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Removal of Lead(II) by *Saccharomyces cerevisiae* AUMC 3875

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THE REMOVAL of lead(II) from artificial aqueous solution using live and dead biomass of *Saccharomyces cerevisiae* AUMC 3875 was investigated. The minimum inhibitory concentration (MIC) value of *S. cerevisiae* AUMC 3875 for lead(II) was 600mg/l. Maximum lead(II) uptake capacities were achieved at pH 5.0 and initial metal ion concentration 300mg/l using 3g/l live and dead cells, respectively. Maximum biosorption capacities were reached after 3h and 20min for live and dead cells, respectively. Fourier Transform Infrared spectroscopy (FTIR) results revealed the important role of C=O, – OH, – NH, protein amide II band, PO_2^-, mannans, sulphur and sulphur-oxygen compounds in lead(II) uptake. Scanning electron microscopy analysis (SEM) showed that the cell surface morphology and surface area/volume ratio changed greatly after lead(II) uptake. Transmission electron microscopy analysis (TEM) confirmed the involvement of both extracellular adsorption and intracellular penetration through the cell wall. X-ray powder diffraction (XRD) analysis revealed the presence of $Pb(SO_4)$, Pb_2OSO_4 in dead yeast cells and $Pb_3O_2(SO_4)$,Pb_2OSO_4 in live biomass. Energy dispersive X-ray microanalysis (EDAX) confirmed the occurrence of sulphur, oxygen and lead(II) on the cell wall. The removal of lead(II) from storage battery industry wastewater was performed by dead yeast cells efficiently.

Keywords: *Saccharomyces cerevisiae,* Lead(II), Biosorption, SEM, TEM, FTIR , EDAX, XRD.

Large amounts of heavy metals are released into the environment due to the technological activities of humans. The impact of these metals in aquatic systems and their accretion throughout the food chain can cause a serious threat to animals and humans, causing a world–wide environmental problem (Machado *et al.*, 2008). Lead(II) is extremely toxic even at low concentrations and accumulates mainly in bones, brain, kidney and muscles and may cause many serious disorders like anemia, nervous disorders, sickness and kidney diseases, particularly in children (Chua *et al.*, 1999).

Conventional methods for removing lead(II) from wastewater include precipitation, ultra filtration and membrane processes including reverse osmosis (Mohammadi *et al.,* 2005). These methods have significant disadvantages, which include incomplete metal removal, high capital cost when applied to high strength wastes with heavy metal concentrations greater than 100 ppm and generation of toxic sludge or other waste products that require disposal (Goyal *et al.,* 2003). These disadvantages, together with the need for more economical and effective methods for recovery of metal, have resulted in development of alternative separation technologies. In this respect, biosorption, a biological method of environmental control, has emerged as an alternative to conventional effluent treatment methods as it has advantages of low operating cost, effectiveness in diluted solutions, generates minimum secondary waste, complexes within short time periods and has no toxicity limit for heavy metals (Volesky, 1990).

Biosorption is an operation that combines the use of biomaterials for sorbing, sequestering and immobilizing organic or inorganic substances from aqueous solutions. This type of uptake may take place by any one or a combination of different processes such as complexation, coordination, chelation, ion exchange or microprecipitation and entrapment. All these mechanisms are associated with either living or dead microbial cells except the last two.

It is necessary to distinguish between active, metabolically mediated metal uptake by living cells as opposed to passive metal sequestering by dead biomass (Naja &Volesky, 2011). Actively metabolizing cells may, in some instances, even actively repel metals ions, particularly the more toxic ones, as a self defense (Naja & Volesky, 2011). When the cells are inactivated the chemical binding sites of the biomass may attract metal ions from solution. Fungal biosorption has been studied extensively because of the availability of large amounts of waste fungal biomass from fermentation industries and the amenability of microorganisms to genetic and morphological manipulation. Biosorption potential of fungi like *Rhizopus*, *Aspergillus*, *Streptoverticillum*, *Phanerochaete*, and *Saccharomyces* have been explored many times (Pakshirajan & Swaminathan, 2010). *Saccheromyces cerevisiae* have found to be the efficient biosorber of heavy metals like Au, Mn, Cu, Co, Pb (Pavarthi *et al.,* 2006 and 2007; Ezzouhri *et al.,* 2008 and Mishra *et al.,* 2009).

