Optimization of Cadmium, Zinc and Copper biosorption in an aqueous solution by *Saccharomyces cerevisiae*

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Abstract: Optimization of Cd (II), Zn (II) and Cu (II) biosorption from contaminated water were performed as function of parameters (pH, contact time, initial metal ions concentration and yeast dose). The experimental results showed that the highest equilibrium adsorption capacity at the optimum pH were 8.5 for Cd (II) and 6 for Zn (II) and 6 for Cu (II). Optimum pH values were carried out to evaluate other parameters. Results demonstrate that removal efficiency increased with increased contact time for the three metal ions. Results indicated that removal efficiency increased with increased yeast dose up to 2 g/ 100ml, and removal efficiency decreased with increased yeast dose from 2.2 g/100ml to 4 g/100ml. The results also showed that increasing removal efficiency from 1 to 20 mg/L concentration for the three metal ions and the removal efficiency decreasing with increasing initial concentration from 25 to 50 mg/L. It is evident that the highest removal efficiency for Cd (II) ion compared to Zn (II) and Cu (II). This study revealed that use of baker's yeast is suitable for removal of these ions from contaminated water in order Cd > Zn > Cu at these conditions. The negative values of the standard free energy change (ΔG°) indicate spontaneous nature of the process. Competitive biosorption of (Zn and Cu) ions was investigated in terms of sorption quantity. The amount of Cu metal ion adsorbed onto unit weight of biosorbent (q_e) decreased with increasing the competing metal ion (Zn), in contrast, the amount of Zn ion adsorbed onto unit weight of yeast has been increased with increasing the competing metal ion (Cu). The binding capacity for Zn (II) is more than for Cu (II). Ion exchange is probably one of the main mechanism during adsorptive process.

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1. Introduction:

Biosorption can be defined as the removal of metal or metalloid species compounds and particulates from solution by biological material Gadd, (1993). Heavy metal pollution has become one of the most serious environmental problems today. Biosorption, using biomaterials such as bacteria, fungi, yeast and algae is regarded as a cost-effective biotechnology for the treatment of high volume and low concentration complex wastewater containing heavy metals in the order of 1 to100 mg/L. Veglio and Beolchini, (1997). Some strong toxic metal ions, such as Hg and Cd (II), are very toxic even in lower concentration of 0.001–0.1 mg/L Alkorta *et al.,* (2004).

There is always a possibility that metals will be taken up by plants such that they end up in the human food chain. Metal studies have shown that various oxidation states of metals are toxic to humans and also that they are associated with several types of diseases Atwood *et al.,* (2000). In order to facilitate the analysis of metal ions in aquatic environments, analytical methodologies that address aspects of sampling; sample clean-up and analyte enrichment are necessary. If possible, such methodologies should be simple, robust and inexpensive as well as address limitations associated with collecting samples from remote sampling sites. Biological substrates including the baker's yeast, *Saccharomyces cerevisiae* has been shown to have good sorption characteristics for several Heavy metals Volesky and May-Phillips (1995). As will as it's abundance of yeast as a by-product of fermentation processes Blackwell *et al.*, (1995). For biosorption of heavy metal ions, pH is one of the most important environmental factors. The biosorptive capacity of metal cations increases with increasing pH of the sorption system, but not in a liner relationship. Esposito *et al*., (2002). Yeast cells of *S. cerevisiae* are able to remove heavy metals from wastewaters between pH 5 and 9 Wang, (2009).

The biosorption capacity and the removal efficiency of metal ions by *S. cerevisiae* became higher with prolonging the contact time Wang, (2006). The uptake rate of the metal ion will be increased with increasing the initial concentration if the amount of biomass is kept unchanged Wang, (2002). Evidenced that the use of a large excess of yeast biomass comparatively to metals allowed an efficient removal of metals from real and synthetic effluents, reducing the amount of metal in solution to the limit of discharge of wastewater in natural waters Machado *et al*.. (2010)

Although copper and zinc are essential trace elements, high levels can cause harmful health effects. Copper is also toxic to a variety of aquatic organisms, even at very low concentration Sheng *et al*., (2004).

