Heavy Metals Bio-Remediation by Immobilized *Saccharomyces cervisiae* and *Opuntia ficus indic*a Waste

Amber S. Gad[1], M. Attia[2] , H.A. Ahmed[3]
[1]Chemistry of Natural and Microbial Products Dept.., [2] Agriculture Microbiology Dept.[3] Biochemistry Dept., NRC, Dokki, Egypt
amber2gad@yahoo.com

Abstract: The working concentrations used in the biosorption medium of *Saccharomyces cervisiae* was 35 mg/l, Cu(II); 15 mg/l,Cd(II) and 25 mg/l, Fe(III) . 18 h equilibrium time needed for maximum metal removal, (100mg/l) metals adapted *S. cervisiae* hasn't prominent enhancing effect, while addition of (4 mg/l) cystine has such effect upon metal removal , with 5.5 initial pH, and 3 %(v/v) inoculums concentration while NaOH treatment resulted in 36.11, 18.11, 33.52% for Cu(II),Cd(II) and Fe(III), respectively .Alginate immobilized *S. cervisiae* cells removed 67.33 %and 45.995 % for Cu(II),Cd(II) and 60.768% for Fe(III),respectively at 30°C and 200rpm from biosorption medium advanced over polyutherene foam immobilized cells . 10%(v/v) *Opuntia ficus indica* polyelectrolyte and 150 rpm are optimum for metal removal in biosorption medium at 30°C . Wastewater treatment with alginate-, polyurethane foam - immobilized cells and natural polyelectrolyte revealed that alginate immobilized cells is the most successful. [Journal of American Science 2010;6(8):79-87]. (ISSN: 1545-1003).

Keywords: Heavy Metals; Bio-Remediation; *Saccharomyces cervisiae; Opuntia ficus indic*a

1. Introduction

The phenomenon of biosorption is defined as a metabolism independent adsorption of pollutants based on the partition process on a microbial biomass (Ringot *et al.* 2006).Or, It is a passive non-metabolically-mediated process of metal binding by biosorbent (Davis *et al.*, 2003). Bacteria, yeasts, fungi and algae have been used as biosorbents of heavy metals. Among these, yeasts are known to be selective metal biosorbents as compared to fungi, actinomycetes and bacteria (Zouboulis *et al.*, 1999, 2001). The yeast *Saccharomyces cerevisiae* as a promising biosorbent has been used to remove Cr(VI), Fe (III) (Goyal *et al*., 2003), Cd (II) (Liu *et al*., 1997), Cu (II) (Jianlong, 2002 and Machado *et al*., 2009) from aqueous solutions. Moreover,It can distinguish different metal species based on their toxicity, such as selenium, antimony and mercury. Microorganism have had to develop different mechanism of metal resistance that include cell membrane metal efflux , intracellular chelation by metallothionine protein and glutathione derived peptides called phytochelatins and metal artmentalization in vacuoles (El Aasar, 2005). Other mechanisms exist for the removal of heavy metals from aqueous solution by bacteria, fungi, ciliates, algae, mosses, macrophytes and higher plants (Holan and Volesky, 1995; Pattanapipitpaisal *et al*., 2002;Wang and Chen,2006 ,and Rehman *et al.*, 2007, 2008).As cellular response to the presence of metals includes various processes such as biosorption by cell biomass, active cell transport, binding by cytosolic molecules, entrapment into cellular capsule, precipitation and oxidation-reduction reactions (Gadd, 1990; Lovely and Coates, 1997) .

Immobilization is either artificial, having to rely on incorporation into a polymer gel, Willaert. and Baron (1996) or natural, relying on the innate properties of microbes to become entrapped in biomass support particles; Atkinson *et al.* (1980) or attached to solid supports, such as coke; Dempsey (1994), Wilkins and Yang (1996) or even polyurethane foam as it is the case of immobilization with bacteria and *Candida* sp., Quek *et al.*, (2006).

Different agriculture residues were used for heavy metal removal (Lee *et al*., 1998; Marshal *et al.*, 1999). *Opuntia,* is another source of viscous natural polyelectrolyte bearing negative surface charges (Forni *et al.*, 1994). Aim of this study is to evaluate some physiological factors of *S. cervisiae* as a biosorbent: concentration of heavy metal, equilibrium time, adaptation to higher concentration, cystine addition, , pH, inoculum concentration, and NaOH - treatment of biomass, to maximize the heavy metal removal and to find effective use of immobilized alginate and PFU as means to offset substrate toxicity. Another trail regarding the use of *Opuntia* natural electrolyte to increase metal removal ;its concentration, and agitation speed were studied, to understand the specificity of both biosorptions for the metal ions involved during the treatment .

2. Material and Methods

Biosorbent:

Culture of *Saccaromyces cervisiae* obtained from the microbial stock collection of Chemistry of Natural and Microbial products lab. NRC. Egypt.

Maintenance medium:

Stocks of strains were maintained on standard yeast extract/peptone/dextrose (YPD) rich medium comprising in (w/v) %: Glucose, 2; Yeast extract, 1 and Peptone, 2, otherwise stated agar, 2 %, Liu *et al*. (1997).