In light of this, the present study has been carried out to investigate the potential of live and dead *Saccharomyces cerevisiae* AUMC 3875 for removal lead(II) from aqueous solution and storage battery industry wastewater. Experimental parameters affecting biosorption process such as pH, biosorbent concentration, initial metal concentration and contact time were studied. The biosorption mechanism was also investigated by using scanning electron microscopy (SEM), transmission electron microscopy (TEM), fourier transform

infrared spectroscopy (FTIR), X-ray powder diffraction (XRD) and energy dispersive X-ray microanalysis (EDAX).

Materials and Methods

Yeast culture

Saccharomyces cerevisiae AUMC 3875 was obtained from Assuit University Mycological Centre.

Chemicals

Stock metal solutions of lead(II) were prepared by dissolving appropriate quantities of lead(II) nitrate salt in double distilled water. The stock solutions were diluted further with deionized distilled water to obtain working solutions of different concentrations.

Preparation of biosorbent

For mass culturing, *S. cerevisiae* AUMC 3875 was cultivated in liquid medium using the shake flask method. The liquid medium used for growth contained 6.0 g yeast extract, 15.0 g sucrose, 0.52 g $MgSO_4$ .7 H_2O, 3.0 g K_2HPO_4, 3.76 g Na H_2PO_4, 3.35 g $(NH_4)_2SO_4$ and 0.017 g $CaCl_2.4H_2O$ per liter of distilled water. The pH of the medium was adjusted to 5.0 with aqueous solution of 0.1 N H_2SO_4 and NaOH. The growth medium was introduced in 500 ml capacity conical flasks each contained 100ml liquid medium. The flasks were sterilized by autoclaving at 121ºC for 20 min. The cooled sterilized flasks were inoculated with cells of *S. cerevisiae* and incubated at 30ºC and 150 rpm. After 24 h, *S. cerevisiae* AUMC 3875 cells were separated from liquid medium by centrifugation at 5000 rpm for 10 min. Cells were washed twice with distilled water and one part was autoclaved to prepare dead cells and other part was directly used as live cells (Suh *et al.*, 1999).

Experimental methods

Determination of minimum inhibitory concentration (MIC)

A sterilized solution of lead(II) nitrate was aseptically added to the sterilized medium to get the final concentration of 0, 200, 400, 500, 600, 700, 800, 900, 1000 and 1100 µg/ml. The plates were inoculated with 100 µl of yeast suspension and incubated at 30°C for 48h: MIC was identified as the minimum concentration of lead(II) that inhibited visible growth of *S. cerevisiae* AUMC 3875.

Batch biosorption procedure

All uptake experiments were performed by suspending the biosorbent in 100ml of metal solution at desirable pH, biosorbent concentration, initial metal ion concentration and contact time. Sorption contact experiments with metal bearing solutions were run in triplicate.

Optimization of pH

To evaluate the effect of pH on metal uptake by yeast cells, the pH of the solution was adjusted in the range between 3 to 6 viz. 3, 4, 5 and 6 before mixing

biomass. The pH was adjusted to the required value with aqueous solution of 0.1N HNO_3 and 0.1N NH_4OH. Initial metal ion concentration 300 mg/l and biosorbent concentration was 3g/l at 28ºC and contact time was 20 minutes in the case of dead cells and 3h for live cells. The concentration of unadsorbed lead(II) in the supernatants was measured by using an atomic adsorption spectrophotometer (Model Unicam 969, Centric Laboratory of Agriculture Faculty, Zagazig University).