Cadmium, Zinc and Copper ions were chosen for biosorption studies with regard to their wide use in industry. Zinc and Copper are very widely used metal in our daily life, but like any other heavy metal it is potentially toxic for all the living organisms. Volesky, (1990). Cadmium can contaminate the environment from anthropogenic sources as well as natural geochemical processes. It can accumulate along the food chain and is not amenable to biological degradation Dursun, (2003).

This investigation aimed to use baker's yeast for adsorption of some metal ions from contaminated aqueous solutions, In addition, to study the influence of the uptake of Cd (II), Zn (II) and Cu (II) by *S. cerevisiae* in different adsorptive conditions and to compare the biosorption behavior of a single-metal system and two-system.

2. Materials and methods

Baker's yeast

The baker's yeast employed in this study was DSM-70460 strain of *Saccharomyces cerevisiae* used in baking industry. It is supplied in the form of dry matter and it's supplied from Deutsche Sammlung Von Mikroorganismen und Zellkulturen GmbH (Germany).

Optimized of pH

In this study, three sets of experiments were conducted to study the effect of pH. The tested solutions 5 mg/L metal ions and yeast dose 0.2 gm/100 ml were adjusted at pH 3, 4.5, 6, 7, 8.5, 9.5 and 11 using 0.1 M HCl or 0.1 M NaOH. All pH measurements were carried out with a HANNA digital pH meter HANNA Instruments and left for 30 min. to reach the equilibrium.

Analytical methods

Samples were analyzed for Cd (II), Zn (II) and Cu (II) using Perkin Elmer (3300) Atomic Absorption-Flame Absorption Spectrophotometer, a Calhodeon Ltd. Hollow Cathode Lamp as light source for Cd (II), Zn (II) and Cu (II) and mixture of air-C_2H_2 as the flame atomizer gas. A standard calibration curves were prepared using Cd (II), Zn (II) and Cu (II) solutions of known concentration in the range 1-100 mg/L.

Evaluation of the effect of various parameters on uptake of metal ions at optimized pH for three metals:

1 - Biosorption experiments

The experiments were conducted with Cd (II), Zn (II) and Cu (II) aqueous solution of initial concentration from 1 to 50 mg/L, yeast dose from 0.2 to 4.0 g/100ml, were mixed at pH range from 3 to 11 and Shaken at a constant speed of 120 RPM in a shaking at temperature 298 K for 15 to 120 min. The samples were centrifuged at 2000 rpm for 15 min. (Hermale Z 200A) the supernated were analyzed and the experiment were carried out at least twice the values used in calculations were mostly the arithmetic average of the experiment data.

2 - Sorption studies

Cd (II), Zn (II) and Cu (II) uptake were calculated by two equations according to Cojocaru *et al.*, (2009).

3 - Effect of contact time on biosorption

Samples were withdrawn at 15, 30, 60, 90 and 120 min. intervals during the biosorption experiments and analyzed for Cd (II), Zn (II) and Cu (II). This factor that influence time was examined in 5 mg/L metal ions and 0.2 g of yeast dose /100 ml.

4 - Effect of yeast dose on biosorption

This factor that influence yeast dose were examined in 5 mg/L for 30 min. yeast dose of 0.2, 0.4, 0.8, 1.0, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6 and 4.0 gm were added to test solution taken in series of 250 ml Erlenmeyer flasks with 100 ml tested solution and biosorption experiments were carried out.

5 - Effect of initial metal ions concentrations on biosorption

This factor that influence initial metal ions were examined in 0.2 g/100 ml for 30 min. Test solutions containing 1, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg/L for $CdSo_4$, $ZnSo_4$and $CuSo_4$ were subjected to biosorption. The salt was chosen a sulphate since sulphate ions exist in most wastewater and mine drainage and thus simulating the potential application of biosorption for Cd (II), Zn (II) and Cu (II) ions removal.

