Diyala Journal For Pure Sciences Vol:6 No:1
Jan. 2010

Biosorption of Hg and Ni ions on Bakers Yeast

BY

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ABSTRACT

The present study evaluates the potential of the yeast *Saccharomyces Cerevisiae* to remove mercury and nickel from aqueous solutions. The effect of pH, initial concentration, contact time, and biosorbent dosage on biosorption capacity is studied in batch experiments. Experiment results show that metal uptake is a rapid process at pH values (5.0-6.0). Sorption isotherms are studied to explain the removal mechanisms of metal ions by fitting isotherm data into Langmuir and Freundlich equations, the biosorption of mercury and nickel from aqueous solutions on live yeast at an initial pH of (5.0-6.0) could be described by both the Freundlich and the Langmuir adsorption isotherms. Pretreatment using NaOH, HCL, and ethanol enhance biosorption capacity of the yeast.

It was concluded that nitric acid with low concentration of 0.05 N is effective in desorbing the biosorbed metal ions. On the other hand, sodium hydroxide solution of 0.2 M is effective in regenerating the yeast; the regenerated yeast could be used for at least six cycles of biosorption, without losing its metal removal capacity. Carboxyl, amine, and phosphate groups present in the yeast were found to be the main biosorption sites for metal ions.

1. Introduction

Heavy metals do not play a role in metabolic processes and are highly toxic even in low concentration [1]. Pollution has become one of the most serious environmental problems today. Heavy metal means the metal ion whose specific weight is usually more than 4or 5g l^{-1} [2]. Heavy metal pollution represents an important environmental problem due to the toxic effects of metals, and their accumulation through out the food chain leads to serious ecological and health problems [3]. Nickel ion intake over the permissible levels (0.2 mg/L) results in different types of disease such as pulmonary fibrosis, renal edema, and skin dermatitis, gastrointestinal

distress(e.g. navsea, vomiting, diarrhea [4]. Mercury, as one of the most dangerous heavy metals, with permissible levels ≤ 0.01 mg/L, in any form introduced to the natural environment from a variety of sources is converted into more toxic form, i.e., methyl mercury chloride by aquatic living-organisms, and accumulated in the tissue of fishes and birds [5].The illness, which came to be known as Minamata disease, was caused by mercury poisoning as a result of eating contaminated fish [6]. Mercury has very high tendency for binding to proteins and it mainly affects the renal and nervous systems [7]. In humans, the initial symptoms include numbness of the lips and limbs. As the Sickness progresses, permanent damage is done to the central Nervous system, and the victim experiences visual constriction, loss of motor coordination, and, in the final stages prior to death, loss of memory, speech, hearing and taste.

Because of these reasons, mercury must be removed to very low levels from wastewater generated in industries such as metal smelting and caustic-chlorine production in mercury cells, metal processing, plating and metal finishing. These effluents require chemical treatment before they can be discharged. Different treatment techniques have been developed to remove either or both dissolved and suspended heavy metal ions from industrial waste waters. Anumber of traditional treatment techniques included precipitation-neutralization, ultra-filtration, reverse osmosis, electro-deposition, solvent extraction, foam-flotation, cementation, complextion/sequestration, filtration and evaporation [5]. The necessity to reduce the amount of heavy metal ions in wastewater stream has led to an increasing interest in selective supports [8].

Biosorption is an alternative technology for the treatment of wastewater containing metal ions, biosorption referred to the pollutants uptake by living or non living biomass [9]. It is also a process in which solids of natural origin are employed for binding heavy metals. It is a promising alternative method to treat industrial effluents, mainly because of its low cost and high metal binding capacity [10]. In biosorption, either live or dead microorganisms or their derivatives are used, which complex metal ions through the functioning of ligands or functional groups located on the outer surface of the cell [11]. Microorganisms including bacteria, algae, fungi and yeasts are found to be capable of efficiently accumulating heavy metals [11, 12, 13]. The mechanisms associated with metal sorption by biological materials are complex and involve both extra cellular and intracellular metal binding. *Saccharomyces cerevisiae* is easy to cultivate at large scale. The yeast can be easily grown using unsophisticated, It should be noted that with different

pretreatment methods and experimental conditions, the capacity of a fungal biomass for a metal varies. The economical and ecological feasibility of biosorption processes depend on the biosorbent metal uptake capacity to reach metal concentration legal limits for wastewater discharge and the ability of elutants to release sequestered metal in subsequent recovery [14]. Recovery allows metal recycling, leading to energy savings and materials conservation [15].