Optimization of biosorbent concentration

The prepared microorganism suspension of live and dead cells (50 ml) with concentrations 2, 3, 4 and 5 g/l (with respect to cell dry weight) were added to 500 ml Erlenmeyer flasks separately. Aliquots (50 ml) of lead(II) nitrate solution prepared at twice the desired concentration (300 mg/l) were added to each flask, and shaken on a rotary shaker incubator at 150 rpm and 30^{o}C (Suh *et al.*, 1999) for 20 min. In the case of dead biomass and 3h in the case of live biomass.

Optimization of metal ion concentration

The batches were set at different initial metal ion concentrations of lead(II). Aliquots (50ml) of 50, 150, 250, 300 and 350mg/l concentrations of lead(II) nitrate were added to 3g/l biomass at 28ºC in 500ml Erlenmeyer flasks.

Optimization of contact time

The biomass suspension (3g/l) was exposed with lead(II) solution (300mg/l) for different periods of time. Samples were analyzed at intervals of 0.17, 0.33, 0.5, 1.0, 1.5, 2.0, 3.0, 6.0 and 12h and the adsorption profile was monitored. For all graphical representations, the mean values of the three replicates of the batch experiments were plotted.

Biosorption data evaluation

The amount of metallic ions biosorbed per gram of biomass (q) was determined using the following equation:

$$\text{Biosorption capacity (q)} = \frac{C_i - C_f}{M} V$$

where, C_i is the initial metal ion concentration (mg/l), C_f is the final metal ion concentration (mg/l), M is the mass of the biosorbent (g), V is the volume of the metal solution and q is biosorption capacity (mg/g).

FTIR spectroscopy

Infrared spectra of live and dead native cells as well as live and treated ones were recorded over the region 400-4000 cm^{-1} with Pelkin-Elmer FTIR 1650 spectrophotometer. The samples were examined in KBr discs containing 3% (w/w) of finely ground powder of each sample.

Scanning electron microcsopy (SEM)

S. cerevisiae AUMC 3875 cells were fixed in 2.5% glutaraldehyde at 4^{o}C for 24h and then post-fixed in 1.0% osmium tetraoxide at room temperature for 1 h (Harely & Fergusen, 1990). The specimens were then dehydrated with ascending concentrations of acetone and finally sputter coated with gold. The Scanning and photographing were done using a Jeol scanning electron microscope (JEM-1200XII).

Morphometric analysis

To calculate cell volume (v) and surface area (A) by the following equation, normal and stressed yeast cell dimension were measured directly from the SEM photographs:

$$V\ (\pi m^3) = \pi\ r^2 h$$

$$A\ (\pi m^2) = 2\pi\ r^2 + 2\pi\ r\ hr$$

where r and h are radius and length of a cell in µm (Neumann *et al.*, 2005). Mean cell dimension of the *S. cerevisiae* AUMC 3875 were measured. Average cellular volume and surface area were calculated from normal cells. Cells showing deformations/depression were not considered.

Transmission electron microscopy (TEM)

S. cerevisiae AUMC 3875 cells were fixed in 2.5% glutaraldehyde for 3h **(**Gupta & Berridge, 1966), washed twice with 0.2M phosphate buffer of pH 7.4 for 30 min, then post-fixed in 1.0% osmium tetraoxide for 2h (Palade, 1952). After that, the cells were washed with phosphate buffer for 30min. All the previous steps of fixation were carried out at 4^{o}C. Samples were dehydrated in a graded ethanol series (50%, 60%, 70%, 80%, 90%, 95% and 100%). They were passed through three changes of acetone: ethanol (1:2, 1:1 and 2:0) for 10 min each and embedded in epoxy medium (Epon 812) (Luft, 1961). Blocks were sectioned with a diamond knife (ultramicrotome RMC USA) into ultrathin section about 70 nanometer. These ultrathin sections were constructed with uranyl acetate (Sptempack & Ward, 1969) followed by lead(II) citrate (Reynolds, 1963) each for 30 min. Transmission and photographing were done using JEOL TEM -1400 electron microscope and optronics AMT CCD camera with 1632 pixel format as side mount configuration.

X-ray powder diffraction analysis (XRD)

X-ray diffraction pattern of powder samples of metal-free control and metal-sorbed live and dead biomass were recorded in a Broker D8 Advanced target Cukα powder diffractometer (λ=1.5418 A^{o}) over the range of 0-80(2θ).