Inoculum preparation:

A loop full of (YPD) slant yeast cells was cultivated in 50 ml liquid YPD medium in 250 Erlenmeyer flask at 30 °C on a rotary shaker for 24h at 200 rpm.

Biosorption medium:

Fifty ml liquid (YPD) medium in 250 ml Erlenmeyer flask adjusted to pH 5.5 by using 0.1M HCl or 0.1M NaOH. sterilized at 121°C for 20 min., inoculated with 2%(v/v) 24h *S. cerevisiae* inoculum suspension containing 10^7 cells /ml , with the sterilized metal salts being added at different concentrations after autoclaving . The flasks were incubated at 30 °C for different time intervals shaking at 200 rpm; Pearce and Sherman(1999) .

Preparation of the heavy metal solutions:

The different metal ions were sterilized by filtration through a pore filter of 0.22 um and were added to achieve final concentrations of 15 35, 25 (mg/l) $CdSO_4.8H_2O$; $CuSO_4.5H_2O$,and $FeCl_3$ respectively in biosorption media , Liu *et al.* (1997) . At the end of incubation period, cultures were harvested, centrifuged at 10,000 x g , final metals concentration were determined and metal removal(%) was calculated .

The effect of type and initial metal concentration on *S. cerevisiae* growth :

Concentrations of heavy metals ions were adjusted between 5- 35 (mg/l) in 50 ml biosorption media in 250 ml Erlenmeyer flasks , inoculated ,incubated for 24h time and were evaluated in relation to growth.

Determination of *S. cerevisia*e growth : At different heavy metals concentrations ,optical density was quantified at 600 nm using spectrophotometer with respect to the control containing no metal at 30°C for 24h; Liu *et al.* (1997).

Effect of equilibrium time on heavy metal removal:

Final metals concentrations in cultures at different incubation time 12, 18, 24,48h at 30°C at 200 rpm were measured, (%) metal removal was calculated. Effect of adaptation to higher metals concentration;: Series of repeated 5 generations sub culturing on YPD agar plates with gradual increased concentrations from 35 mg/l of $CdSO_4.8H_2O$;$CuSO_4.5H2O_4$ and $FeCl_3$ to 100 ppm incubated for 48h at 30ºC on .The resistant. *S. cervisiae* inoculums prepared as mentioned before and used to inoculate 50 ml biosorption media in 250 ml Erlenmeyer flasks incubated for 18h. Control and higher concentrations adapted flasks were centrifuged and final heavy metals concentrations were measured in the filtrate, (%) metal removal was calculated; El Aasar (2005) .

Effect of cystine on heavy metal removal:

Different concentrations of sterilized cystine (2-6 mg/l) were added to the sets of 50 ml biosorption media flasks in 250ml Erlenmeyer flasks after autoclaving. Treated and control flasks were 2%(v/v)inoculated ,incubated for 18h at 200 rpm at 30°C , centrifuged, final metals concentrations were measured,(%) metal removal were calculated; Pearce and Sherman (1999) .

Effect of initial pH on heavy metals removal : *S.cervisiae* culture in biosorption media at 30ºC,200 rpm for 18h adjusted to different pH values (2.5 – 6.5)were harvested, centrifuged ; final metals concentrations in different flasks were measured . (%) metal removal was determined; Pearce and Sherman (1999):

Effect of inoculums concentration on heavy metal removal:

Different biosorbent inoculums concentrations (2, 3, 4, v/v %,) flasks containing biosorption media were harvested after18h , centrifuged and final metals concentrations were measured for (%) metal removal calculation.

Effect of NaOH treated biomass;

Centrifuged *S. cerevisiae* biomass was divided into 2 portions ,one part was heated in 0.75 M NaOH at 70–90°C for 10–15 min ,the cells were converted to non viable and the cell wall microstructure was altered ,washed thoroughly till the washing becomes near neutral, suspended in 5 ml sterilized water and I ml was used for inoculating 50 ml biosorption media in 250 ml Erlenmeyer flasks .Similarly, the other part was suspended in 5 ml water, 1ml suspension of untreated biomass were inoculated in biosorption media as control flasks, incubated as mentioned, harvested, and centrifuged. Final metals

concentrations in all media were evaluated and (%) metal removal were calculated; Göksungur *et al.* (2005).

S. cervisiae cells immobilization:
Calcium alginate :

S. cervisiae cells were separated by YPD centrifugation at 10,000 rpm for 10 min,. The cells were washed and re-suspended in sterile 50 ml of acetate buffer (pH , 4.0) and 10ml suspension was mixed homogeneously with 90ml volume of aliquot of Na-alginate suspension to contain 2%(w/v) Na-alginate. Alginate-biosorbent suspension was added drop wise to 500 ml of 2%(w/v) $CaCl_2$ with a pipette. Alginate drops solidified upon contact with $CaCl_2$, forming beads and thus entrapping biosorbent particles. The beads were allowed to harden for 30 min and were then washed with sterile physiological saline solution (0.85%, w/v,NaCl) to remove excess calcium ions, Equal number of control beads without cells and immobilized cell beads were used to inoculate 50 ml portions of biosorption media at 200 rpm at 30°C. After 18h, filtration to harvest the beads, centrifugation at 10,000 x g for the escaped cells and determination of final heavy metals concentrations in the filtrates by atomic absorption were carried out for control beads flasks and beads with entrapped yeast cells flasks .(%) metal removal were calculated; Ksunger *et al*.(2003)

Determination of beads dry weight according to Aloiu *et al*. (2008).
Polyurethane foam (PUF):

Preparation of Polyurethane foam (PUF): Commercial type of different densities with different pore sizes PUF was obtained from glass Industries, Egypt. PUF were washed with distilled water and then dried at 30 °C overnight and weighed; Quek *et al.* (2006).

