.990
35	7.70	0.0032	0.987
45	8.23	0.0026	0.989

Table 6.4 The adsorption constants obtained from the Freundlich adsorption isotherms for nickel (II) ions at different temperatures

Temperature (C^o)	K	$1/n$	R^2 (regression coefficient)
15	0.043	0.470	0.937
25	0.069	0.634	0.935
35	0.002	0.715	0.931
45	0.001	0.748	0.947

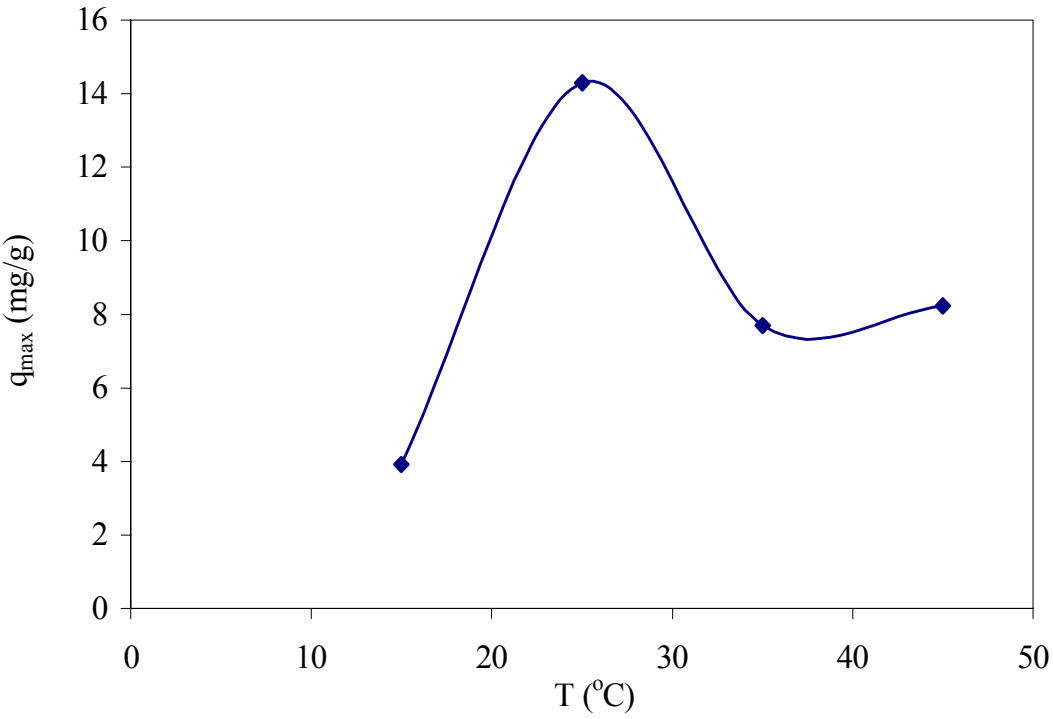

Figure 6.12 The Langmuir parameters of q_{max} at different temperatures

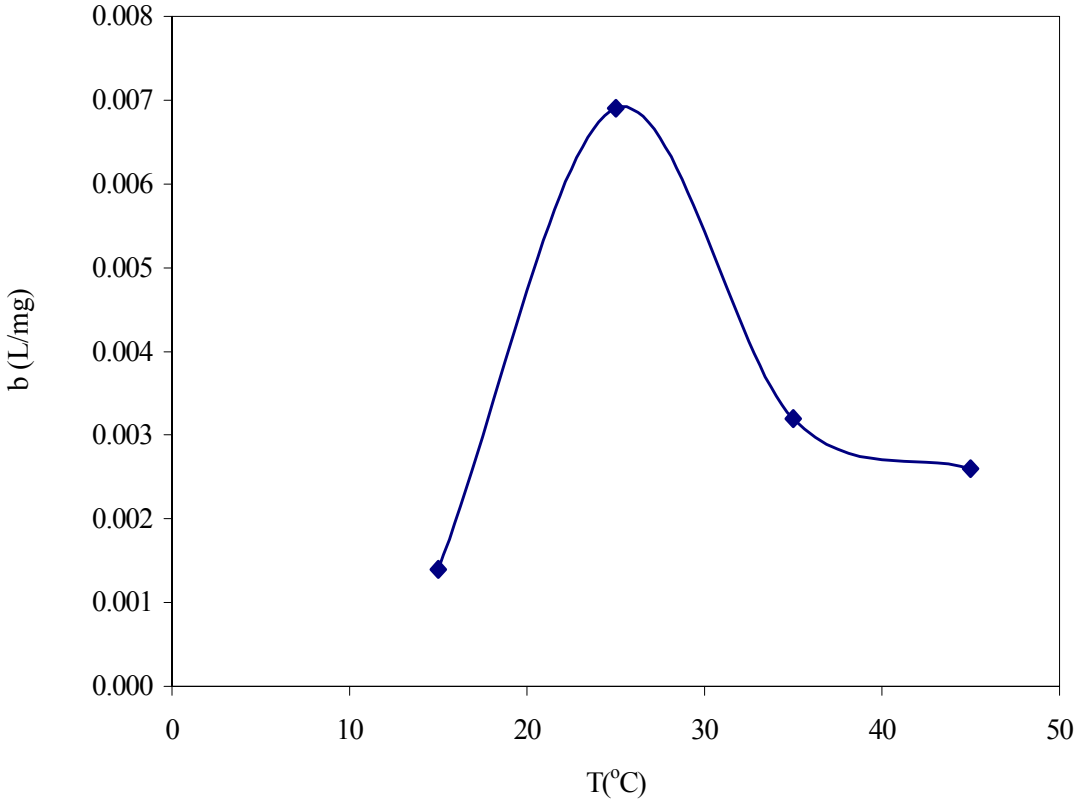

Figure 6.13 The Langmuir parameters of b at different temperatures

6.3 Determination of Biosorption Enthalpy Change of Ni(II) Ions

The thermal properties of the biosorption system are not well known. However, the overall enthalpy of the interactions between the cell wall and the heavy metal ions is temperature dependent in the biosorption process. The temperature changes can affect the number of factors which are important in heavy metal ion biosorption. The change of the temperature can affect the microorganism cell wall configuration and the ionization of chemicals on the cell wall. Although, it is known that the magnitude of the heat effect for the biosorption process is the most important criterion to develop the thermodynamic and kinetic relationship between the metal – microorganism interaction process (10,22,25).

Adsorption process, especially physical adsorption, is generally assumed to be an exothermic process. The adsorption of metal ions increases with increasing temperature which is explained on the basis of thermodynamic parameters. The enthalpy change for the biosorption of nickel ions on *S. cerevisiae* was calculated using the Langmuir constant *b* that related to the energy of adsorption. According to the Arrhenius equation, the *b* has the form:

$$b = bo\, e^{(-\Delta H/ R T)}$$

The enthalpy change was obtained by calculating the slope of a plot of ln *b* versus 1/T (8,11). The negative values of slope or the positive values of enthalpy change show the adsorption to be endothermic. On the other hand, the positive values of slope or the negative values of enthalpy change show the adsorption to be exothermic. The change of the Langmuir constant *b* with temperature for the biosorption of nickel ions on *S. cerevisiae* at optimum pH and biomass concentration were represented by Figure 6.14. The value of the enthalpy change for the biosorption of nickel ions by *S. cerevisiae* and the regression coefficients (R^2) were given in Table 6.5.

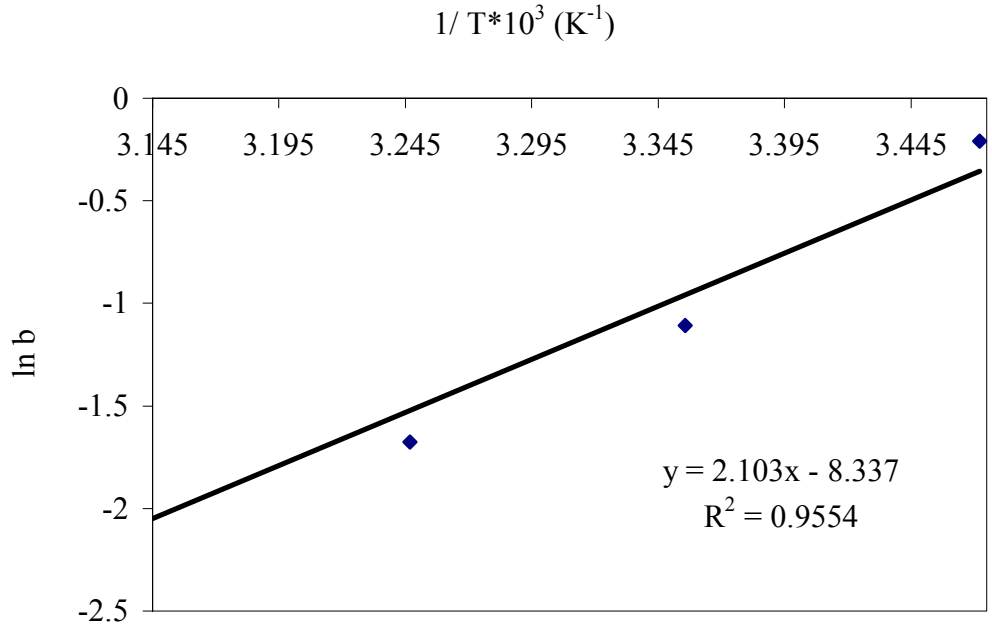

Figure 6.14 The change of the Langmuir constant b with temperature for the biosorption of Ni(II) on *S.cerevisiae* (pH=5.0; biomass concentration =1.0 g/L ; agitation rate = 150 rev min^{-1})

Table 6.5 The value of enthalpy change for the biosorption of nickel ions by *S.cerevisiae*.