Finally, biosorbent regeneration for use in multiple adsorption–desorption cycles, contributes to process cost effectiveness. The efficiency of metal recovery depends on choice of eluent and elution conditions, as various eluants presenting different desorption mechanisms may be used. Lowering pH (e.g. with mineral acids) causes metal desorption [16], resulting from competition between protons and metal ions for binding sites [14]. Mineral acids such as HCl, H_2SO_4 and HNO_3 and the organic acid CH_3COOH are efficient desorption agents [17]. The purpose of this work is to study optimal conditions for Hg and Ni ^{sorption} on the yeast *Saccharomyces cerevisiae*.

2. Materials and methods

2.1. Microorganism

Saccharomyces cerevisiae type (DCL. SM1. 4SP) from market was used in this investigation.

2.2. Metal solutions

Solutions were prepared by dissolving $HgCl_2$ and $NiCl_2.6H_2O$ in DDW. Metal solution was adjusted to different pH with 0.1 M NaOH and 0.1N HCL, prior to mixing with the yeast. Stock solutions were used for preparing different test solutions with varying metal ion concentration.

2.3. Elutants

Various chemical solutions were used to desorb metal ions biosorbed. The elutants used include de ionized water, 0.05N HNO_3, and $CaCl_2$.

2.4 Metal uptake

The metal uptake q was calculated from the mass balance as follows:

$$q = \frac{v\,(C_o - C_e)}{m * 1000} \qquad (1)$$

where q is the quantity of metal uptake by biomass (mg per gram); C_0 and C_e are the initial and final (after sorption at equilibrium) metal concentration, respectively; V is the volume of solution in ml and m is the dry weight of the biomass added.

To describe and analyze adsorption equilibrium, a number of adsorption isotherm models have been developed. The Langmuir and Freundlich are the models commonly applied in the field of environmental engineering [18]. The Langmuir mode1 has the following form:

$$q = q_m \frac{b\ C_e}{1+\ b\ C_e} \qquad (2)$$

Where,

q = amount of adsorbate adsorbed per unit weight of adsorbent (g/mg);

q_m = constant related to the energy or net enthalpy of adsorption; (mg/g);

b = amount of adsorbate adsorbed per unit weight of adsorbent; L/mg.

C_e = concentration of adsorbate in solution at equilibrium (mg/L),

Linearized Langmuir model is:

$$\frac{1}{q} = \frac{1}{q_m b}\ \frac{1}{C} + \frac{1}{q_m} \qquad (3)$$

The Freundlich isotherm model was developed for heterogeneous surfaces and is empirical and described in a nature as.:-

$$q = K\ \ (C_e)^{1/n} \qquad (4)$$

Where:

k = equilibrium constant indicative of adsorption capacity ($mg^{1-1/n}.g^{-1}.L^{1/n}$);

n = adsorption equilibrium constant (dimensionless).

By converting the above equation to a linear form and using the graphical method, Eq. (4) is re-written:

$$Log\,(q) = Log\,(k) + \frac{1}{n} Log\,(C_e) \quad (5)$$

2.5 Metal binding experiments

2.5.1 Effect of pH

Experiments were conducted at 25 ^{0}C to study the effect of solution pH on metal ions biosorption by contacting (0.1 g) of live or pretreated yeast with 100 ml of 10 mg/L metal ions solution. The mixture was agitated on a rotary shaker (DUBNOOT BSD/DCE) at 200 rpm for 3 h. The pH was adjusted to the required value using 0.1 M NaOH and/or 0.1N HNO_3 before the addition of the sorbent. The studies were conducted at pH 2, 3, 4, 5.5, 6, and 8. In the meantime, a control without yeast was set up. PH in the reaction mixture was not controlled. Samples were withdrawn at pre-determined time intervals (5, 15, 30, 50, 80, 120, 150, and 180 min), centrifuged at 3000 rpm for 10 min. and the final pH were recorded. The residual metal ions concentration in the solution was analyzed by an atomic absorption spectrophotometer**.** These experiments were repeated and the mean values were used.

2.5.2. Biosorption studies

Equilibrium sorption experiments were carried out at best pH of each metal ion which was determined throughout this work. By contacting 0.1, 0.5, 1.0, 2.0, 2.5, and 3.0 g of sorbent with 100 ml of metal ion solution, the ion concentrations were varied over the range 10–100 mg/L. The mixture was agitated on a rotary shaker at 200 rpm for 3 h. The sorbent was separated from the solution and the supernatant was analyzed for metal ions. All experiments were conducted in duplicates and at the room temperature around (25^{o}C). In the meantime, a control without yeast was set up.