Energy dispersive X-ray microanalysis

Metal-loaded live and dead biomass were used for energy dispersive X-ray microanalysis using X-ray micro analyzer (Model Oxford 6587 INCA x-sight) attached to JEOL JSM-5500 LV scanning electron microscope at Regional Center of Mycology and Biotechnology, Cairo, Egypt.

Application of lead(II) removal capacity by dead biomass of S. cerevisiae AUMC 3875

Wastewater was obtained from storage battery factory in Industrial area of Tenth of Ramadan, Sharkia, Egypt. Physicochemical parameters of wastewater viz temperature, pH and the concentration of other metal ions present at the wastewater were measured (Table 1). The concentrations of other metal ions present at the tested wastewater were so small that they could not affect the removal of lead(II) from the wastewater.

TABLE 1. Characteristics of used wastewater.

Characters	Value
Temperature	35^{o}C
pH	1.7-1.8
Lead(II)(mg /l)	3.77
Chromium	0.011
Cadmium	___

Results and Discussion

Lead(II) tolerance by S. cerevisiae

Results showed high yeast cells tolerance to lead(II) concentration up to 600 mg/l of lead(II) which is 1000 fold higher than the concentration permitted in the industrial effluents (0.5 mg of lead(II) per litre of effluent). Comparable results were obtained by Wang & Chen (2006). Biological mechanisms implicated in yeast cells tolerance include extracellular precipitation, complexation and crystallization, the transformation of metal species, biosorption to cell walls, intracellular chelation by the generation of metallothioneins and phytochelatins, and metal localization / sequestration within vacuoles (Gadd, 1992 and Liu & Culotta, 1999). Colonies developed in the presence of high lead(II) concentrations become much darker than control ones. Such dark colour is probably due to the intensification of pigments on cell wall as response to the stress applied by lead(II) (Ezzouhri *et al.*, 2008).

Effect of biosorbent concentration

Results on the influence of biomass concentration showed that when the amount of biosorbent increased from 2.0 to 3.0g/l, the uptake capacity of lead(II) by live and dead cells increased (Fig.1). This could be due to the available more binding sites for the biosorption to occur (Cruz *et al.*, 2004 and Yahaya *et al.* , 2009). Also it can be seen that biosorption capacity decreased from 27 to 18mg/g and 19 to 12mg/g for dead and live biomass respectively, as the biomass concentration increased from 3 to 5g/l (Fig.1).This decrease might be due an increase in electrostatic interactions between cells at higher concentrations and

this cause cells to agglomerate, which contribute to a decrease in the amount of binding sites available (Cho *et al.*, 2010). It was reported that higher specific sorption at lower biomass concentrations could be due to an increased metal to biosorbent ratio (Puranik & Paknikar, 1999). Mashitah *et al.* (1999) reported higher biosorbent concentration could produce a "screening" effect on the cell wall, protecting the binding sites, thus resulting in lower lead(II) uptake. However, Fourest & Roux (1992) reported that the reduction in metal sorption with increasing biomass concentration is due to an insufficiency of metal ions in solution with respect to available binding sites.

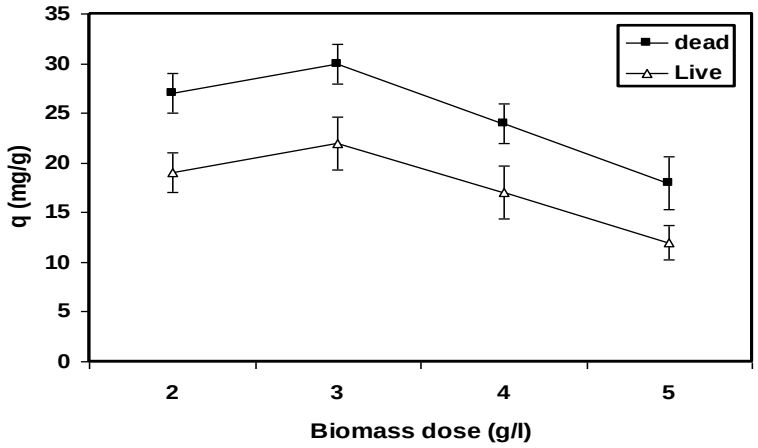

Fig.1. Effect of biosorbent concentration (m) on lead(II) uptake capacity of live and dead biomass of *S. cerevisiae*.