	ΔH (kJ/mol)	R^2 (regression coefficient)
S. cerevisiae- Ni(II)	-17.10	0.9554

The adsorbed Ni(II) ions quantities at equilibrium decreased with increasing temperatures in the range of 25 – 45^{o} C as can be seen in Figure 6.6. The positive values of slope or the negative values of enthalpy change were obtained from the curve seen in Figure 6.14. The biosorption of nickel (II) ions on *S.cerevisiae* was determined to be exothermic as seen in Table 6.4. It is known that the heat of physical adsorption is typically between of 2.1 and 20.9 kJ/mol (22). Physical adsorption phenomenon is associated with the presence of weak Van der Waal's forces. Equilibrium between the cell surface and the metal ions is usually rapidly attained and easily reversible, because the energy requirements are small (8). Volesky hypothesized that uranium, cadmium, zinc and cobalt biosorption by dead biomass of algae, fungi and yeasts takes place through electrostatic interactions between ions in solution and cell wall (10,35). The

same result was found for marine algae was studied by Schiemer and Wrong who suggested that nickel ions were bound predominantly by electrostatic attraction (23).

Moreover, the bound energies of various mechanism for adsorption may be approximately ranked from strongest to weakest. Covalent or electrostatic chemical bonding is higher than 41.80 kJ/mol, dispersion interactions and hydrogen bonding vary between 8.36 and 41.80 kJ/mol and dipole-dipole interactions are small than 8.36 kJ/mol. Although, the heats of chemisorption generally change from 80 to 200 kJ/mol (8,11).

In this study, the heat of biosorption was compared with the heats of physical and chemical adsorption. The value that is of the same order of magnitude for physical adsorption was observed. The heats of biosorption (ΔH) of chromium (VI) and lead (II) ions by *Z. Ramigera* and nickel (II) ions by *R. arrhizus* were found by Sağ and Kutsal as 16.0 kJ/mol, 18.9 kJ/mol and –21.4 kJ/mol, respectively. They assumed that these values being of the same order of magnitude as the heat of physical adsorption. Although, the heat of nickel biosorption by *R. arrhizus* was found to be negative, it was indicated that the adsorption was an exothermic process. Increase in adsorption of nickel (II) ions with a rise temperature have been explained on this basis by Sağ and Kutsal (22). From Table 6.5, the heat of nickel biosorption on *S.cerevisiae* was determined as –17.10 kJ/mol which is close to the heat of nickel biosorption on *R.arrhizus* found by Sağ and Kutsal (22). However, it was considered that the proposed mechanisms for the heavy metals uptake process were mainly both microrganism and metal dependent because of specific surface properties of the microorganisms, cell physiology and different solution chemistry of metal ions. The complexity of the microorganism's surface structure implies that there may be many ways for the metal to be captured by the cell wall. Therefore, biosorption mechanisms are still not very well understood (22).

6.4 Kinetics of Nickel (II) Biosorption

In this study, experimental kinetic data were obtained using waste *S.cerevisiae* for nickel(II) biosorption over a range of operating pH values. These results were obtained from batch experiments in well-stirred vessels and shown in Figure 6.15. As can be seen, the initial rate of adsorption was very fast, and this was followed by a much slower phase for four different pH values. There seems to be an initial period to be less

than a few minutes of rapid adsorption responsible for about 60 % of total final adsorption at pH 5.0. After this rapid initial uptake further biosorption by waste yeast occured slowly and reached an equilibrium after 2 hours. No obvious increase in Ni(II) uptake was observed thereafter upto 24 hours.

This result suggested that the slow and metabolism-dependent uptake of metal ions into intracellular organelles was not important in this study. The maximum uptake of Ni(II) ions was obtained 6.30 mg Ni (II) g^{-1} dry biomass at 100 mg/L initial metal concentration, at pH 5.0 and 25 ^{o}C. *S. cerevisiae* took up 3.95 mg Ni(II) g^{-1} dry biomass after 1-min biosorption, nearly 60% of the total amount of Ni (II) accumulated throughout the whole 24-h treatment process. The residual Ni(II) concentration dropped rapidly in the first few minutes, decreased gradually in the first hour and no further decline was found after one hour of biosorption for the other pH values of 3.0, 4.0 and 5.0. The decline in Ni concentration remaining in the solution corresponded to the increase in cellular nickel concentration (Figure 6.15). The uptake values of nickel by the cells were 1.21, 1.26, 3.95 and 6.78 mg Ni(II)/ g biomass at the first minute of biosorption at the pH values of 3.0, 4.0, 5.0 and 6.0, respectively. After 24 hours the uptake of nickel increased and 1.40, 3.66, 6.3 and 10.3 mg Ni(II)/ g biomass values were obtained for pH 3.0, 4.0, 5.0 and 6.0, respectively.

As it is clear from these values, the maximum uptake was observed at pH 6.0. However, as it was discussed in Section 6.1.2, the precipitation could be occured and that the value could not be attributed to the adsorption experiments. Therefore, pH 5.0 was selected as the optimum value. Because of the same reason, the rate calculations were only done for the pH values of 3.0, 4.0 and 5.0.

This initial rapid mechanism that is known as passive uptake. It is considered as reversible accumulation step and is also called biosorption. Biosorption can be considered as a collective term for a physical and chemical adsorption, ion exchange, coordination, complexation, chelation and microprecipitation (1,27,42,45,49). The functional groups such as phosphate, carboxyl, amine and sulphoxide groups can form complexes with the metal ions. Chitin and chitosan present in fungal cells can also sequester metal ions (33,34,36). For example, Brady and Stoll suggested that the yeast cell wall components bind heavy metals in the order of protein> mannose > chitin> glucan. On the other hand, most of the metal uptake was due to ion-exchange (33).