2.5.3 Desorption of Metal Ions and Regeneration and Reuse of Yeast

Various chemical solutions were used to desorb metal ions biosorbed. After biosorption of Ni and Hg, yeast samples (0.1 g dry weight) (separated from metal solution by centrifugation) was contacted with 25 ml of various elutants for one hour on a rotary shaker at 125 rpm. The elutants used include de ionized water, 0.05N HNO_3, and $CaCl_2$. The mixture was centrifuged at 3000 rpm for 10 min. and measured the supernatant for metal ions concentration.

For the regeneration of yeast eluted using acidic elutants, two methods were used. The first method is to wash the yeast with deionized water to remove H^+ ions from yeast till pH of the wash solution reaches a range of 5.0 to 5.4. The second method is to use 0.2N NaOH solution at

a solid/liquid ratio (S/L) of 1g/L of yeast to condition the yeast for 30 minutes. Afterwards; a generous amount of deionized water is used to rinse the regenerated yeast till pH in the wash solution reached the range of 7.0 to 8.0. Yeasts regenerated or conditioned with these two methods are dried in an oven at 60 ^{o}C for 6 hours and then reused for two cycles of biosorption-elution-regeneration to evaluate the performance of yeast in retaining metal biosorption capacity**.**

2.5.4 Pretreatment Methods

Live yeast in batches of 5 g (dry weight) was pretreated in eight ways listed in Table (1). In each pretreatment, the yeast was slowly stirred in the chemical solution for a suitable period of time. After each pretreatment the yeast was washed with generous amounts of de ionized water and then dried in an oven at 60 ^{O}C for 6 hours. For the untreated control sample, yeast was directly used in the biosorption experiments.

Table (1): Pretreatment methods applied to *Saccharomyces cerevisiae*

Type	Solution	Duration (min)	Autoclave(*)
1	Raw Yeast	20	X
2	100 ml ethanol of 700 g/L	15	+
3	100 ml of 1 mol l^{-1} NaOH	15	+
4	100 ml DDW	15	+
5	100 ml of 0.1 N HcL	15	X
6	100 ml of 0.1 N HcL^{+}	15	+
7	100 ml 0f 0.2 N $CaCl_2$	20	X
8	100 ml 0f 0.5 N $NaCl_2$	20	X

* **Autoclaved for 30 min at l2l°C (15) psi; (+) applied; (x) not applied**.

3. Results and discussion

3.1. Effect of Different Variables on Biosorption Process

3.1.1 PH

Figures (1) shows the relation between metal uptake and pH indicates that at low pH, protons would compete with metals for the active sites responsible for the biosorption which would decrease the metal sorption. However, at an initial pH of 4.0 or less, lower biosorption was occurred. It should be noted that at pH 2.0 the metals biosorption has not been observed. The low biosorption capacity at pH values below 4.0 was attributed to hydrogen ions that compete with metal ions on the sorption sites.

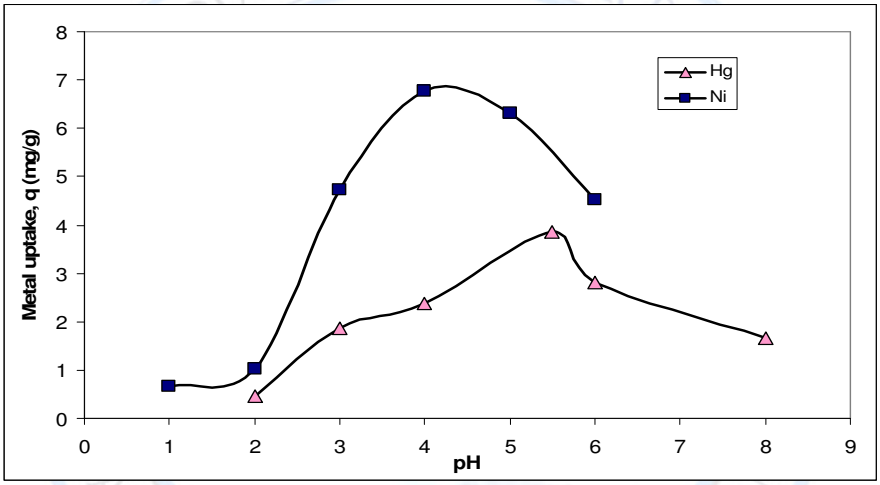

Figure (1): Effect of pH on biosorption of mercury and nickel at room temperature. Reaction volume= 100 ml, yeast weight=0.1 g, T=25 ^{0}C.