6 - Competitive adsorption in binary metal system

In this group of experiments, competitive adsorption of Zn (II) and Cu (II) ions from their binary solutions was investigated by following a similar procedure as described above. These studies were performed at a initial pH of 6 at 298 K. Experiments of competitive adsorption of Zn (II) and Cu (II) included two parts: (i) the competitive adsorption of Zn (II) and Cu (II) in the total metal concentration was fixed (5 mg/L); (ii) in a serious of two metal ions solution, the initial concentration of Zn (II) was fixed to 5 mg/L, where as, concentration of Cu (II) were varied from 0 to 4 mg/L. In another binary system, initial concentration of Cu (II) was constant in 5 mg/L, and concentrations of Zn (II) were varied from 0 to 4 mg/L.

3. Results and Discussion:

The biosorption has attracted the attention as low-cost treatment technology for the removal of heavy metals from wastewaters.

Optimized of pH

The charge of the adsorbate and adsorbent often depends on the pH. The adsorption of Cd (II), Zn (II) and Cu (II) as a function of pH were measured. As shown in Table (1) there were increases in biosorption uptake equilibrium adsorption capacity (q_e) of Zn (II) and Cu (II) with increasing pH from 3 to 6 for both metal ions. On the other hand maximum Cd (II) uptake observed at pH 8.5. The lowest metal uptake values were determined at pH 3 for three metal ions. At pH values above the isoelectric point, there is a net negative charge on the cell wall components, which will be promate reaction with metal ions. As the pH is lowered, however, the overall surface charge on the cells will be positive, which will inhibit the approach of positively charged metal ions Goksungur *et al.*, (2005).

Table (1): The optimization initial of solution pH on biosorption for Cd (II), Zn(II) and Cu (II) at 5 mg/L metal ions and yeast dose 0.2 (gm) after 30 min and 25 ^oC.

	pH	**C_o mg/L**	**C_e mg/L**	**q_e mg/g**	**q_{max} mg/g**	**Y %**
Cd	3	4.99	3.67	0.660	1.32	26.45
	4.5	4.93	2.79	1.070	2.14	43.41
	6	4.97	2.6	1.185	2.37	47.68
	7	4.90	1.69	1.605	3.21	65.51
	8.5	4.95	0.22	2.365	4.73	95.55
	9.5	4.91	0.53	2.190	4.38	89.20
	11	4.98	0.92	2.030	4.06	81.16
Zn	3	4.95	2.633	1.1555	2.317	46.66
	4.5	4.89	2.60	1.145	2.29	46.83
	6	4.96	1.498	1.731	3.462	69.80
	7	4.90	2.14	1.380	2.76	56.32
	8.5	4.88	2.878	1.005	2.01	41.18
	9.5	4.91	3.10	0.905	1.81	36.86
	11	4.92	3.20	0.860	1.72	34.95
Cu	3	4.98	3.957	0.515	1.033	20.89
	4.5	4.91	2.859	1.0205	2.041	41.54
	6	4.90	2.206	1.347	2.694	54.98
	7	4.96	2.968	1.0063	2.0025	40.32
	8.5	4.92	3.16	0.880	1.76	35.77
	9.5	4.90	3.323	0.7905	1.58	32.24
	11	4.95	3.46	0.746	1.49	30.10

C_o : initial metal concentrations (mg /L)
C_e : final metal concentrations (mg /L)
q_e : equilibrium adsorption capacity (mg /g)
q_{max} : amount of bioaccumulated ions in yeast (mg/g)
Y : removal efficiency yeast (%)

Similar results were detected by Volesky (1990) who found that the optimal pH value is 5 – 9 for Cu (II) biosorption by *S. cerevisiae*. Also Mapolelo and Torto, (2004) proved that the optimal pH values are greater than 5 for Cu^{2+} and Zn^{2+}. In this study maximum Cd (II) uptake was observed only at alkaline pH 8.5.

However; Mullen *et al.*, (1992) have reported an increased trend in biosorption of Cd (II) by *A. niger* to the tune of 2.9 times when pH was increased from 4 to 7. Also Parvathi *et al.*, (2007) found that biosorption of Mn (II) increased with rise in pH, and maximum manganese uptakes were observed at initial pH 9 by *S. cerevisiae*.

Generally, an increase of pH causes deprotonation of metal ions binding sites exposed by cellular surface. However, a decrease of pH causes competition between protons and positively charged metal ions. However these rules concern only cations .Since biosorption is reversible process, decreasing pH would result in deprotonation. This property is used in regeneration of biosorbents Naja and Volesky, (2010).