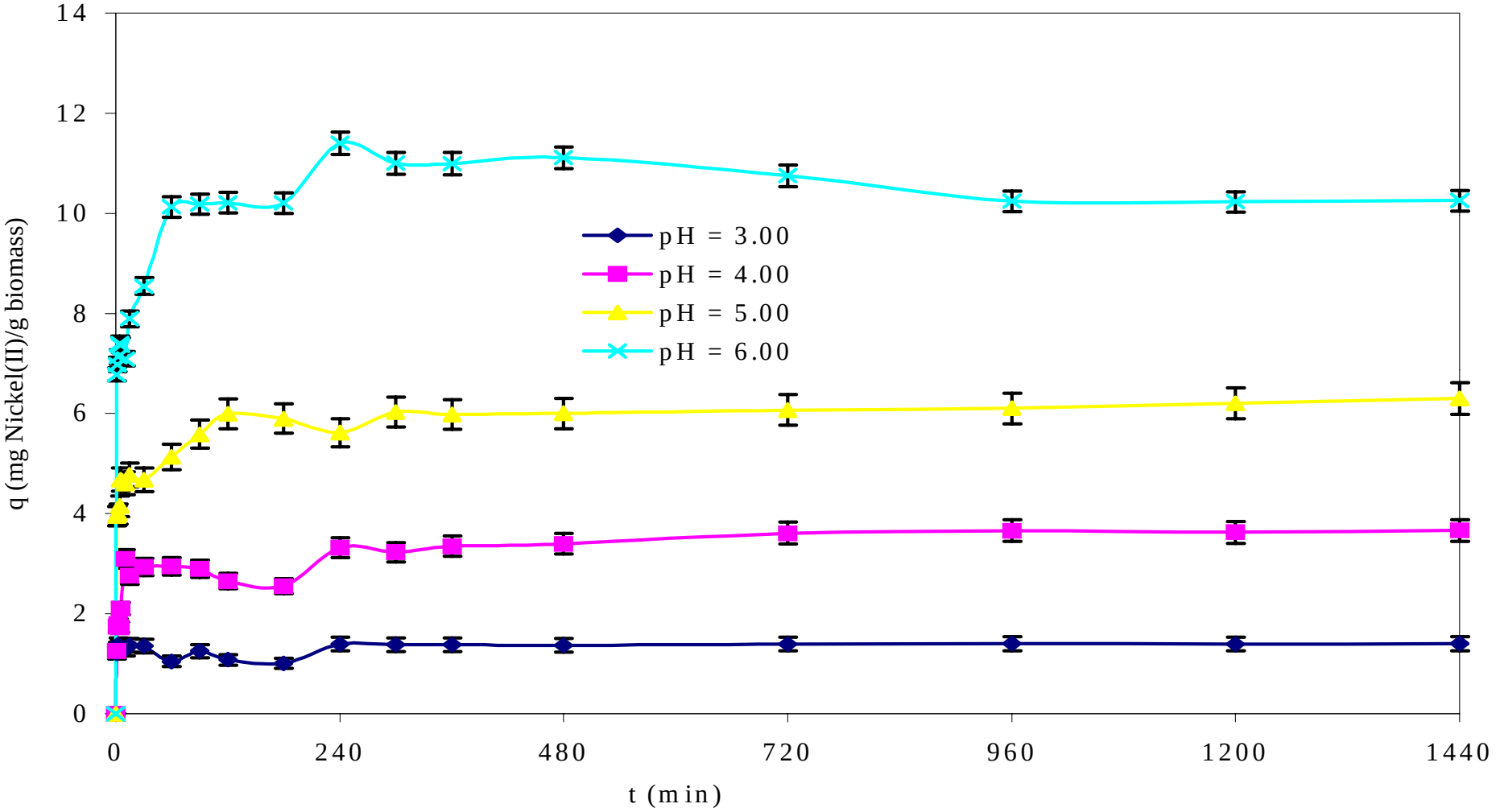

Figure 6.15 The adsorption curve for the biosorption of Ni (II) ions on *S. cerevisiae* at different pH values (C_i = 100 mg/L; temperature= 25^{o}C; biomass concentration= 1.0 g litre^{-1}; agitation rate = 150 rev min^{-1})

The displacement of calcium, magnesium, potassium and hydrogen ions by biosorption of metals on *S.cerevisiae* have been documented (1,10,34-36,39-42,48).

Nickel uptake by different microorganisms have been reported such as nickel uptake by *Streptomyces noursei* and by *C. vulgaris* have been observed as 0.8 mg Ni(II) g^{-1} dry biomass and 1.28 mg Ni(II) g^{-1} dry biomass , respectively (19, 43). Maximum nickel uptake by *Aspergillus niger* has been found only 1.75 mg Ni(II) g^{-1} dry biomass and these values are smaller than that of the results of this study. However, nickel removal by immobilized biofilm of *Citrobacter* , by *Rhizopus arrhizus* and by biowaste of fruit juice industry were obtained as 8.8 mg Ni(II)/ g dry biomass, 10.32 mg Ni(II)/ g dry biomass and 13.0 mg Ni(II)/ g dry biomass , respectively (21,22,25,26,27,38).

In this study the kinetic results were given as the initial adsorption rates, *r* (mg/g-min.). The initial biosorption rate was obtained by calculating the slope of a plot of the adsorbed metal ion quantity *q* per gram of dry biomass (mg/ g) versus time (min) at $t = 0$ (Figure 6.15). Figure 6.16 shows the initial biosorption rates of Ni(II) ions by *S.cerevisiae* for different pH values. As shown in this figure, biosorption rates were increasing with increasing pH. The initial rates of biosorption were found to be 0.65, 1.26 and 3.65 mg Ni(II) / g-min for pH values of 3.0, 4.0 and 5.0, respectively. The highest rate of biosorption of nickel (II) by waste yeast was obtained at pH 5.0 (Fig. 6.16).

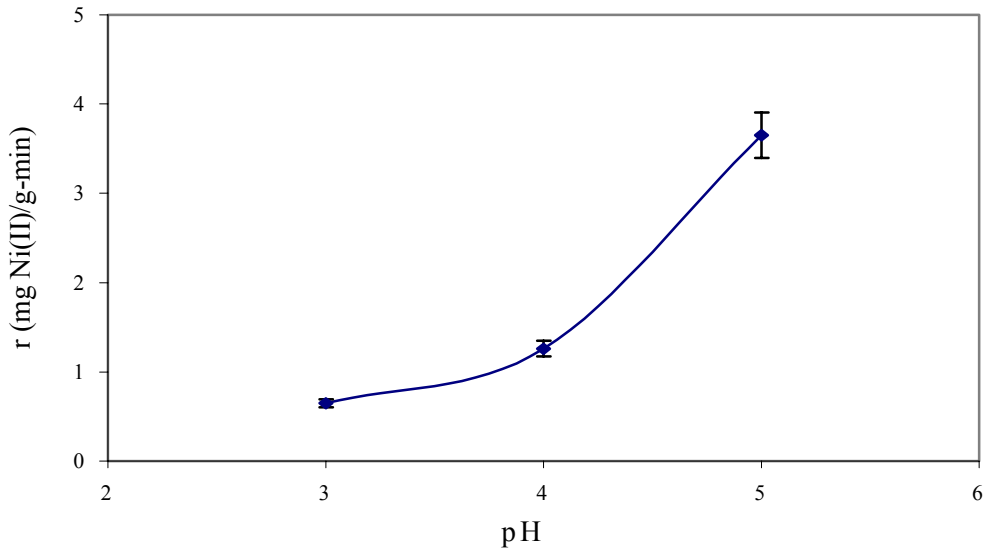

Figure 6.16 The effect of pH on initial adsorption rates. (C_i = 100 mg/L; temperature = 25°C; biomass concentration = 1.0 g $litre^{-1}$; agitation rate = 150 rev min^{-1})

Chapter VII

CONCLUSIONS AND RECOMMENDATIONS

In this study, the adsorption of nickel (II) ions using the waste baker's yeast was investigated in a batch system. The baker's yeast, a mass of viable yeast cells of the *Saccharomyces cerevisiae* genus, was obtained from the Pakmaya (Baker's Yeast Industry) as a waste biomass.

Biomass was pretreated with sodium hydroxide, formaldehyde, nitric acid and ethanol and biosorption capacities of pretreated cells were compared with live cells. Results indicated that pretreatment of *Saccharomyces cerevisiae* reduced biosorption of nickel as compared to live cells. The better nickel removals by live biomass could be due to the presence of chelating ligands that may be damaged by the chemicals during pretreatment or by microorganisms taking up nickel intracellulary. It is advantageous to use live biomass instead of pretreated biomass by expensive chemical substances. Therefore, all biosorption studies were conducted by using the live cells.

Nickel biosorption resulted in an increase of pH. The nickel ion uptakes by waste baker's yeast were obtained to be 1.39, 3.66, 6.3 and 10.3 mg Ni(II)/g biomass for the pH values of 3.0, 4.0, 5.0 and 6.0, respectively. At above pH values above 5.0, nickel ions precipitated because of high OH^- ions. On the other hand, protonation of the cell wall component of yeast adversely affected the biosorption capacity at low pH values.

The optimum biomass concentration was observed as 1.0 g/L. Above this value the amount of adsorbed nickel(II) ions on the yeast cell decreased because high biomass concentrations led to interference between binding sites of the cell wall. On the other hand, at low biomass concentration the biosorption decreased since the required surface area for metal binding decreased. The biosorption experiment of nickel (II) ions was repeated with different initial metal concentration, from 50 mg/L to 250 mg/L. It was observed that biosorption increased with increasing metal concentration upto 150 mg/L. At higher concentrations, the adsorption did not change. Biosorption reached an equilibrium state at 150 mg/L and this type of reaction was termed as saturation type reaction.

Another parameter affected the biosorption of nickel(II) ions was the temperature. Maximum biosorption of nickel(II) ions by waste yeast was obtained at 25^{o}C. The adsorptive capacity of the microorganisms for nickel(II) ions decreased with increasing temperatures between 25 and 45^{o} C. It has been reported that at low temperatures, the binding of heavy metal ions to the microorganisms occurred by a physical adsorption process. The metal ions were usually rapidly bound and easily reversible because of small energy requirement. As a result, the optimum values of pH, temperature, yeast concentration and initial metal concentration for nickel biosorption by waste yeast was observed as 5.0, 25^{o}C, 1.00 g/L and 150 mg/L,respectively. At this optimum conditions the maximum amount of adsorbed metal ions on waste yeast was determined as 7.82 mg Ni(II)/ g biomass.

The applicability of the Langmuir model for metal-microorganism system was tested at different temperature and at different pH values. The biosorption of nickel(II) ions by waste baker's yeast did not fit the Freundlich adsorption isotherms. The Langmuir constants *b* and q_{max} were found to be maximum under the optimum conditions. The q_{max} and *b* values for nickel (II) ions were determined as 14.30mgNi(II)/gbiomass and 0.0069 L/mg, respectively. The result showed that q_{eq} (7.82 mgNi(II)/g biomass) was smaller than q_{max} which can be explained that the adsorpion of nickel ions on *S. cerevisiae* may be a monolayer type adsorption and the surface of the yeast is not fully covered. The highest *b* values that is according to the affinity between the binding sites was observed at optimum biosorption condition. Although the enthalpy change for the biosorption process was evaluated by using the Langmuir constant *b*, related to the energy of adsorption. Nickel(II) biosorption was determined to be an exothermic process since decreased binding occurs as the temperature is increased in the range of 25–45^{o}C.