3.1.2 Initial Concentrations of Metal Ions

On the other hand, figure (2) shows the relation between metal uptake and initial metal concentration, biosorption has been observed to increase as initial concentration increases; this may be attributed to the active binding sites available for available sorbate ions [19]. Figures (3 and 4) shows that biosorption is very fast for the metal ions in the first 5 minutes, while for the remaining time period, the metal concentrations in the liquid continued to diminish and reach an equilibrium concentration value. The faster first phase of metal biosorption may be attributed to

the surface adsorption due to the action of ion exchange with the participation of some functional groups; while the second lower phase may represent diffusion of metal ions into the cell.

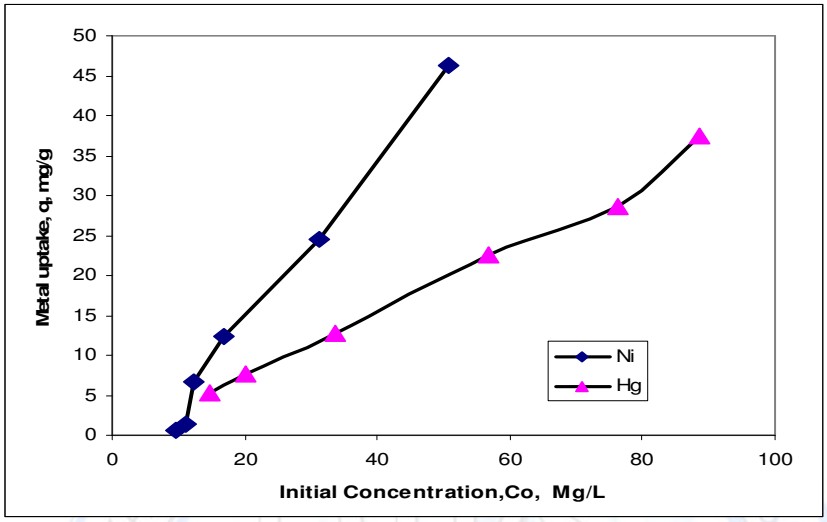

Figure (2): Effect of different initial concentrations on metal uptake, reaction volume= 100 ml, yeast weight=0.1 g, T=25 ^{0}C.

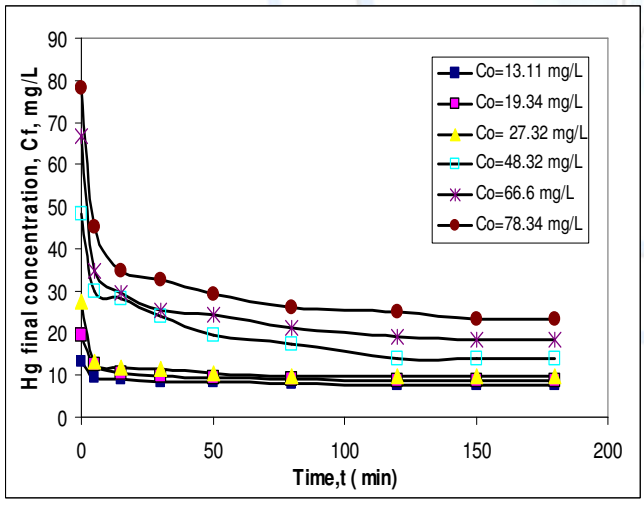

Figure (3): Relation of mercury concentration with time at different initial concentrations, reaction volume= 100 ml, yeast weight=0.1 g.,

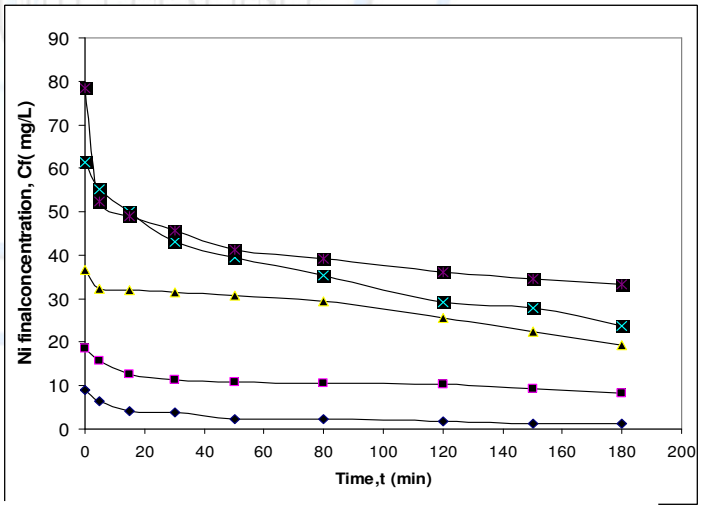

Figure (4): Relation of nickel concentration with time at different initial concentrations, reaction volume= 100 ml, yeast weight=0.1 g.,