Effect of time:

As shown in Fig. (1); It was found that the equilibrium adsorption capacity increased as the contact time increased. The biosorption of Cd (II), Zn (II) and Cu (II) on yeast was rapid for first 15 min. and equilibrium was nearly reached after 90 min. Results revealed that the highest removal efficiency Cd (II) occurred after 15 min., then the increased removal efficiency occurred slowly. This trend emphasizes that sorption times have an important effect on recovery efficiency, which decreases with increase biosorbent contact time with metal solution. Metal accumulation inside the cell may resulted from bioaccumulation, slow metabolic dependent removal mechanism, or by simple metal diffusion Gaad, (1990).

Tavares *et al*., (1995) mentioned that, biosorption kinetics with an initial rapid metal uptake followed by slow uptake was observed, this kinetic model has been accepted for various biosorbents such as bacteria and fungi (yeast) under similar operation conditions. A similar trend was observed for the uptake of three metal ions.

The data shown in Fig. (1) indicate that selected sorption time for biosorption capacity was 120 min., this sorption period equilibrium adsorption capacity reached maximum at 90 min. for Zn (II) and Cu (II). The highest Cd (II) biosorption capacity was observed compared with Zn (II) and Cu (II), the explanation based on it's most noxious valency, may readily participate in cell metabolism Salem, (1994).

Goyal *et al*., (2003) reported that the uptake of metal ions by microorganisms in batch systems has been shown to occur in two stages: an initial rapid stage (passive uptake), followed by much slower process (active uptake). The first stage is physical adsorption or ion exchange at the surface of the biomass, which is biosorption. The biosorption equilibrium occurs at the end of rapid physical adsorption stage (first-stage). Adsorption isotherm equation is frequently used to represent this equilibrium. The same behavior was observed by Han *et al.*, (2006a) equilibrium time for Zn (II) and Cu (II).

Özer *et al.*, (1999) found that the adsorption equilibrium of Cd (II) on *S. leibleinii* was established in 15 – 20 min. and the adsorbed amount did not change with time.

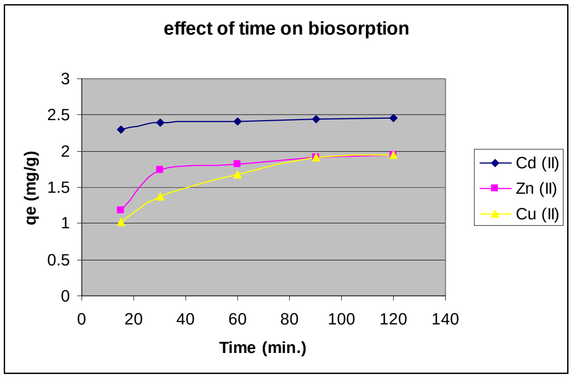

Fig. (1) Effect of time on biosorption for Cd (II), Zn (II) and Cu (II) at 5 mg/L and yeast dose 0.2 (gm) at 25^{o}C.

Effect of yeast dosage

The effect of yeast dosage on removal efficiency (Y) % showed in Fig. (2). Results showed that increasing metal removal as increased yeast up to 2 g/ 100 ml solution. As expected (Fig. 2), the uptake of Cd (II) was much more sensitive to increase amount of yeast in solution compared to that Zn (II) and Cu (II). The increase in metal uptake with yeast dosage confirms the increase in binding sites for metal ions. On the other hand, it was observed that the metal uptake decreased with increasing the amount of yeast dose from 2.2 to 4.0 g/ 100 ml. Decreased removal efficiency may be due to aggregation of yeast. Al-Asheh and Duvnjak, (1995) reported that higher uptake at lower biomass dosage could be due to metal ions and biosorbent ratio, which decrease upon an increase in biomass dosage due to of high biomass dosage resulted aggregates of biomass and may cause interference between binding sites at higher biomass dosage or insufficiently of metal ions in the solution with respect to available binding sites. It is likely that protons will then combine with metal ions for the ligands and thereby decrease the interaction of metal ions with the cell components Saĝ and Kutsal, (1996). It should be mentioned that the cadmium ion adsorption capacity decreased with increase of biosorbent dosage Vasudevan *et al.*, (2002). Their theory is similar to that advanced by Zou *et al.* (2006) to account for the cell surface remaining unsaturated at higher biosorbent dosage.Uslu and Tanyol, (2006) found that the initial adsorption rates of Cu (II) decreased with increased biosorbent concentration. Fig. (2) shows that the uptake of Zn (II) reached a maximum at 2 g/ 100 ml yeast dosage. These data probably indicate that low uptake values reflect a sluggish adsorption process. Wilhalmi and Duncan, (1996) reported that the Zn (II) is preferable that the adsorption process on the membrane take places over short period of time to avoid interalisation of metal ions which would result in lower ions would result in lower desorption and enrichment of metal ions.