Biosorption kinetics influenced from the pH changes in the solution. The fastest rate was obtained at pH 5.0 with the overall uptake value of 6.3 mg Ni(II)/ g biomass after 24 hours. The equilibrium was reached within 2 hours, very rapid adsorption was observed during the early stages of time course. The initial rates of nickel biosorption were 0.65, 1.21 and 3.65 mg Ni(II)/ g-min for pH values of 3.0, 4.0 and 5.0, respectively.

The results obtained in this study indicated that electrostatic interactions are important in metal biosorption processes. Biomass particles may have an overall negative charge the magnitude of which increases with increasing pH as more sites are

deprotonated. The cell wall of *Saccharomyces cerevisiae* contains polysaccharides, proteins and lipids. The outer layer of the cell wall of *S. cerevisiae* consists of a coat of protein. Thus, the variations of surface charge on the cell wall with pH would be somewhat similar to that of protein. A charge develops on a protein molecule by the dissociation of ionizable side groups of the constituent amino acids (33).

Moreover, the biosorption of nickel on yeast cells was occured as monolayer adsorption since the equilibrium isotherms can be described by the Langmuir model. The biosorption kinetics were very fast which may indicate a rapid binding to negatively charged groups on the cell surface. It can be concluded that nickel(II) ions binding on live cells of *S. cerevisiae* was a result of passive uptake and active transport of metal ions inside the cells and metabolic uptake were negligible.

Since the studies in the literature focusing specifically on nickel removal is rare, the results of this study may contribute to the following research on nickel biosorption by *S. cerevisiae*. Due to the time limitations of the present work, some aspects could not be studied. For future studies effect of ionic strength on the equilibrium isotherms, immobilization of yeast cells on a suitable support material and nickel removal from the electroplating wastewater could be worth to investigate.

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APPENDIX A

ICP – AES Axial Liberty Performance Data

The performance data of ICP – AES were given in Table A.1. The calibration was done using 2, 5, 10 and 20 ppm Ni standard solutions. The correlation coefficient of the calibration curve, illustrated in Figure A.1, was 1.000

Table A.1 ICP – AES performance data

Calibration response curve	y = 534.5x (r=1.000)
Limit of detection (3x)	0.023 ppm (1 ppm Ni)
	0.053 ppm (2 ppm Ni)
Relative standard deviation (RSD %)	0.3 % (5ppm Ni standard solution)
	0.15 % (10 ppm Ni standard solution)

The 3x detection limit of the system was 0.023 and 0.053 ppm for 1 and 2 ppm Ni standard solution, respectively. As seen in Table A.1, the measurement of 5 ppm and 20 ppm Ni standard solutions have RSD % less than 1.

Different wavelenghts 221.647 nm, 231.604 nm, 232.003 nm, 341.476 nm and 352.452 nm were tested at the beginning of the experiments. The wavelength 231.604 nm is in agreement with the literature was used for Ni ions (Figure A.2).

APPENDIX B

Amount of Adsorbed Ni(II) Ions

The amount of adsorbed metal ions (mg) per g biomass was calculated by using the following equation:

$$q = (C_i - C_{eq})* V / m$$

Where q is the amount of metal ions adsorbed on the biomass. C_i and C_{eq} are the initial and equilibrium metal concentration in the solution, m is the amount of biomass and V is the volume of biosorption medium. The experimental and calculated data are given in Tables B.1 – 2 – 3 – 4. At pH 5.00 and 25^o C, the amount of adsorbed nickel ions is calculated below :

C_i = 151.34 mg Ni(II) /L (see Table B.4)
C_{eq}= 140.12 mg Ni(II)/L (see Table B.4)
m = 0.144 g
V = 0.10 mL
q = (151.34 – 140.12) * 0.100 / 0.144
q = 7.78 mg Ni(II) / g biomass

Rate calculations

The initial biosorption rate is obtained by calculating the slope of a plot of the adsorbed metal ion quantity q per gram of dried biomass (mg/g) versus time (min) at t=0. For example the initial biosorption rate at pH 5.0 was found to be 3.65 from the slope of the curve as seen in Figure B.1.

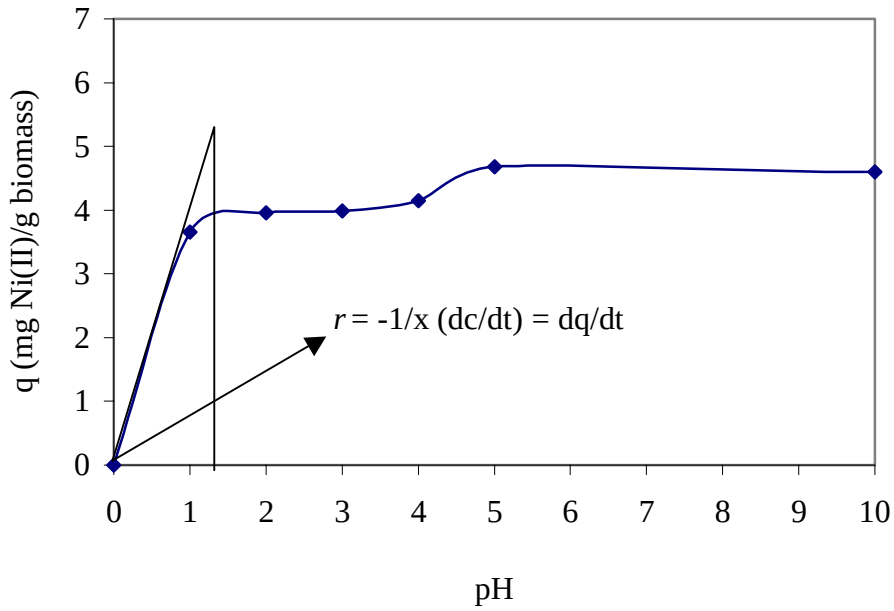

Figure B.1 The adsorption curve for the biosorption of Ni(II) ions on *S.cerevisiae* at pH 5.0. (temperature = 25^{o}C; biomass concentration = 1.0 g/L; agitation rate= 150 rev min^{-1})

Table B.1 The experimental data of the equilibrium for nickel(II) biosorption on *S.cerevisae* for different pH values. (*C*i=100 mg/L; biomass concentration= 1.0 g/L, temperature= 25^{o}C, agitation rate =150 rev min^{-1})

Time (min)	q (mg Ni(II) /g biomass)			
pH:	3.00	4.00	5.00	6.00
1	1.21	1.26	3.95	6.78
2	1.30	1.74	3.97	7.14
3	1.38	1.78	3.98	7.20
4	1.33	1.73	3.99	7.40
5	1.34	2.10	4.00	7.10
10	1.29	3.10	4.68	7.90
15	1.37	4.46	4.60	8.55
30	1.36	2.79	4.80	10.13
60	1.10	2.94	4.98	10.18
90	1.25	2.90	5.13	10.20
120	1.33	2.66	6.00	10.21
180	1.31	2.55	5.99	11.40
240	1.39	3.32	5.62	11.10
300	1.38	2.30	6.03	11.00
360	1.37	3.35	5.98	11.11
480	1.39	3.40	6.00	10.75
720	1.39	3.61	6.07	10.24
960	1.40	3.66	6.10	10.23
1200	1.39	3.63	6.20	10.26
1440	1.40	3.66	6.30	10.30

Table B.2 The experimental data of nickel(II) biosorption on *S.cerevisae* for different biomass concentrations and temperature. (pH=5.0; agitation rate=150 rev.min^{-1})

Temperature (^{o}C)	X (g/L)	C_i (mg/L)	C_{eq} (mg/L)	q_{eq} (mg Ni(II) /g biomass)
15	0.50	100.00	98.00	1.96
	1.00	102.97	100.96	2.00
	1.53	100.00	95.25	3.07
	2.00	98.50	93.50	2.48
	5.00	97.75	88.75	1.78
25	0.10	105.17	101.75	3.87
	0.54	101.28	98.79	4.58
	1.00	104.76	97.17	6.00
	1.54	100.78	94.40	4.12
	2.02	101.57	95.06	3.20
	3.03	101.17	92.91	2.72
	5.02	100.47	90.37	2.00
35	0.54	97.75	96.75	1.90
	1.00	103.60	101.21	1.98
	1.51	100.50	99.27	1.85
	2.98	97.50	96.61	1.77
	5.00	98.25	97.91	1.69
45	0.52	97.24	96.00	2.05
	1.00	102.75	100.83	1.85
	1.53	95.75	92.50	2.10
	2.00	94.00	90.51	1.74
	5.00	95.25	87.50	1.53

Table B.3 The experimental data of nickel(II) biosorption on *S.cerevisae* for different metal concentrations and pH values. (biomass concentration= 1.0 g/L, temperature=25^{o}C agitation rate= 150 rev min^{-1})

pH	C_i (mg/L)	C_{eq} (mg/L)	q_{eq} (mg Ni(II) /g biomass)
3.00	60.10	59.22	0.72
	101.70	100.21	1.38
	151.21	148.51	2.50
	175.70	173.10	2.55
	249.87	247.10	2.57
4.00	52.30	49.67	2.51
	78.15	74.21	3.50
	103.13	97.67	5.07
	159.51	152.79	6.11
	255.00	249.50	6.18
5.00	51.03	45.69	3.37
	100.90	91.94	5.80
	151.34	140.12	7.78
	199.98	186.64	7.82
	253.60	241.24	8.00