PUF Immobilization of *S.cervisiae* cells
Weighed PUF are placed into a 100 ml conical flask containing 18 ml of YPD broth and autoclaved at 121 °C for 15 min. Two ml of 2-day old liquid cultures were used for inoculation. Control foam flask kept un-inoculated The flasks were incubated at 30 °C with shaking at 200 rpm for 48h .YPD biosorption media was inoculated under aseptic conditions with equal number of PUF with immobilized yeast cells and control foam cubes flasks without cells , incubated at 30°C at 200rpm,for 18h;Quek *et al.* (2006).

Determination of PUF immobilized cells dry weight:. The content of the flasks was separated by filtration, using a Whatman No. 1 filter paper, dried overnight and weighed. An average control foam cube dry weight was subtracted from dry weight of foam plus cells to determine the dry weight of the attached cells .The filtrates were centrifuged at 10,000 x g for escaped cells, and the concentrations of the metals ions in the filtrates was determined for control flasks (no yeast) and immobilized cells flasks using an atomic absorption spectrophotometer and (%) metal removal was determined; Tsekoa and Petrov (2002).

The extraction of *Opuntia* natural polyelectrolytes:

The cactus waste was cut in small pieces, and approximately 132 g of cactus pieces with 750 ml of tap water were transferred to a 2 l flask and stirred for 30 min. The extraction of viscous natural polyelectrolyte (soluble sugars) was performed by decantation and keeping at 4ºC until use. Different mucilage concentrations (5-2o%,v/v) and different agitation speed (50-200rpm) were tested for maximum metal removal (%)with a control in biosorption medium and also in treating wastewater under the same incubation conditions;Olivera *et al.(*2001).

Wastewater samples were collected in screw capped Sterilized bottles from a stagnant fresh water port in Helwan , Egypt. Some physicochemical parameters of wastewater as biological and chemical oxygen demands (COD, BOD), and total suspended matter were measured according to Apha (2001). Cu (II),Cd(II), Fe(III) concentrations were determined using atomic absorption .

Wastewater treatment with immobilized yeast cells and natural polyelectrolytes:

Alginate- and PUF- immobilized cells (≈0.25, 0.12 dry weight /flask) were used to inoculate 50 ml of wastewater in 250 ml Erlenmeyer flasks that adjusted to pH 5.5, respectively, another set of 250 ml Erlenmeyer flask with 50 ml wastewater were treated with 10% (v/v) natural polyelectrolyte and a control wastewater flask kept untreated, incubated at 30ºC and 150 rpm for 18 h. and different previous parameters were evaluated.

Determination of heavy metal concentration in the filtrate:

Following metal treatment, culture filtrates were taken at certain intervals , centrifuged at 10,000 x g for 5 min and the clear supernatant liquids was used to determine heavy metals ions concentrations by using atomic absorption spectrophotometer, Varian absorption spectra AA20-NRC.,Egypt; Ksunger, *et al.*(2003).

-Metal removal (%)=Initial metal conc.(mg/l) - Residual conc.(mg/l)/Initial metal conc. (mg/l)x100; Berekaa and Hussein (2005).

3. Results and Discussion

Effect of type and concentration of heavy metal ion on growth of *S. cervisiae*:

As it could be seen from fig (1), The O.D. measurement indicated that selected working concentrations, were (35,15,25,)mg/l for Cu(II),Cd(II), and Fe(III), respectively, as it moderately inhibit the growth of *Saccaromyce*s when assayed singly compared to the biomass growth obtained under control conditions on agitation at 200 rpm for 24h at 30°C . Above 35, 15, and 25 mg/l of Cu(II),Cd (II) and Fe(III) ,respectively yeast decreased gradually in growth as indicated by O.D,fig (1). The role of the microbial cell wall in the biosorption process is to adsorb metal ions in the cell wall itself or pass through the cell membrane into the vacuoles. To balance the stimulatory or inhibitory effects of essential ions and to counteract the toxicity of nonessential metals, all organisms possess homeostatic mechanisms that properly control the cellular accumulation, distribution, and detoxification of metals, .*S. cerevisiae* provides an ideal system. Gadd (1988) stated that microorganisms can take up nickel intracellular or the presence of chelating ligands that may be present on the cell surface in trace amount even after washing the biomass thoroughly and before using in biosorption experiments. Hence, both type of the heavy metal and its concentration affect behavior of *Saccaromyces* biosorption. .