3.1.3Concentrations of Yeast

The effect of different initial concentrations of yeast on biosorption of the metal ions of Ni and Hg is shown in figure (5). It can be seen that as amount of yeast increases, the metal uptake decreases. This is due to interaction of binding sites. In order to assess a wide range of yeast concentration to see its impact on biosorption, figure (6) show the concentration gradients of metal ions with different amounts of yeast. Figures (7,8) show the metal uptake, q (mg/g) with time at different initial concentrations.

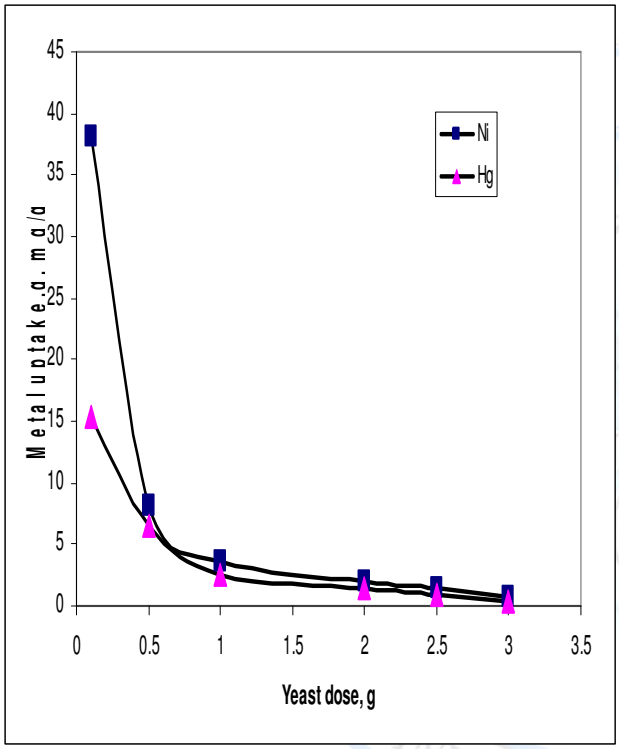

Fig.(5):Metal uptake at different yeast concentrations,

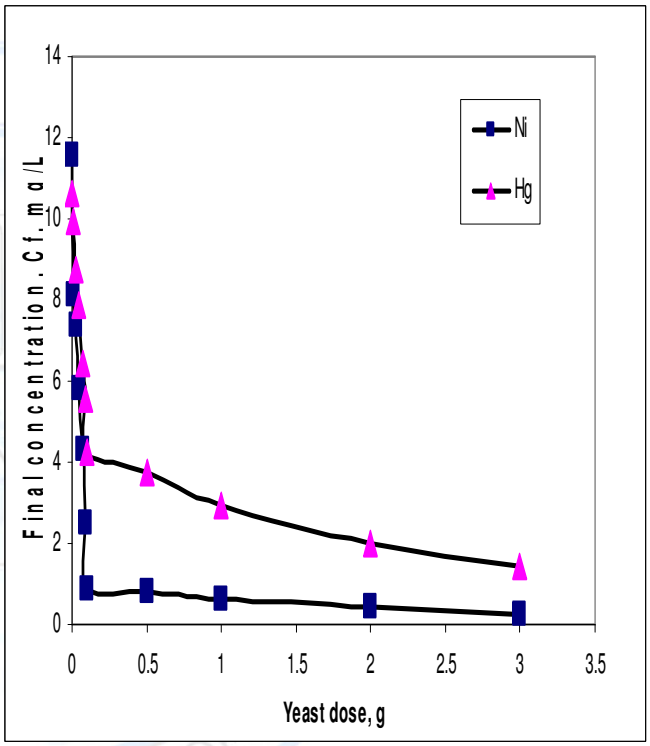

Fig.(6):Concentration gradient with amount of yeast , and reaction volume= 100 ml, T=25