Table B.4 The experimental data of nickel(II) biosorption on *S.cerevisae* for different metal concentration and temperature. (biomass concentration=1.0 g/L, pH=5.0 agitation rate =150 rev min^{-1})

Temperature (^oC)	C_i (mg/L)	C_{eq} (mg/L)	q_{eq} (mg Ni(II) /g biomass)
15	51.08	49.25	1.65
	99.75	97.75	2.00
	152.75	149.75	2.85
	203.50	199.75	3.07
25	51.03	45.69	3.37
	100.90	91.94	5.80
	151.34	140.12	7.78
	253.60	241.24	8.00
35	51.21	50.00	1.05
	103.60	101.21	1.98
	153.00	149.83	2.67
	205.00	201.25	2.70
45	51.75	50.83	0.95
	102.75	100.83	1.85
	155.00	149.50	2.34
	205.00	201.84	2.61

APPENDIX C

Determination of the Langmuir model parameters

The Langmuir equation can be represented by the following equation:

$$q = q_{max}\, b\, C / (1+bC)$$

Where q is the amount of adsorbed per unit mass adsorbent, q_{max} is the maximum amount of adsorbed per unit mass adsorbent or the monolayer capacity, b is an empirical constant that reflects the affinity between adsorbent and adsorbate and C is the concentration of adsorbate in solution at equilibrium. The experimental data can be plotted to estimate q_{max} and b with rearranging the Langmuir equation as:

$$1/q = 1/ q_{max} + 1/(b\, q_{max} C)$$

so that plot of $1/q$ versus $1/C$ has slope $1/b\, q_{max}$ and intercept $1/ q_{max}$. The linearized Langmuir and Freundlich adsorption isotherms for nickel ions at different conditions are given in Figures C.1–14. For example, at pH 5.0 and 25^{o}C the Langmuir parameters b and q_{max} are obtained by using the Figure C.2. As seen in Figure C.2, the slope and intercept are given below:

slope = $1/ (b\, q_{max})$ = 10.128
intercept = $1/ q_{max}$ = 0.0701
q_{max} = 14.265 mg Ni(II)/g biomass
b = 0.0069 L/mg

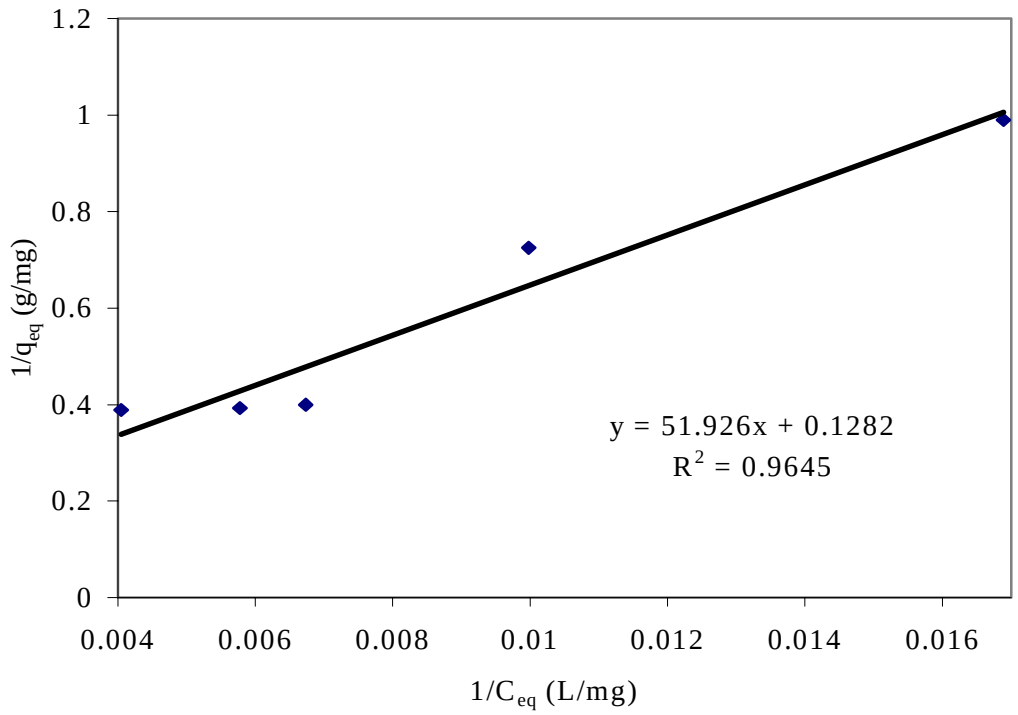

Figure C.1 The linearized Langmuir adsorption isotherm obtained at pH=3.0 (Biomass concentration =1.0 g/L; temperature =25^{o}C; agitation rate= 150 rev min^{-1})

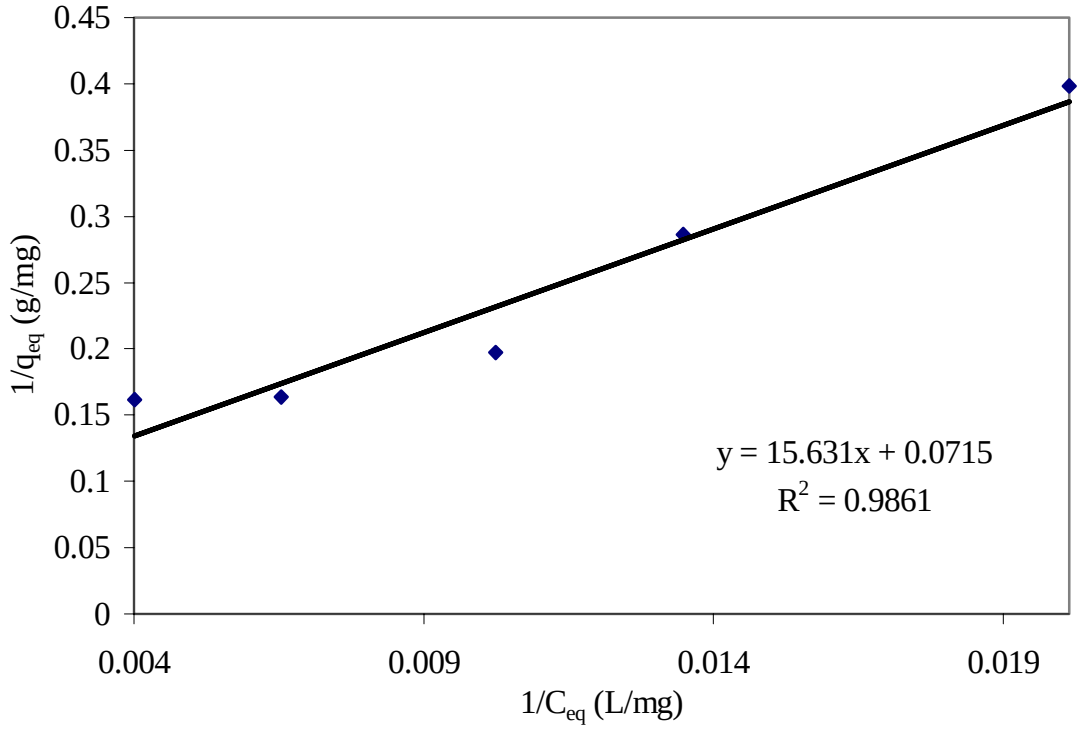

Figure C.2 The linearized Langmuir adsorption isotherm obtained at pH=4.0 (biomass concentration 1 g/L; temperature 25^{o}C; agitation rate=150rev min^{-1})