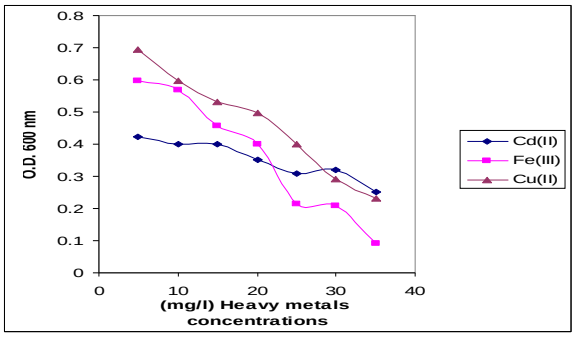

Fig.(1).The growth of *S cervisia* in the presence of cadmium, copper and iron different concentrations after 24h at 30°C and 200rpm.

Effect of equilibrium time on biosorption of heavy metal ion *by S. cervisiae*:

Table (1) indicated that with equilibrium time18 h, (%) metal removal was 14.532 in biosorption medium with Cu(II), 10.433 in Cd(II) medium and 15.594 biosorption medium with Fe(III) culture .This is may be due to the ratio of protein to hydrocarbon or as Park and Choi (2002) stated that after 24 h at 200 rpm at 30°C there is no serious metal accumulation of cadmium in the cell which was related to the cell metabolism. Equilibrium time varied according to the biosorption conditions as it was attained after 30 and 60min for dead and live cell used for removal of Cu^{2+},Machado *et al.(*2009).

Table(1). Effect of incubation equilibrium time on (%) metal removal ion by *S. cervisiae*.

Time in (h.)	(%) metal removal		
	Cu(II)	Cd(II)	Fe(III)
12	12.031	9.011	12.191
18	14.532	10.433	15.594
24	12.411	11.313	14. 356
48	11.087	12.058	11.081

The effect of adaptation of *S. cervusiae* on removal of heavy metal:

As it could be seen from fig (2), increased adaptation to 100 mg/l, for Cu (II), Cd (II) and Fe (III), has no enhanced effect on *S. cervisiae* as it showed no much advanced difference in the process of heavy metals removal compared to that of the non adapted *S. cerevisiae*. under the same incubation conditions as it exhibited (%) removal of 15.99,10.67,and 14.0 for Fe(III), Cd(II) and Cu(II) for control (untreated),respectively compared to 16.75,11.56and 14.87% for adapted biosorbent in the same order .

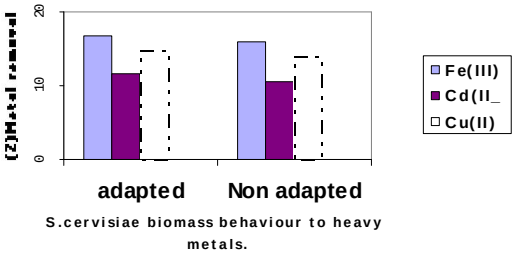

Fig.(2).Effect of (100mg/l)adapted *S.cervisiae* on (%)heavy metals removal .

Effect of cystine on the biosorption of heavy metals :

Table (2) suggested that , sensitivity to Cu(II), Cd(II), and Fe(III) can be reversed by the addition of cystine to the media to enhance the ability to synthesize this amino acid, so, a somewhat high level of cystine was required to reverse the metals toxicity .These results indicate that the intracellular cystine alleviates the toxicity of Cu (II), Cd II), and Fe (III),.As, 4 mg/l cystine resulted in (%)metal removal of 18.311in presence of copper and 15.311Cd(II) and 19. 996 % in iron biosorption medium after 18h at 200rpm and 30°C .Engle and

Kunz (1995) stated that removal carried out in three steps; rapid binding to the negatively charged groups on the cell wall and passive transport of the metal ion through the cell wall within short time 3-5 min , penetration though cell wall to the cytoplasm and accumulation of the heavy metal in the cytoplasm,. Similarly, Pearce and Sherman (1999) revealed that histidine binds divalent metals, and is routinely exploited by insertion of polyhistidine tracts into proteins, so that the protein can be bound to resins with bound divalent metals ions such as Co^{2+} and Ni^{2+}.

Table (2). Effect of cystine on(%) metal removal by *S. cervisiae*.

cystine (mg/l)	(%) metal removal		
	Cu(II)	Cd(II)	Fe(III)
2	12.332	12.413	15.454
4	18.311	15.311	19. 996
6	12.087	14.098	12.018

Initial pH effect on heavy metal removal by *S. cervisiae*:

Table(3) revealed that, pH of the solution was a critical parameter for the adsorption process (%)metal removal was 18.911 in Cu(II) medium at pH 5.5 and 15.213 in Cd(II) medium and 19.256 in Fe(III) medium after 18h at 200rpm and 30°C,in presence of 4 mg/l cystine. On increasing pH, the biosorption capacity increased as there is an increase in ligands with negative charges which results in increased binding of cations because the ligands on the cell are closely associated with the hydronium ions. With increased pH, the hydronium ions are gradually dissociated and the positively charged metal ions are associated with the free binding sites ;Ahlya *et al*.(2003). However, at pH 6.5, (%) metal removal of Cu (II),Cd(II) and Fe (III) decreased to 15.187,10.198,14.198 due to its partial precipitation. Little metal removal was observed at pH 2.5 is an indication of competition of excess of protons for the same binding sites on the cell wall. The iron, cadmium and copper uptake increased with increasing pH, to the maximum near pH 5.5 then decreased at 6.5 pH .At pH values higher than 6.5, metal ions precipitated, so it is not conducted, due to the high concentration of OH- ions in the adsorption medium to avoid possible hydroxide precipitation. and biosorption . Ahlya *et al*.(2003) indicated the importance of pH in the biosorptive process: it affects the solution chemistry of the metals, and the activity of the functional groups in the biomass. The more rapid increase in metal removal in accordance with pH may be presumed by larger degree of deprotontion of the cell wall at higher pH because the hydrolysis degree of heavy metal in the solution at a given pH was nearly constant regardless the microbial species.

Table (3). The pH effect on (%) metal removal of *S . cervisiae*.

pH	(%) metal removal		
	Cu(II)	Cd(II)	Fe(III)
2.5	3.131	1.211	2.091
4.5	12.132	11.123	12.154
5.5	18.911	15.213	19. 256
6.5	15.187	10.198	14.198

Effect of inoculum concentration on sorption of heavy metals by *S. cervisiae* :

As it could be seen from table (4), decreasing the biomass resulted in the increase of the metal removal due to exhaustion of metal ion in the biosorption medium or the interaction between the metal ion binding sites; Park and Choi(2002). The biosorption capacity profiles of Fe (II) and Cu (II) are somewhat similar. Also, it is clear that the carboxyl group, the phosphoric and amino groups in the cell wall reported to be involved on metal ion adsorption group, El Aasar (2005). For 3%,(v/v) inoculum concentration 28.44, 17.47 and 25.57% of the Cu (II),Cd (II) and Fe(III) ,respectively were biosorbed after 18 h at 200rpm and 30°C in presence of 4 mg/l cystine; the auto-aggregation of yeast biomass is rapid. High biosorbent concentrations at (4%,v/v) are known to cause cell agglomeration and consequent reduction in inter-cellular distance resulted in decrease in metal removal (%) as indicated in table (4) 20.24, 17.36. and 16.33 for Cu(II),Cd(II) and Fe(III), respectively . In other words, metal removal is higher when the inter-cellular distance is more (at low biosorbent concentration), as this condition ensures optimal electrostatic interaction between cells, a significant factor for biosorption (Itoh *et al.* 1975). The affinity for cadmium ion penetration of the cell wall during biosorption although thicker outer wall of *S.cervisiae* may inhibit the penetration through the cell wall like ex-polysaccharide pullan from *A. pullanus*. However , Park and Choi(2002) stated that many ligands on the outer cell wall mannan layer seemed to contribute to the high cadmium removal capacity by *S.cervisiae*. However, the ligand in microorganisms accumulates heavy metals on the cell wall. On the cell wall sorption, complexation, ion exchange , precipitation and accumulation of the heavy metal occurred according to the species of the microorganisms , the formation rate of the cell wall during cultivation , the ultra structure of cytoplasm and the creation rate of polysaccharide can vary ; Park and Choi(2002).

Table (4). Effect of inoculum concentration on(%) metal removal by *S. cervisiae*.

S. cervisiae cells conc. (%)v/v	(%) metal removal		
	Cu(II)	Cd(II)	Fe(III)
2	18.34	15.26	19.34
3	28.44	17.47	25.57
4	20.24	17.36	16.33

NaOH effect on heavy metal removal cells of S. *cervisiae*

Fig (3) indicated that pretreatment of biomass by NaOH enhanced metals removal 18.11% of Cd(II) and 36.11% for Cu(II) and 33.52% for Fe(III) from biosorption media after 18h at 200 rpm at 30°C,compared to untreated biosorbent which resulted 11.45,15.47,14.98 % for metals in the same order in biosorption media without additives , this is because *S.cervisiae* has the toughest cell wall structure among microorganisms hitherto with characteristic combination between glucan and mannaoproteins ,Meena and Raja (2006). So, non-living biomass appears to present specific advantages in comparison to the use of living microorganisms. Killed cells may be stored or used for extended periods at room temperature, they are not subject to metal toxicity and nutrient supply is not necessary. So, living cells can be pretreated using chemical means with the objective of increasing the metal biosorption capacity such as treating the cells with acid, or alkali chemicals showed enhancement in removal of heavy metals. As increased biosorption after pretreatment may be due to chemical modification of cell wall components or the exposure of active metal binding sites embedded in the cell wall. Ahlya *et al*. (2003) showed that alkali treatment of biomass may destroy autolytic enzymes that cause putrefaction of biomass and remove lipids and proteins that mask reactive sites. Also, Besides, the pre-treatment could release polymers such as polysaccharides that have a high affinity towards certain metal ,Ahlya *et al*.(2003).Also, Norris and Kelly (1979) revealed that the adsorption between microorganisms and heavy metals occurred initially in the cell surface by ion exchange reaction and that the plasma membrane which play a role in transmitting metal selectively must be destroyed for heavy metal to accumulate in the cell.