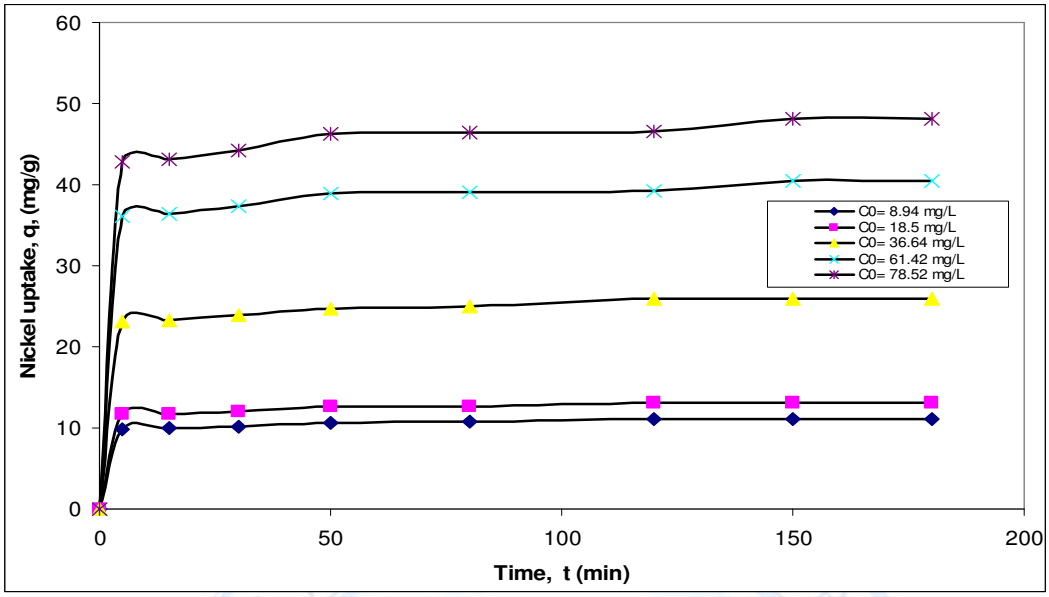

Fig. (5.19): Nickel uptake with time at different initial concentrations, reaction volume= 100 ml., yeast=0.1 g, pH=6.0.

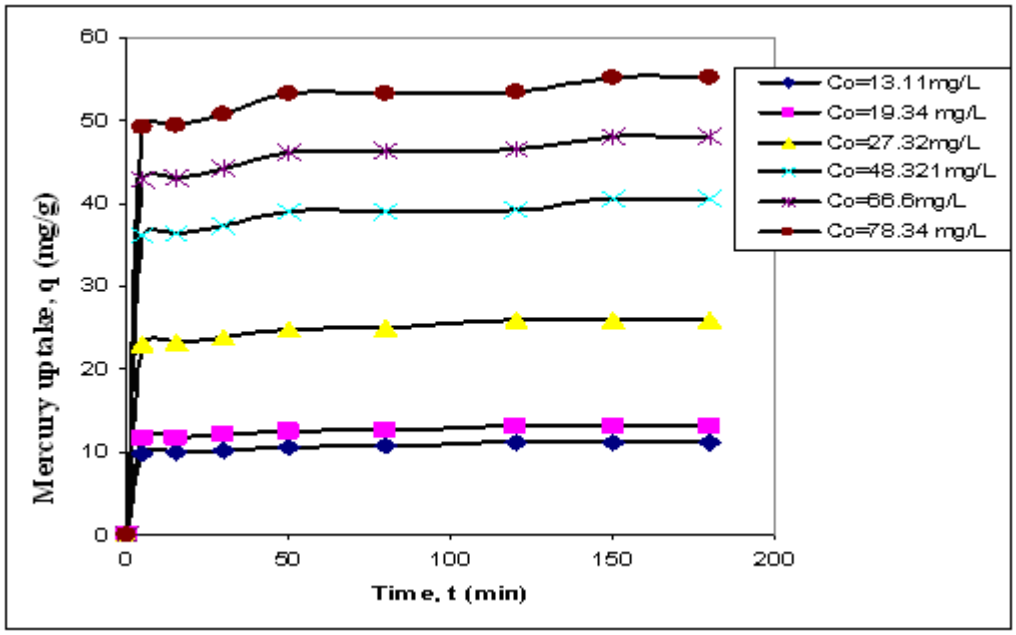

Fig. (8): Mercury uptake with time at different initial concentrations, reaction volume= 100 ml., yeast=0.1 g, pH=6.0.

The (q) versus (C) sorption isotherms relationship is mathematically expressed by linearized Langmuir and Freundlich models (Eq. (2 and 4)). As the values of (k) and (n) are high

and value of (b) small, the affinity of the yeast is large [20]. Tables (2, 3) show Langmuir and Freundlich parameters at different initial concentrations.