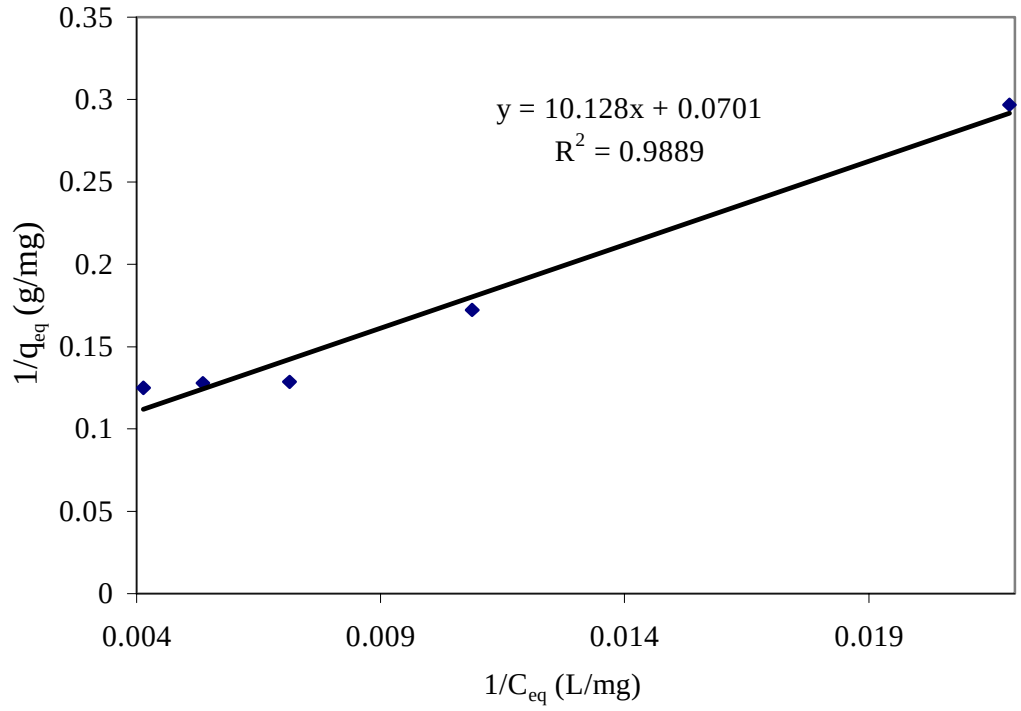

Figure C.3 The linearized Langmuir adsorption isotherm obtained at pH=5.0 (biomass concentration=1 g/L; temperature=25^{o}C ; agitation rate = 150 rev min^{-1})

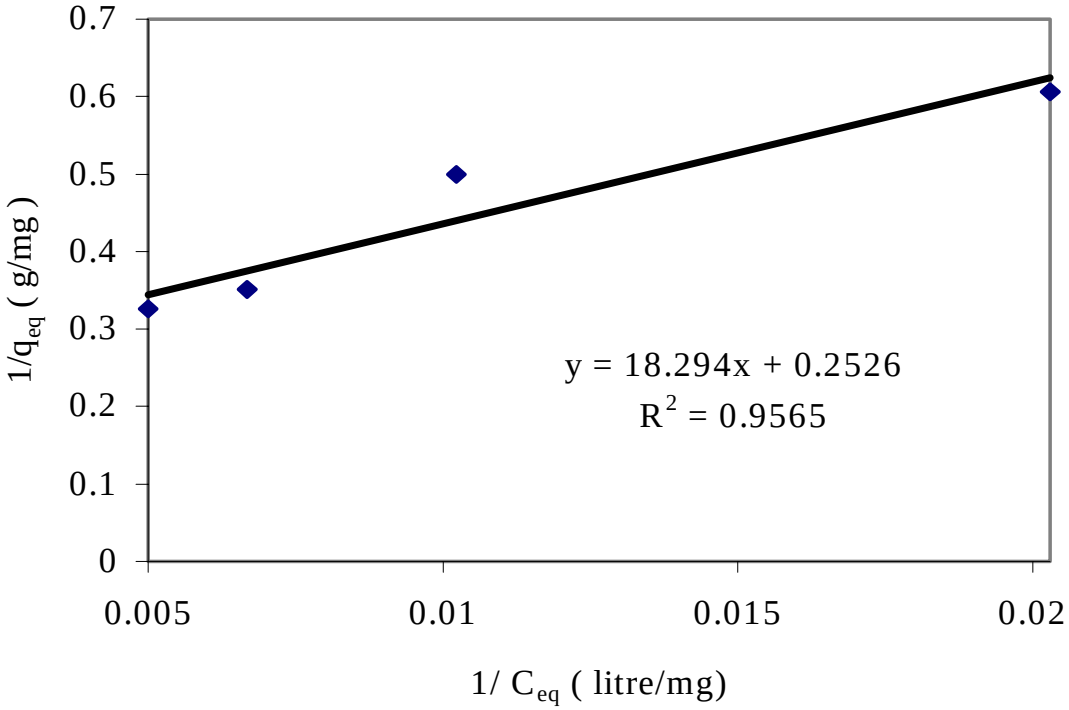

Figure C.4 The linearized Langmuir adsorption isotherms obtained at 15^{o}C (pH=5.0; biomass concentration=1.0 g/L ; agitation rate = 150 rev min^{1})

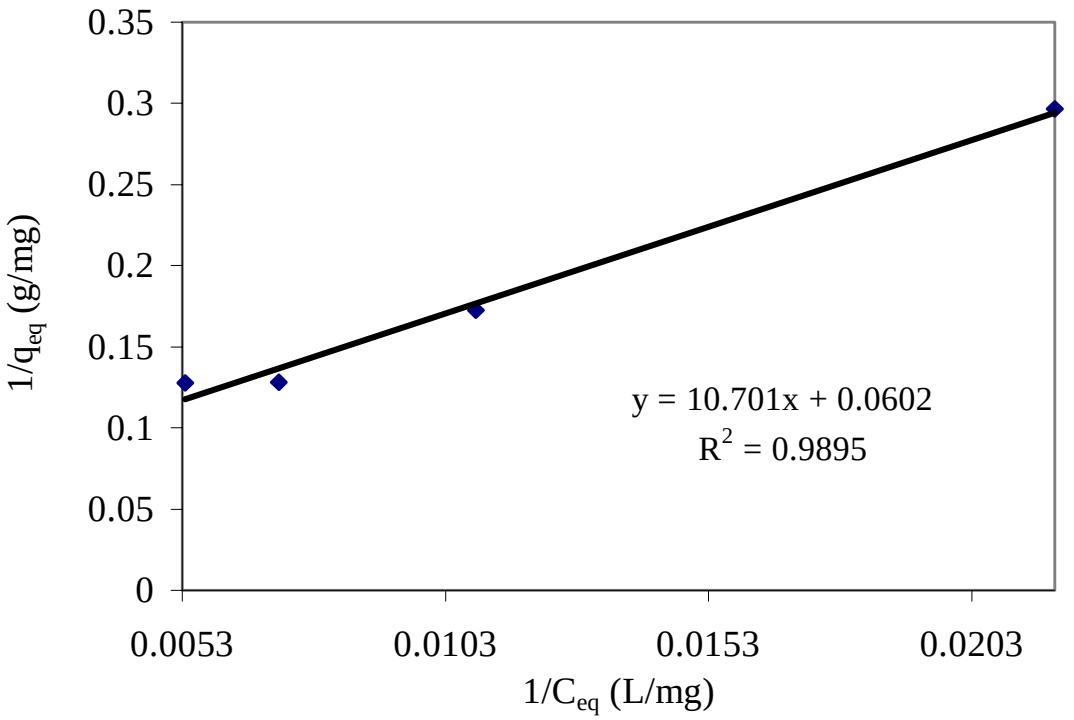

Figure C.5 The linearized Langmuir adsorption isotherms obtained at 25°C (pH=5.0; biomass concentration=1.0 g/L ; agitation rate=150 rev min^{1})

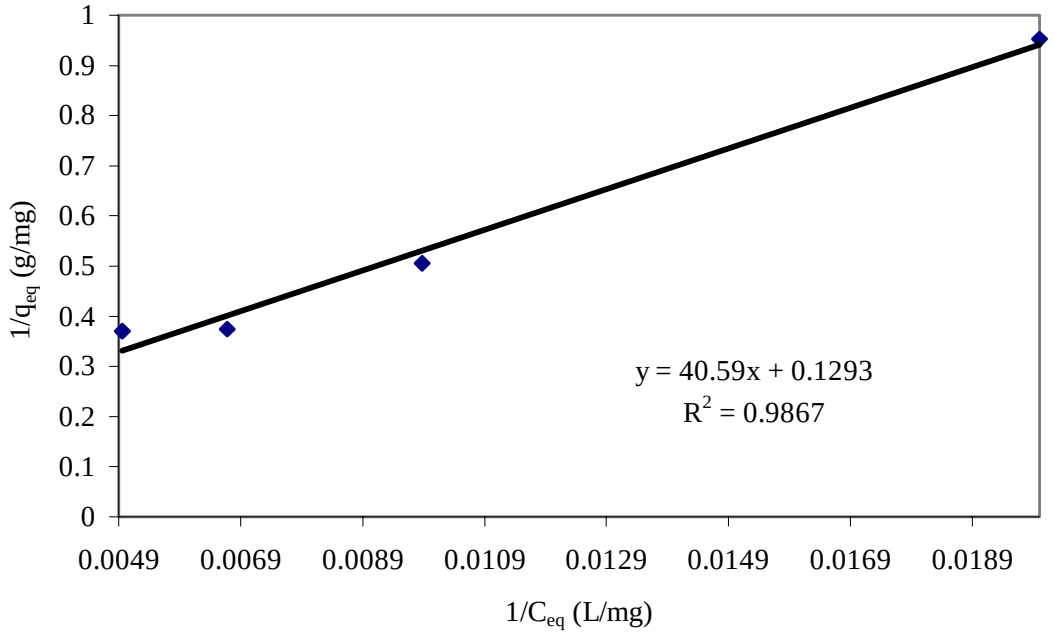

Figure C.6 The linearized Langmuir adsorption isotherms obtained at 35°C (pH=5.00 biomass concentration=1.0 g/L ; agitation rate = 150 rev min^{-1})