Biosorption conditions: initial metal ion concentration (C_i) = 300mg/l; pH = 5.0; contact time (t) = 20min (for dead cells) and 3h (for live cells)

Effect of initial metal ion concentration

Figure 2 indicated that the initial metal ion concentration had a strong effect on the biosorption capacity. It can be seen that as the metal ion concentration increased, biosorption capacity also increased and reached a saturation value of 30 and 22mg/g for dead and live biomass respectively, which were achieved at 300 mg /L concentration of lead(II) ions. The uptake capacity was reduced with an increase in initial metal ion concentration to 350 mg/L. At lower initial solute concentration, the ratio of the initial moles of solute to the available surface area was low; subsequently, the fractional sorption become independent of the initial concentration. At higher concentration, the sites available for sorption become fewer compared to the moles of solute present and, hence, the removal of solute was strongly dependent upon the initial solute concentrations (Binupriya *et al.*, 2007). The reduction In uptake capacity with an increase in initial lead(II) concentration

probably due to the saturation of the binding sites of the biosorbent (Ray *et al.*, 2005). Comparable results were reported by Parvathi *et al.* (2006), Ezzouhri *et al.* (2008) and Dhankhar *et al.* (2011).

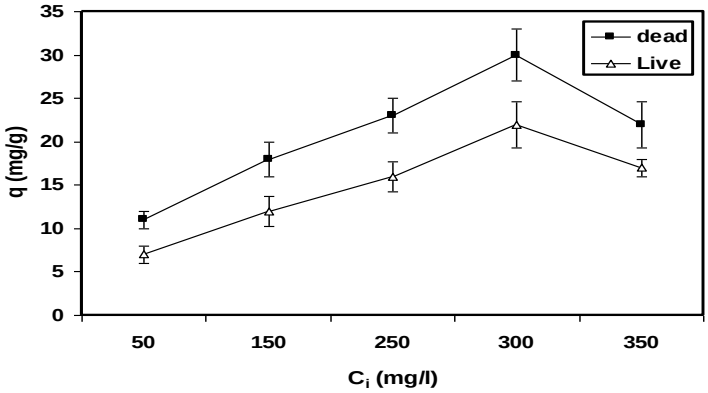

Fig. 2. Effect of initial metal ion concentration (C_i) on lead(II) uptake capacity of live and dead biomass of *S. cerevisiae*.

Biosorption conditions: m = 3.0g/l; pH = 5.0 and t = 20min (for dead cells) and 3h (for live cells).

Effect of initial pH

Initial pH value of the solution is an important controlling factor in the biosorption process. The results showed that the adsorptive capacity of dead and living *S. cerevisiae* biomass increased with the rise of pH from 3.0 to 5.0 and decreased at pH 6.0 (Fig.3). Low pH (3.0) had more drastic effect on the adsorption capacity of live cells (7.0mg/g) than of dead ones (20mg/g). This is due to protein denaturation at low pH (Rothschild & Mancinelli, 2001).

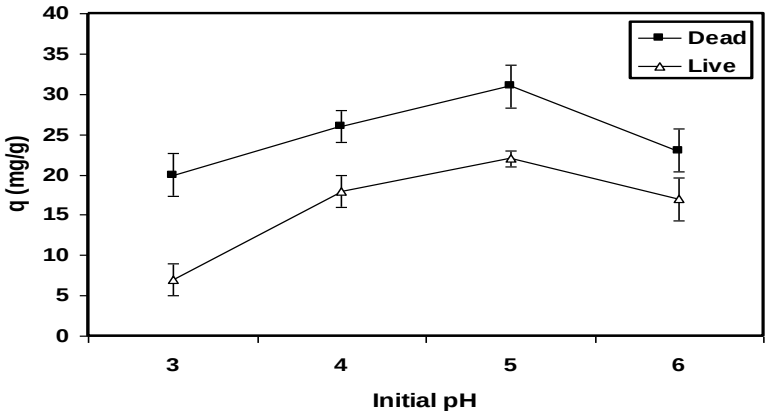

Fig. 3. Effect of initial pH on lead(II) capacity of live and dead biomass of *S. cerevisiae*.