Table (2): Langmuir and Freundlich parameters of Nickel ions at different Initial concentrations

Nickel Ions conce. (mg/L)	Langmuir parameters		Freundlich parameters	
	q_m (mg/g)	b (l/mg)	K (l/mg)	n
78.52	69.54	2.45	58.65	28.56
61.42	55.2	7.65	56.06	24.12
36.64	46.45	6.05	31.8	22.55
18.5	17.5	6.98	11.54	35.46
8.94	7.96	7.54	6.85	28.56

Table (3): Langmuir and Freundlich parameters of mercury ions at different initial concentrations

Merrycu Ions conce. (mg/L)	Langmuir parameters		Freundlich parameters	
	q_m (mg/g)	b (l/mg)	K (l/mg)	n
78.34	69.54	2.45	65.65	30.52
66.60	55.2	7.65	55.06	27.12
48.32	46.45	6.05	42.8	25.55
27.32	27.65	1.78	24.54	40.46
19.34	18.33	6.95	12.85	34.56
13.11	11.75	4.89	11.36	19

3.3 Elution of Biosorbed Metal Ions

Various elutions were used to desorb the metal ions loaded on yeast. Table (4) shows the elution of biosorbed metals by various reagents. It is clear that HNO_3 proved to be a more effective elutants than $CaCl_2$ and distilled water. Bruno [21] showed that more than 95% of lead could be desorbed from nonliving *Sargassam sp.* with the use of mineral acids. The mineral acids are proton exchange agents. HNO_3 was able to effectively elute biosorbed metal ions from *Aspergillus niger* [22].

Table (4): Elution of biosorbed metals by various reagents

Chemical reagent	% recovery Ni	% recovery Hg
Distilled water	4.65	5.34
0.05 N HNO_3	92	89
$CaCl_2$	54	60

3.4 Effect of Pretreatment on Yeast

As shown in figures (9, 10); live yeast was observed to possess a nickel and mercury biosorption capacity (14.52, 4.22 mg/g) at pH 6 with 51, 27 % removal of metal ions respectively. Pretreatment using ethanol increases biosorption capacity of nickel and mercury from (14.52-22.32,4.22-8.02 mg/g) at pH 6 with 79, 51 % removal of metal ions, The higher metal uptake values obtained by ethanol treated yeast cells may be explained by the increase in the availability of binding sites and thereby the improvement in the access of metal ions to the metal binding sites of yeast cells. While using caustic treatment and boiling water gives (14.52-18.36, 14.52-17.35, and 14.52-16.56 mg/g) with 64, 60, and 58 % removal respectively for nickel and for mercury(4.22-7.91, 8.91, and 9.23) with 51,57, and 59% respectively. The highest metal uptake was obtained with caustic treated yeast cells and this effect of caustic treatment on metal uptake was explained by the removal of protein groups of the cell wall that make non-

absorbable protein complexes with metal ions. In other wards, when proteins are dissolved from the cell wall of yeast cells, the protein molecules in the liquid phase compete for metal ions with the protein molecules on the cell wall and these metal ions —protein complexes were not adsorbable, thereby impending the nickel and mercury binding. By fixing the soluble protein in the cell wall by some denaturation processes such as heat and ethanol treatment gives better results; Using 0.1 N HCl with autoclaving gives result as (14.52-22.94, 4.22-8.12 mg/g) with 80, 52 % removal of metal ions and 74, 57% without autoclaving respectively. The reduction of biosorption capacity when using $NaCl_2$ and $CaCl_2$ in comparison with live yeast may be attributed to the loss of intracellular uptake or loss of amino functional groups on the yeast surface through the non-enzymic browning reaction with 38, 34% removal for nickel and 7, 13% for mercury.