However, Yan and Viraraghavan, (2001) indicated in the contrary, acid pretreatment of *Mucor rouxii* significantly decreased the bioadsorption of heavy metals , this is attributed to the binding of H^+ ions to the biomass after acid treatment may be responsible for the reduction in adsorption of heavy metals, this comes in agreement with the findings of Michado *et al*. (2009).

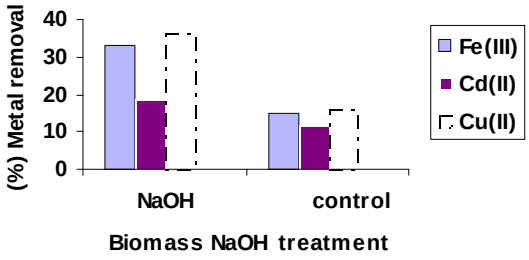

Fig (3). The effect of NaOH -treated *S. cervisiae* cells on (%)metal removal .

Effect of immobilized *S.cervisiae* cells on sorption of heavy metals:

Table (5) indicated the moderate adsorption affinity between the PEU on inert supports foam as sorbent and metals sorbets .However, with still low efficiency than alginate immobilized cells of *Saccharomyces* as (%) metal removal was 21.33 for Cu(II), 23.995 for Cd(II) and 26.768 for Fe(III) ,respectively in comparison with 67.33, 45.995, and 60.768, in the same order with alginate because of both yeast cells and the polymeric matrix of alginate beads itself in absorbing the metals, moreover, alkaline earth metal-alginate entrappers matrix is a known procedure, Meena and Raja (2006) . Stoll ,and Duncan,.(1997)stated that medium accumulation of heavy metals by microbial biomass with high surface area-to-volume ratio holds great potential for heavy metal removal in both soluble and particular forms, especially when the heavy metal concentrations are low (<50 mg/L) Immobilization offer advantages such as easier separation and reuse of cells, maintenance of higher cell concentrations and stabilization adhesion to supports ; Aloiu *et al*. (2008).

Table (5).(%) Metal removal by immobilized *S. cervisiae*.

Metal ion	(PUF) cells dry wt. (g/culture)	Alginate cells dry wt. (g/culture)	%Metal removal alginate	%Metal removal (PFU)
Cu(II)	0.211	0.576	67.33	21.33
Cd(II)	0.113	0.546	45.995	23.995
Fe(III)	0.101	0.454	60.768	26.768

Effect of agitation speed on heavy metal settlement by *Opuntia* extract:

Table (6) indicated that, 150 rpm is the optimum agitation for settlement of heavy metals in biosorption media as (%) removal of Cu(II) 32.13., 12.54 Cd(II) ,Fe(III) 22.51(mg/l) on using 20% (v/v) *Opuntia* natural polyelectrolyte under the same incubation conditions .This is may be due to mucilage hydrophilic character, several hydrogen bonds are formed between polyelectrolyte and water molecules. This association tends to occupy larger surface area causing its very high viscosity; Oliveira *et al.* (2001). This comes in agreement with La Mer and Healy (1963) and Nozaki, *et al.* (1993) who stated that natural polyelectrolyte have been used as auxiliary of flocculation and coagulation in wastewater treatment and water cleaning process .However, Olivera *et al*. (2001), stated that optimum conditions for flocculation and coagulations were: 30 sec. of strong shaking but with addition of aluminum sulfate and natural polyelectrolyte, followed by 15 min. of slow shaking, and then 30 min. for complete settlement .Iron and copper settlement increased more than 2-fold with rise in agitation speed from 50 to 150 rpm, beyond which there was no further increase.

Table (6).Effect of agitation speed on (%) metal removal by *Opuntia* polyelectrolyte.

Agitation speed(rpm)	Metat Removal(%)		
	Cu(II)	Cd(II)	Fe(III)
50	13.54	10.67	10.55
100	22.14	11.55	17.33
150	32.13	12.54	22.51
200	32. 65	12.54	22.54

Effect of natural electrolyte concentrations on heavy metal settlement:

Table (7) showed the mucilage efficiency for reducing heavy metals from biosorption media ,also, proved its flocculation with the efficiency increase with10 %(v/v) mucilage concentration resulted in 38.50%Cu (II) 16.12;Cd(II) 30.12 Fe(III).Olivera *et al.* (2001), revealed that the flocculation process induced by anionic polyelectrolyte such as the natural polyelectrolyte extracted from *Opuntia ficus indica*. The positive metals ion serves to form a bridge among the anionic polyelectrolyte and negatively charged functional groups on the colloidal particle surface. Cactus mucilage is a neutral mixture of approximately 55 high-molecular weight sugar residues composed basically of arabinose, galactose, rhamnose, xylose, and galacturonic acid. This natural product was characterized for its use as a flocculating agent. Table(7) shored that 10%, v/v of mucilage provided the optimal effectiveness for metals removal in biosorption media,. Table(7)also ,suggested that *Opuntia* mucilage proved the feasibility of applying mucilage as a method for heavy metals removal as a natural flocculating agents, as covalent bonds in vector compounds, or on cell cross-linking, that are innovative, environmentally benign, and cost-effective.

Table (7). (%)removal of heavy metals by *Opuntia* polyelectrolyte different concentrations.