Biosorption conditions: C_i = 300mg/l; m = 3g/l and t = 20 min (for dead cells) and 3h (for live cells)

Additionally, at low pH, H^+ ions would compete with metal ions and binding sites are available to metal ions only in a deprotonated state (Ofomaja & Ho, 2007). With an increase in initial pH, protonation effect becomes minor and the negative charge density on the biomass surfaces increases and more metal binding sites become available (Say *et al.*, 2001). The optimum pH value was 5.0 at which the adsorption capacities were 31 and 22 mg/g for dead and live cells, respectively. pH values can affect metal speciation. An increase in pH can result in the precipitation of metal hydroxides. Consequently, less soluble amounts of metals will be available for accumulation in the yeast cells (Sheng *et al.*, 2004).

Effect of contact time

The biosorption capacity of dead and live biomass as a function of contact time is presented in Fig.4. It can be seen that the primary fast phase of biosorption by dead biomass within the first 20 min, and this was followed by second slow phase until equilibrium. A similar trend was observed by Wang *et al.* (2010). Equilibrium time was found to be 1h.

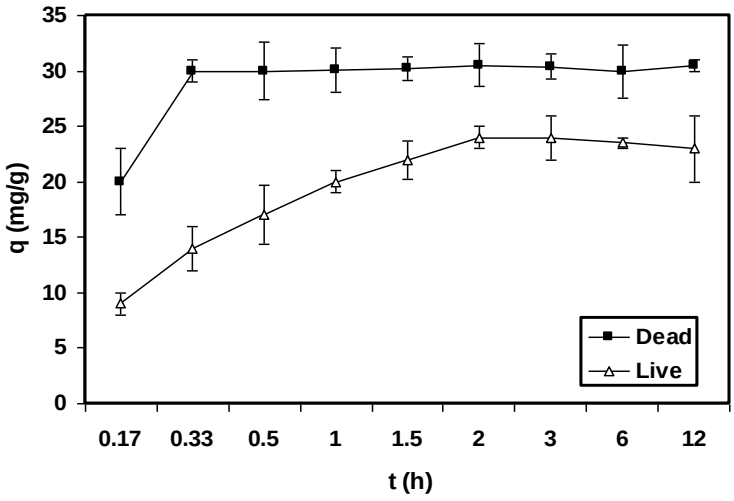

Fig. 4. Effect of contact time (t) on lead(II) capacity of live and dead biomass of *S. cerevisiae*.

Biosorption conditions: C_i = 300mg/l; m = 3g/l and pH = 5.0 .

In contrast, the rate of biosorption by live biomass was slow and reached the equilibrium within 3h. Metal ion uptake in yeast is known to involve an initial rapid phase (passive uptake), followed by much slower phase (active uptake). The first stage is physical adsorption or ion exchange at the surface of the biomass and accounted for the major part in total metal uptake, while the second stage contributes small part (Goyal *et al.*, 2003).