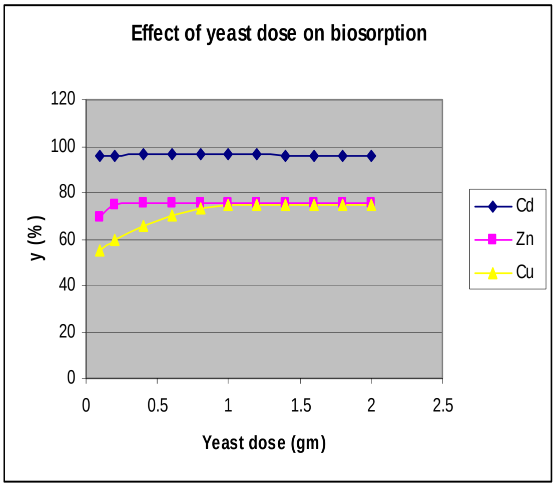

Fig. (2) Effect of yeast dosage on biosorption for Cd (II), Zn (II) and Cu (II) at 5 mg/L after 30 min and 25 oC.

Effect of initial concentration ions:

As shown in Fig. (3); Removal efficiency increased with increasing the metal ions concentration from1 to 20 mg/L in three metal ions. On the other hand, after 20 mg/L of Cd (II), Zn (II) and Cu (II), ions decreasing uptake for three metal ions. As increase initial concentration from 25 to 50 mg/L decreasing removal efficiency from 84.80, 66.14 and 45.65 to 77.52, 38.44 and 33.52 for Cd (II), Zn (II) and Cu (II), respectively. This could be attributed to the strong effect of initial concentration, which is very high comparing with the relatively low sorption capacity of biosorbent. This agrees with Cojocaru *et al.*, (2009) who found that increase in density of the negative charge on the cell surface, causing proton removal from solution, thereby decreasing biosorption capacity of Cu (II) because of competitively adsorption of protons. A similar observation was reported by Ferraz and Teixeirain, (1999) who suggested an increase of electrostatic interaction at high biomass concentration inhibited metal biosorption. When the biomass concentration is low, metal ions in the solution would not only be adsorbed to the surface of the biomass, but also enter into intracellular part through facilitating the concentration gradient of metal ion Wang, (2002).

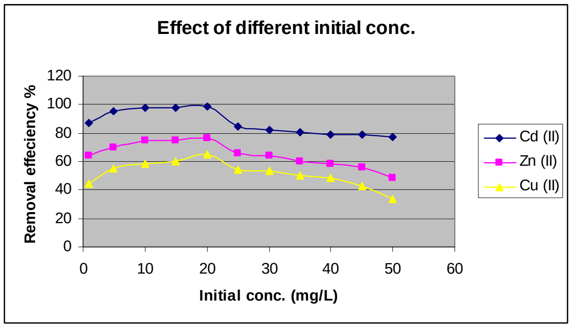

Fig. (3) Removal efficiencyof Cd (II), Zn (II) and Cu (II) at different initial concentrations.