Polyelectrolyte conc.% (v/v)	Metal removal(%)		
	Cu(II)	Cd(II)	Fe(III)
5	14.30	19.43	22.44
10	38.50	16.12	30.12
20	30.40	16.43	25.23

Finally, The use of this type of green chemistry shows *Opuntia* mucilage as a resource for achieving potable water, as the use of natural environmentally benign agents in the treatment of drinking water is rapidly gaining interest due to their inherently renewable character and low toxicity. As a gum-like substance, cactus mucilage, which shows excellent flocculating abilities, is an economically viable alternative for low-income communities and overcoming many problems and its use beside PUF- and alginate- immobilized cells, which have the advance of the ease of cell separation, for efficient metals removal and in decreasing the parameters indicated in table (8): total suspended solids , Cu(II),Cd(II) and Fe(III) concentrations , chemical and Biological oxygen demands(COD,BOD). Thus suggesting clarifying a local port sample regarding environmental impacts as permissible Egyptian limits Laws in 44/2000 and 48/1982 lows ,which may grant the possibility of its applications.

Table(8) .Bio-treatment of a fresh water port sample with immobilized yeast and *Opuntia* polyelectrolyte

Factor(mg/l)	Permissible limit (mg/l)		Final conc. polyelectrolyte	Final conc. immobilized Alginate	Final conc. immobilized Foam	Initial conc.
	48 /198 2	44 /200 0				
Cu(II))	1	0.2	0.106	0.065	0.101	0.512
Cd(II)	1	1.5	0.311	0.105	0.299	0.325
Fe(III)	-	1	0.321	0.177	0.349	0.633
COD	1100	60	800	900	900	900
BOD	600	80	600	700	700	700
Total suspended solids	60	800	600	500	1100	1200

4. References

1. Ahalya, N.; Ramachandra, T.V., and. Kanamadi, R.D (2003).Biosorption of Heavy Metals. Res. J. of Chem. And Env., 7 (4):71-79.
2. Alaoui, S.M.; Merzouki, M.; enninckx, M .J. P, and Belemlih , M. (2008). Relationship between cultivation mode of white rot fungi and their efficiency for olive oil mill waste water treatment. E lect. J. of Biotech., 11: 4 .
3. APHA, (2001). Standard Methods for Water and Wastewater Examination, 20 th ed. American Public Health Association, Washington.
4. Atkinson, B.; Black, G.M., and Pinches, A. (1980). Process intensification using cell support systems.Process Biochem., 15(4): 24-&.
5. Berekaa,M.M., and Hussein, H. (2005). Impact of phenotypic variation on rubber degradation and metal resistance in *Goronia wesfalic*a variants.J.Biot: 21: 433-466.
6. Davis, T. A.; Volesky, B. and Mucci, A.(2003),A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.*,37(18) : 4311-4330.
7. Dempsey, M.J.(1994). Biofilms and fluidized bed fermentation. International Biodeterioration & Biodegradation, 34(3-4): 237-244.
8. Engl,A., and kunz,B. (1995). Biosorption of heavy metals by *Saccharomyces cervisiae*. Effect of nutrient conditions. J.Chem. Tech . Biotch., 63;257-61.
9. El Aasar ,S.A. (2005). Adaptive tolerance of *Trichoderma hamatum* in cadmium, copper and lead heavy metals.Egy.J. Biotech.., 21 278-294.
10. Forni, E.; Penci, M.,and Polesello, A..(1994); Carbohydr. Polym, 23, 231.
11. Gadd, G.M. (1990). Heavy metal accumulation by bacteria and other microorganisms. Experientia, 46: 834-840.
12. Goksungur, Y.; Üren, S. and Guvence,U. (2005). Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass. Biores. Technol., 96(1):103-109.
13. Goyal, N.; Jain, S.C. and Banerjee, U.C(2003).Comparative studies on the microbial adsorption of heavy metals. Adv. in Env. Res. , 7(2), : 311-319.
14. Holan, Z.R., and Volesky, B. (1995). Accumulation of Cd, lead and nickel by fungal and wood biosorbents. Appl. Biochem. Biotechnol. 53: 133-146.
15. Itoh, M.; Yuasa, M. ,and Kobayashi,T. (1975) .Adsorption of metal ions on yeast cells at varied cell concentrations. Plant and Cell Physiology, 16(6): 1167-1169.
16. Jianlong, W.(2002). Biosorption of copper (II) by chemically modified biomass of *Saccharomyces cerevisiae*. Process Biochem., 37(8) : 847-380
17. Ksungur, Y., G.; Ren; S., and .Ven, U. G.(2003). Biosorption of Copper Ions by caustic treated waste Bakers yeast biomass.Turk.J.Biol.,27:23-29.
18. La Mer, Y . and Healy,T. (1963); Rev. Pure Appl. Chem, 13, 112.
19. Lee, S.H.; Jung, C.H.; Chung, H.; Lee, M.Y., and Yang, J. (1998). Removal of heavy metals from aqueous solutions by apple residues. Process Biochem. 33, 205-211.
20. Liu X. F.; Supek F., Nelsoni N., and Culotta V. C. (1997).Negative control of heavy metal uptake by the *Saccharomyces cerevisiae* BSD2 Gene. The J. of Biol. Chem.272,(18) : 11763–11769.
21. Lovely, D.R , and Coates J.D. (1997). Bioremediation of metal contamination. Curr. Opin. Biotechnol. 8: 285-289.
22. Marshall, W.E.; Wartelle, L.H.; Boler D.E.; Johns, M.M.,and Toles, C.A. (1999). Enhanced metal adsorption by soybean