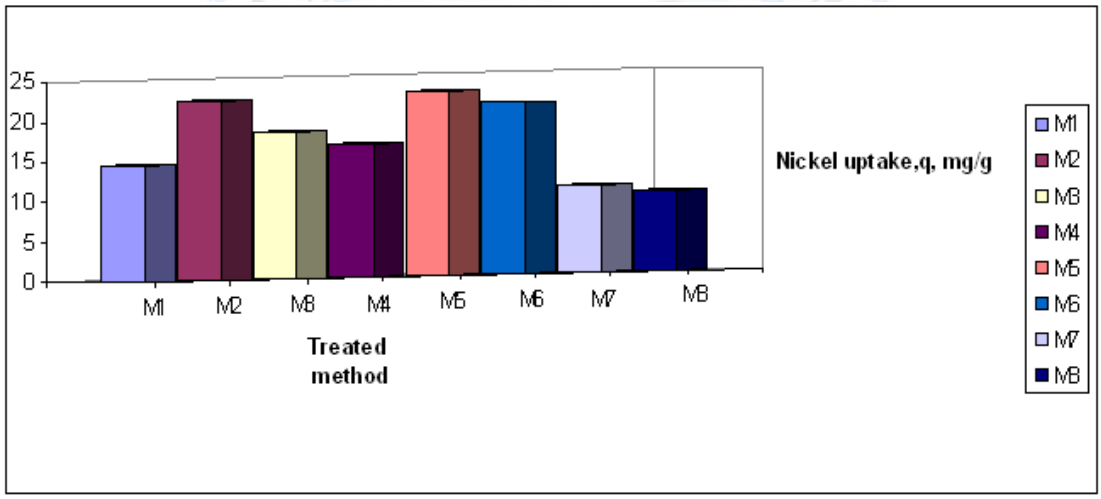

Fig. (9): Effect of different treated methods on metal uptake of nickel at pH 6.0, 0.1 g yeast, v=100 ml, M1= raw yeast, M2= ethanol, M3= 100 ml of 1 mol/L NaOH, , M4= 100 ml DDW, M5= 100 ml of 0.1 N HCl^+, M6=100 ml of 0.1 N HcL, M7= 100 ml 0f 0.2 N CaCl2, M8= 100 ml 0f 0.5 N NaCl2.

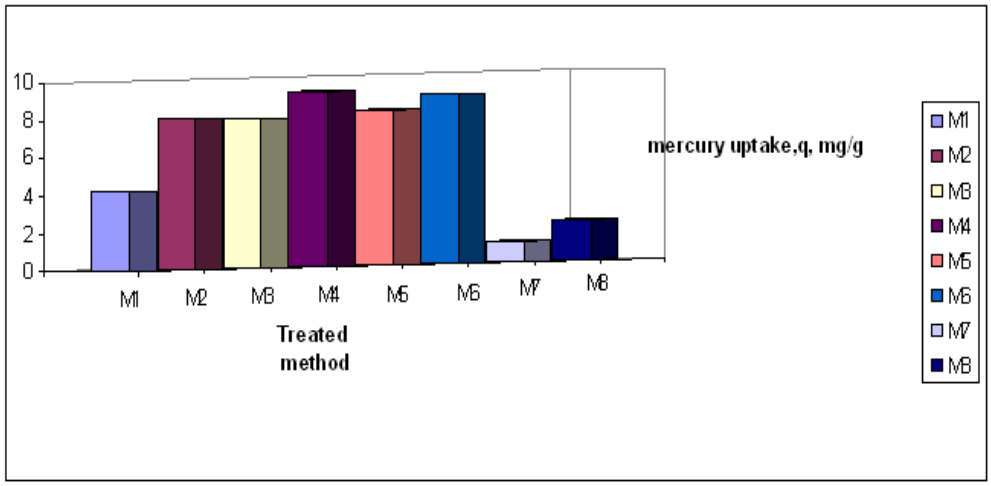

Fig. (10): Effect of different treated methods on metal uptake of mercury at pH 6.0, 0.1 g yeast, v=100 ml, M1= raw yeast, M2= ethanol, M3= 100 ml of 1 mol/L, , M4= 100 ml DDW, M5= 100 ml of 0.1 N HCl^{+}, M6=100 ml of 0.1 N HCl, M7= 100 ml 0f 0.2 N CaCl2, M8= 100 ml 0f 0.5 N NaCl2.

Conclusions

The biosorption characteristics of *Saccharomyces cerevisiae* were studied for nickel and mercury. The results indicated that this yeast may be used as an inexpensive, and effective for the removal of nickel and mercury from aqueous solutions. The biosorption process was affected by experimental conditions such as pH, initial metal ion concentration, contact time, and amount of yeast. Sorption data of nickel and mercury followed the Langmuir adsorption model with high coefficient of determination than Freundlich adsorption model. The uptake capacity of nickel and mercury increases with increasing of initial metal concentration and decreases with increasing of biosorbent weight. The kinetics of sorption show three distinct stages, the initial process of external mass transfer is fast and confined to the first few minutes and is termed first stage of sorption. The second and third stages of sorption are found to be clearly separated by a plateau depending on the concentration or availability of metal ions in the solutions for sorption. Among the pretreatment methods which have been used to increase the biosorption capacity of the yeast, alkaline treatment was found to be superior to the others. Desorption studies conducted showed that the metal ions sorbed onto the yeast could be desorbed effectively using 0.05 N nitric acid and the spent yeast could be regenerated with 0.2 N sodium hydroxide solution.

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