Adsorption equilibrium

Adsorption isotherms show the distribution of solute between the liquid and solid phases equilibrium conditions. Many different isotherm models have been proposed for the adsorption of solutes in a liquid solution onto a solid surface. Langmuir model is probably the most popular isotherm models due to its simplicity and its good agreement with experimental data. The Langmuir model, the saturated monolayer isotherm, can be described by the linear form Langmuir, (1916). Although the Langmuir constant q_{max} is dependent on experimental conditions such as solution pH and temperature, it is a good measure for comparing different sorbents for the same (metal) sorbate. Another important factor in evaluating sorbent performance is the initial gradient of the adsorption isotherm, since it indicates the sorbent affinity at low metal concentrations. In the Langmuir equation, this initial gradient corresponds to the affinity constant (b). A high value of the affinity constant is thus desirable Sheng *et al.*, (2004). The equilibrium established between the adsorbed metal ions (q_e) and that remained free in the solution (C_e) was also represented by the Freundlich adsorption isotherm, the linear equation as following Freundlich, (1906).The equilibrium data of (Cd), (Zn) and (Cu) ions adsorption by baker's yeast obtained at 25 ^{o}C (298 K) was applied to Langmuir and Freundlich models. The relative parameters and correlation coefficients (R^2) were listed in Table (2). The mono-component Langmuir constant, q_{max}, represents the monolayer saturation at equilibrium or the total capacity of yeast for (Cd), (Zn) and (Cu) ions. From table (2), the maximum loading capacities of yeast was obtained as 4.545 mg/ g for (Cd) 0.6676 mg/ g for (Zn) and 0.4833 mg/ g for (Cu). So the ability of Cd (II) adsorption on yeast is biggest than Zn (II) and Cu (II). From Table (2), the values of k_f showed easy uptake of metal ions with high adsorption capacity of baker's yeast. As $0.1 < 1/n < 1.0$, indicating that (Cd) and (Zn) ions are favorably adsorbed by yeast at temp 25 ^oC. studied Aksu , (2002). The Gibbes free energy of biosorption determined Han *et al.*, (2005). The free energy changes for Cd (II), Zn (II) and Cu (II) sorption onto yeast were determined at 25 ^oC and at optimum pH, are -7.60, -2.075 and -0.495 and kJmol^{-1}, respectively. The negative values of ΔG^o valid at the feasibility of the biosorption process Cd (II), Zn (II) and Cu (II) the spontaneity of biosorption.

Effect of coexistence ions on the biosorption of zinc and copper ions on baker's yeast:-

Table (2): Constants of Langmuir and Freundich isotherms for Cadmium, Zinc and Copper biosorption by *S.cerevisiae* at optimum pH and 25 ^oC.

Metals	pH	Langmuir			Freundich		
		B L/mg	qmax mg / g	R2	1/n	kf mg/L	R2
Cd	8.5	4.545	2.368	0.981	0.322	1.62	0.810
Zn	6	0.6676	1.731	0.952	0.497	0.384	0.793
Cu	6	0.4833	1.347	0.999	0.982	0.524	0.999

1 - The effect on biosorption of Cu ion with the presence of Zinc ion in the solution

As shown in Fig (4 down curve); with increasing Zn (II) concentration the biosorption quantity of Cu (II) decreased. Also, the adsorptive quantity of Cu (II) decreased with the increasing Zn (II) initial concentration. The biosorption quantity of copper ion decreased from 1.374 mg/g to 1.132 mg/g. Thus it can be seen that the existence of Zn (II) has greater effect on the biosorption of copper on yeast. When the Zinc ion existence in the solution it will be adsorbed by yeast and compete to the copper ions and it will hold some activated sites on yeast, hence the adsorption capacity of copper will decrease. So when the ions of Zn (II) existence it will be a disadvantage condition to the biosorption of copper ion by yeast. Furthermore, the concentration of Zn (II) is more, the effect is serious. The biosorption capacity of one metal ion is interfered and reduced by co-ions, including other metal ions and anions presenting in solution, however the gross uptake capacity of all metals in solutions remains almost unchangeable Wang, (2006).

2 - The effect on biosorption of Zn ion with the presence of Copper ion in the solution

As shown in Fig. (4 upper curve); with increasing the copper concentration, the biosorption quantity of Zn ion increased. Also, the adsorption quantity of Zn (II) increased with the increasing of Cu (II) concentration. The biosorption quantity (q_e) of Zn (II) increased from 1.731 to 1.905 mg/g when the concentration of Cu (II) ranged from o to 4 mg/L. As the copper ions existence in solution, similarly, it will be adsorbed by yeast and compete to the Zn ions. So where the ions of copper existence, it will be advantage condition to the biosorption of Zn ion on yeast, Furthermore, the concentration is higher, the effect is also serious. The decrease of metal uptake in competitive conditions was thought to be a response to increased competition between same charged species for binding sites of the yeast cells Goksungur *et al.*, (2005).