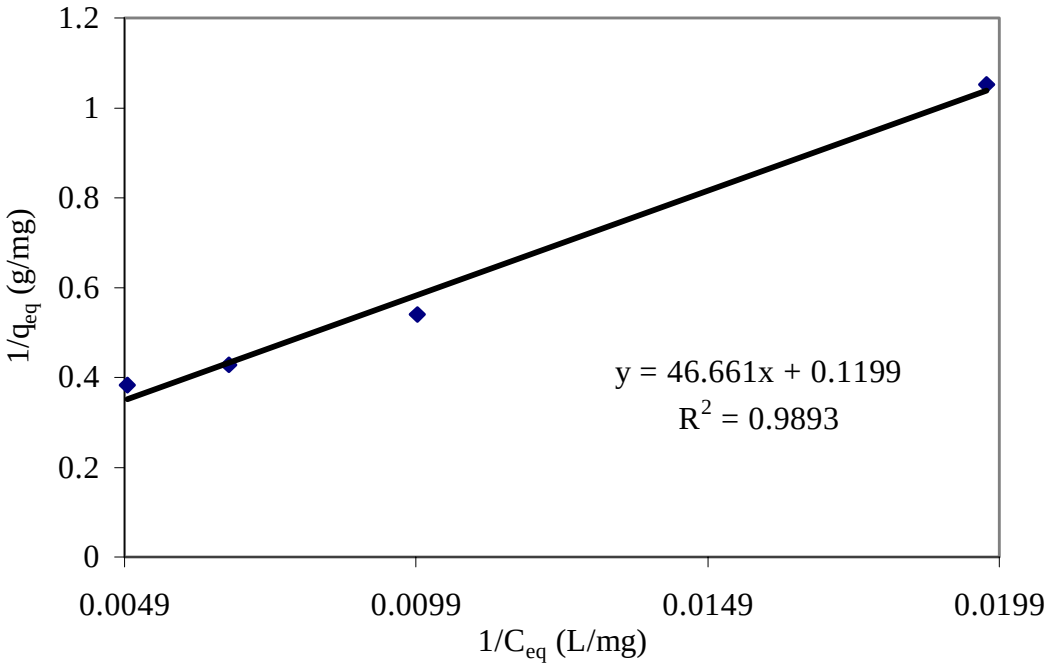

Figure C.7 The linearized Langmuir adsorption isotherms obtained at 45°C (pH 5.00; biomass concentration 1 g/L ; agitation rate : 150 rev min^{-1})

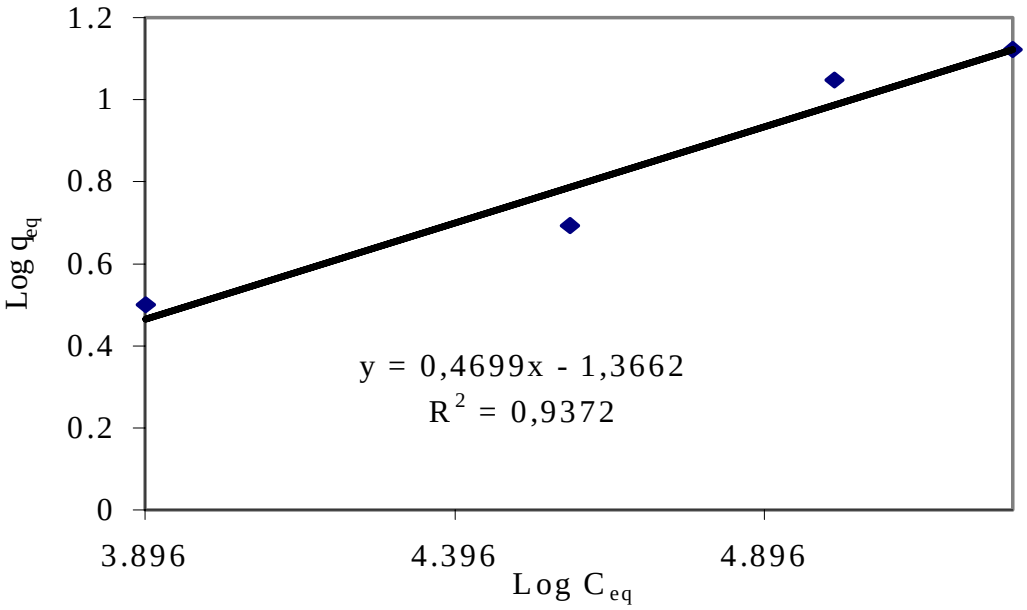

Figure C.8 The linearized Freundlich adsorption isotherms obtained at 15°C (pH=5.00; biomass concentration=1.00 g/L ; agitation rate=150 rev min^{-1})

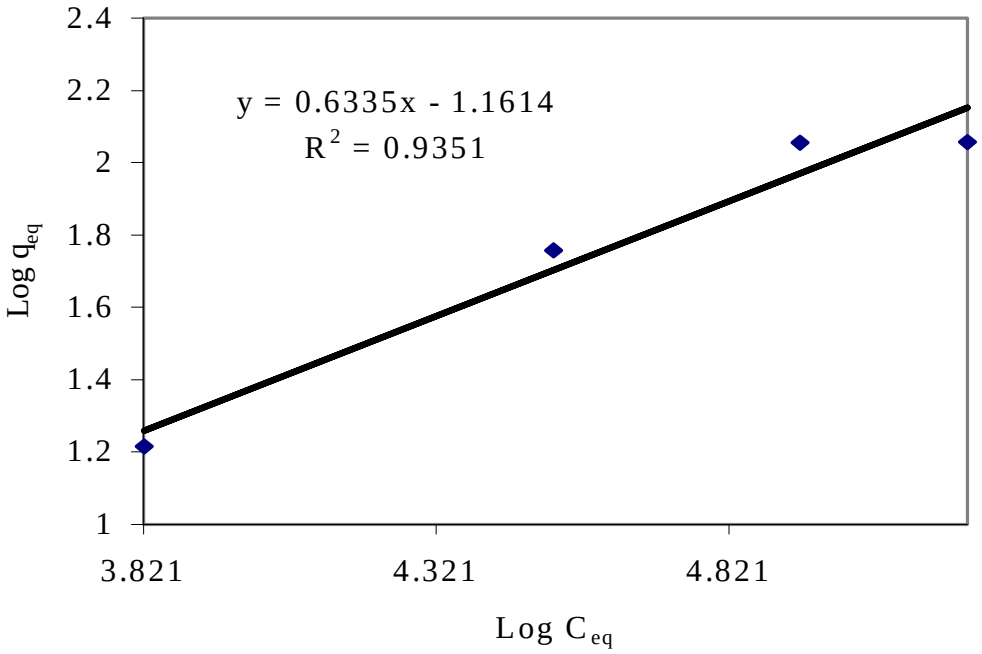

Figure C.9 The linearized Freundlich adsorption isotherms obtained at 25°C (pH=5.00; biomass concentration=1.0 g/L ; agitation rate=150 rev min^{-1})

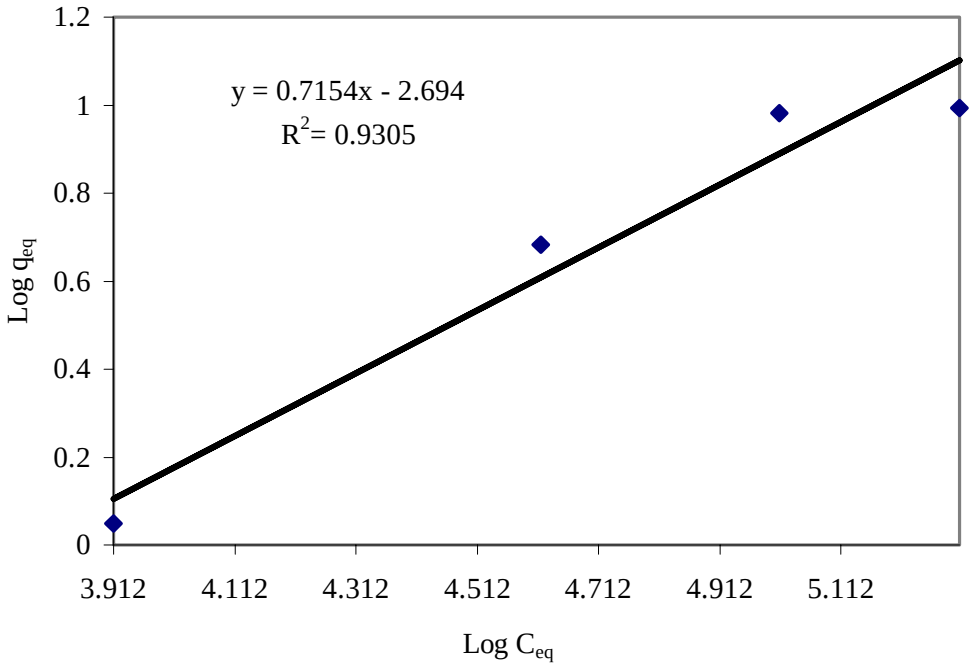

Figure C.10 The linearized Freundlich adsorption isotherms obtained at 35°C (pH=5.00; biomass concentration=1.0 g/L ; agitation rate=150 rev min^{-1})