hulls modified with citric acid. Bioresour. Technol. 69, 263-268.
23. Machado, M.D.; Janssens, S.; Soares, H.M.V.M.; Soares, E.V. (2009). Removal of heavy metals using a brewer's yeast strain of *S. cerevisiae*: advantages of using dead biomass .J of Appl. Microbiol., 106 (6) : 1792-1804(13).
24. Mena, K. and Raja, T.K. (2006). Immobilization of *Saccharomyces cervisiae* cells using various metal alginate. W. J. of Microbiol. and Biotechnol.22:651-652.
25. Norris, and Kelly, D.P. (1979). Accumulation of metals by bacteria and yeast Dev.Ind.Microbiol.20:247-51.
26. Nozaki, J.; Messerschimidt, I.,and Rodrigues, D.G.(1993). Arq.Biol. Tecnol. 36, 761.
27. Oliveira, M.A; Rodrigues, Reis,C. E. M.dos and, Nozaki,J. (2001).Production of fungal protein by solid substrate fermentation of *Cactus cereus peruvianus* and *Opuntia ficus indica* .Quím. Nova .24 (3).
28. Pattanapipitpaisal, P.; Mabbett, A.N.; Finlay,J.A , et al. (2002).Reduction of Cr (VI) and bioaccumulation of chromium by Gram-positive and Gram-negative micro-organisms not previously exposed to Cr-stress. Environ.Technol. 23: 731-745.
29. Park,J.K.,and Choi,S.B.(2002).Metal recovery using immobilized cell suspension from brewery .Korean J. Chem. Eng. 19:68-74.
30. Pearce, D. A., and Sherman, F. (1999). Toxicity of Copper, Cobalt, and Nickel salts is dependent on Histidine metabolism in the yeast *Saccharomyces cerevisia*e J. of Bacteriol., 181(16): 4774-4779.
31. Quek, E.; Ting, Y. P., Tan , H.M. (2006). *Rhodococcus* sp. F92 immobilized on polyurethane foam shows ability to degrade various petroleum products. Biores. Technol., 97: 32–38.
32. Rehman ,A; Shakoori F.R., and Shakoori, A.R. (2007). Heavy metal resistant *Distigma proteus* (Euglenophyta) isolated from industrial effluents and its possible role in bioremediation of contaminated wastewaters.World J. Microbiol. Biotechnol. 23: 753-758.
33. Rehman, A.; Shakoori, F.R., and Shakoori, A.R. (2008). Heavy metal resistant freshwater ciliate, Euplotes mutabilis, isolated from industrial effluents has potential to decontaminate wastewater of toxic metals. Biores. Technol. 99: 3890-3895.
34. Ringot, D; Lerzy, B.; Chaplain, K. Bonhoure, J.P.; Auclair, E. and Larondele, Y. (2006). In vitro biosorption of ochratoxin A on the yeast industry by-products: Comparison of isotherm models. Biore. Technol.
35. Stoll, and Duncan, Jr. (1997). Comparison of heavy metal sorptive properties of three types of immobilized non viable *Saccharomyces cervisiae* biomass. Process biochem, 32; 467-72.
36. Tsekoa, K., and Petrov. G. (2002). Removal of Heavy Metals from Aqueous Solution Using Rhizopus delemar mycelia in Free and Polyurethane-Bound Form.Z. Naturforsch. 57c, 629 D 633
37. Wilkins E. A. and Yang, Q. (1996). Comparison of the heavy metal removal efficiency of biosorbents and granular activated carbonsJ.Environ.Health,A-31(9):2111-2128.
38. Wang, J., and Chen, C.(2006). Biosorption of heavy metals by *Saccharomyces cerevisiae*: A review. Biotechnol.Adv., 24, (5):427-451.
39. Willaert, R.G., and Baron, G.V. (1996). Gel entrapment and micro-encapsulation: Methods, applications and engineering principles. Reviews In Chemical Engineering. 12(1-2): p. 5-205
40. Yan, G., and Viraraghavan, T., (2001). Heavy metal removal in a biosorption column by immobilized *M. rouxii* biomass. Bioresour. Technol. 78, 243-249.
41. Zouboulis, A. I.; Rousou, E. G.; Matis, K. A., and Hancock, I. C.(1999). Removal of toxic metals from aqueous mixtures. Part 1: Biosorption. J. of Chem. Technol. and Biotechnol., May, 74(5): 429-436.
42. Zouboulis, A. I.; Matis, K. A., and Lazaridis, N.K. (2001). Removal of metal ions from simulated wastewater by *Saccharomyces* yeast biomass: combining biosorption and flotation processes. Separation Sci. and Technol, 36(3): 349-365.

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