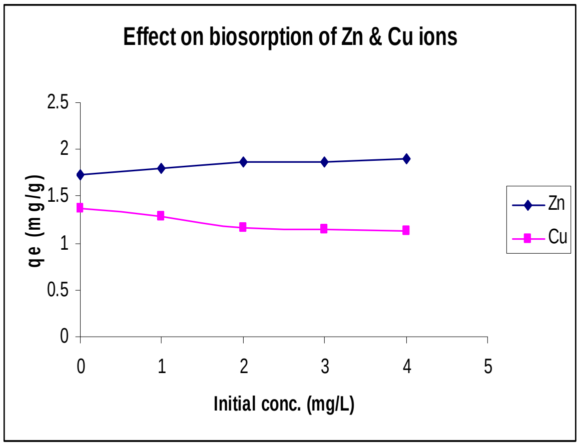

Fig. (4) The effect on biosorption of zinc when copper in existence (upper curve) and the effect on biosorption of copper when zinc in existence (down curve).

3 - The competitive adsorption of Cu (II) and Zn (II) in the total concentration fixed

As shown in Fig (5); it was observed that when increased the concentration of copper, reduced the concentration of zinc, the uptake increased for Zn (II) while decreasing for Cu (II) when there was two metal ion existence, the value of q_e about zinc was bigger than copper, so the binding ability for zinc is strong than copper. The baker's yeast can be used to remove copper and zinc ions in solution when there was two metal ions coexistence. Above all, competitive biosorption of two metals was investigated in terms of sorption capacity and found that the biosorption capacity of biomass decreased with increasing the competing metal ion concentration. Han *et al.*, (2006b) found that the amount of one metal ion adsorbed onto unit weight of biosorbent decreased with increasing competing metal ion concentration, but from our investigation showed in Fig. (5) it can be concluded that the total capacity for adsorbing copper ion decreased while zinc ions increased.

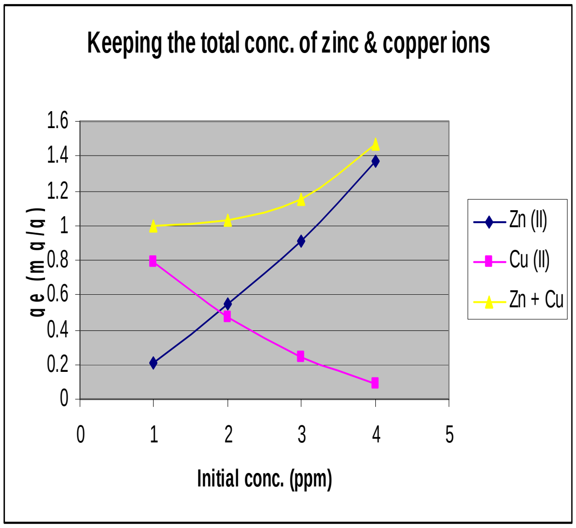

Fig. (5) Keeping the total concentration of zinc and copper ions fixed, the equilibrium level of zinc and copper ions onto baker's yeast.

4. Conclusion:

The analysis of our results shows the possibility used of baker's yeast in dried form for remove Cd, Zn, Cu ions from contaminated aqueous solutions .Results obtained for the removal of initial metal ions concentration in an aqueous solution using *Saccharomyces cerevisiae* showed a decrease of biosorption efficiency at low and high value initial pH. The removes percentage for Cd (II),is the highest removal efficiency in optimal condition (pH=8.5)compared with Zn (II) and Cu (II) (pH=6). The degree of removal of this hazards ions were depend on the pH, contact time and initial concentration of the contaminated water.

Competitive biosorption of two metals (Zn & Cu) ions was investigated in terms of sorption capacity and found that the biosorption capacity for Cu ion decreased with Zn metal ion concentration.

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