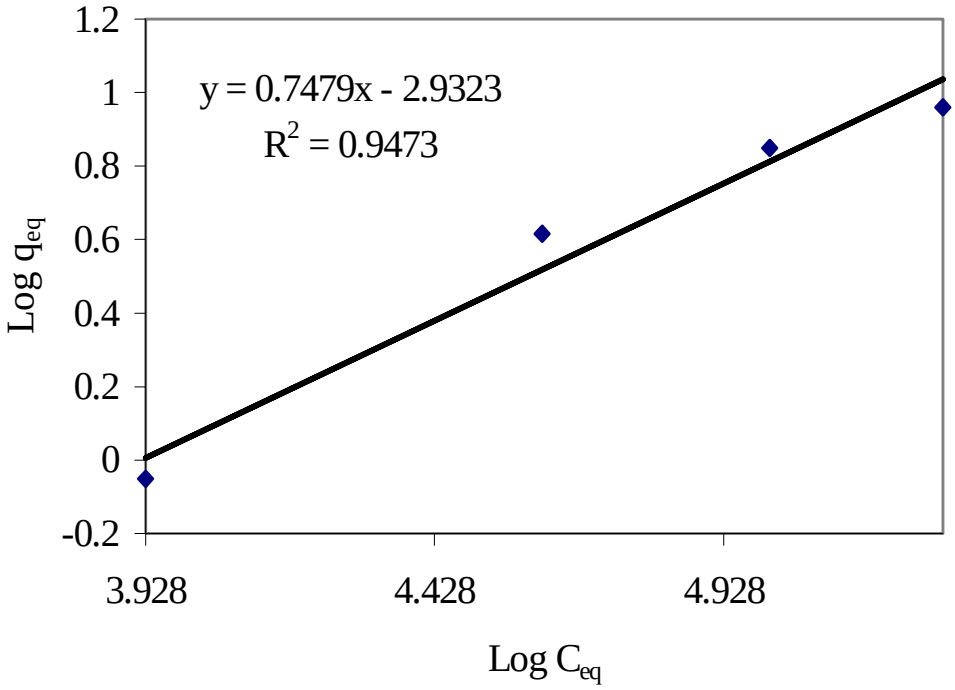

Figure C.11 The linearized Freundlich adsorption isotherms obtained at 45°C (pH=5.00; biomass concentration=1.0 g/L ;agitation rate=150 rev min^{-1})

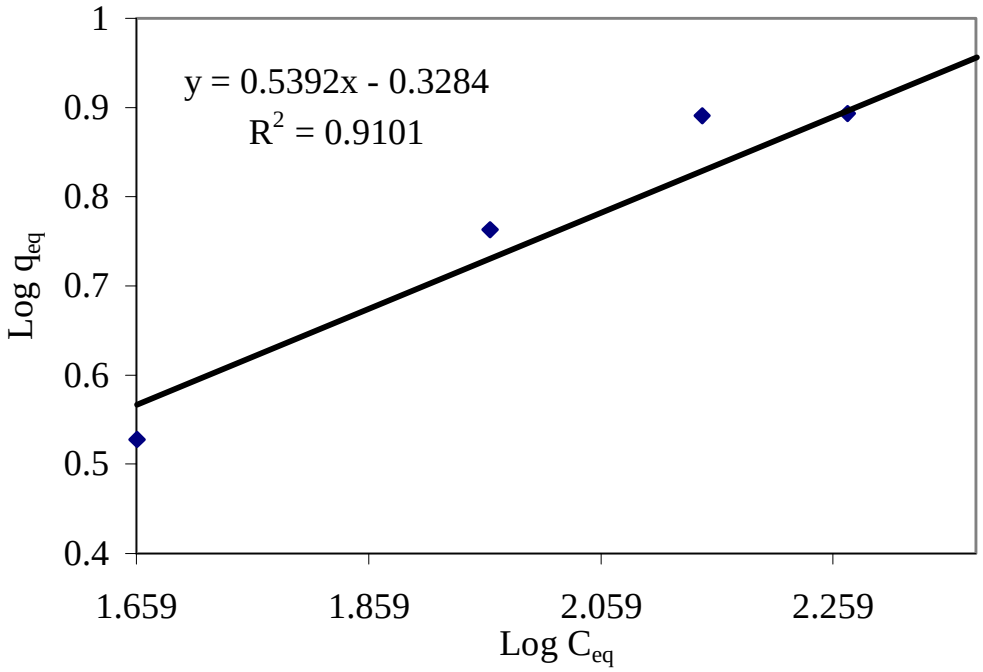

Figure C.12 The linearized Freundlich adsorption isotherm obtained at pH=5.0 (biomass concentration=1 g/L;temperature=25°C;agitation rate=150 rev min^{-1})

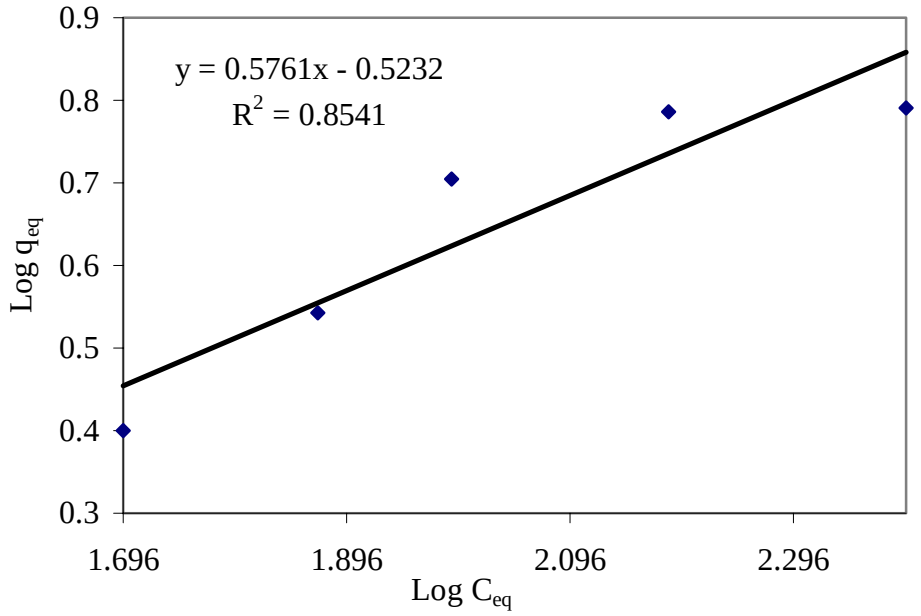

Figure C.13 The linearized Freundlich adsorption isotherm obtained at pH=4.0 (biomass concentration=1 g/L; temperature=25^{o}C; agitation rate=150 rev min^{-1})

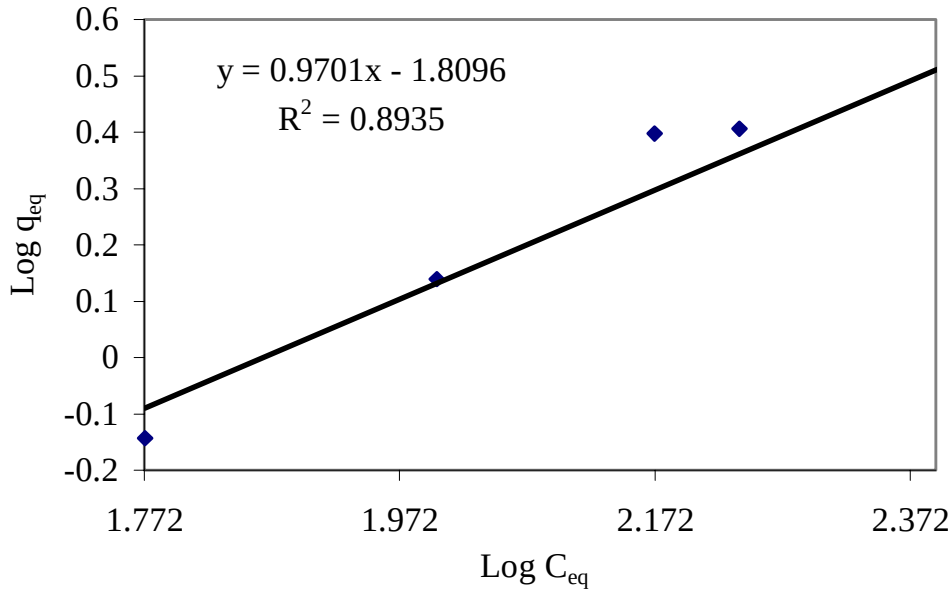

Figure C.14 The linearized Freundlich adsorption isotherm obtained at pH=3.0 (biomass concentration=1 g/L; temperature=25^{o}C; agitation rate=150 rev min^{-1})