Electron microscopy analysis

SEM analysis was carried out to observe the differences in the surface morphology after the metal uptake by dead and live biomass (Fig. 5). Prior to lead(II) accumulation, *S. cerevisiae* AUMC 3875 had smooth cell surface with no membrane identitions and uniform electron dense area throughout the unicellular body of the stain (Fig. 5a). Also the cells were overlapped with each other. After lead(II) uptake, the dead cells become rough due to the aggregates of metal complex deposited in the form of granules (Fig. 5b). This confirmed the important role of *S. cerevisiae* AUMC 3875 cell wall in adsorption process. Similar findings were observed by Limin *et al.* (2009). Additionally, the dead cells become loosely packed. In live cells, the rupture of cell wall was very evident; consequently, the cells showed wrinkled and rough appearance (Fig.5d). Also, the cell shape was progressively deformed. The disruption of cellular and organelle membranes are among toxic effects of heavy metals on fungi (Gadd, 1992). Similar observations were reported by Suh *et al.* (1999), Dai *et al.* (2009) and Lin *et al.* (2010). Average dimension of normal live cells was 3.8 – 3.9μm by 1.2–1.13 μm (Fig. 5) and the surface area /volume ratio was 9.4. In lead(II) stressed cells average dimension was 2.5 – 2.6 μm by 1.02 –1.04 μm the surface area/ volume ratio was 8.4. The relative decrease in cell volume plays the key role in the consequent reduction in attachment /uptake sites on the cell surface for the heavy metals and lowering the toxic effects of environmental stress factors (Neumann *et al.*, 2005 and Chakravarty & Banerjee, 2008).

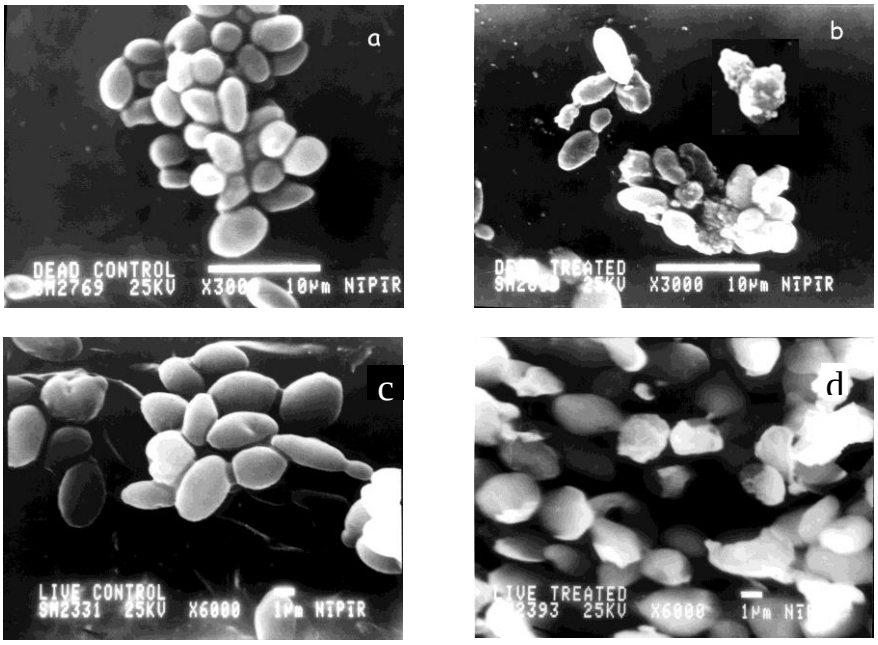

Fig. 5. SEM of *S. cerevisiae* AUMC 3875: (a) Native dead cells, (b) After lead(II) biosorption, (c) Native live cells, (d) After lead(II) Biosorption (25kv).

TEM observations of original and lead(II) loaded dead and live cells of *S.cerevisiae* AUMC 3875 are represented in Fig. 6. Lead(II) was deposited on the inside and outside of dead (Fig. 6b) and live cells (Fig. 6d) *S. cerevisiae* AUMC 3875. From TEM micrographs of dead cells, it can be seen that extracellular adsorption of lead(II) occurred mainly in the parts with complete cell walls (Fig. 6d). Various ligands located on the cell wall were known to be involved in metal biosorption (Remacle, 1990). The presence of lead(II) deposits within the dead cells may be due to the loss of membrane integrity that leads to the enhancement of metal removal (Machado *et al.*, 2008). Fomina *et al.* (2005) reported that the fungal biomass can act as a metal sink by metal biosorption to biomass cell walls, intracellular sequestration and accumulation and precipitation of metal compounds onto and /or around hyphae.

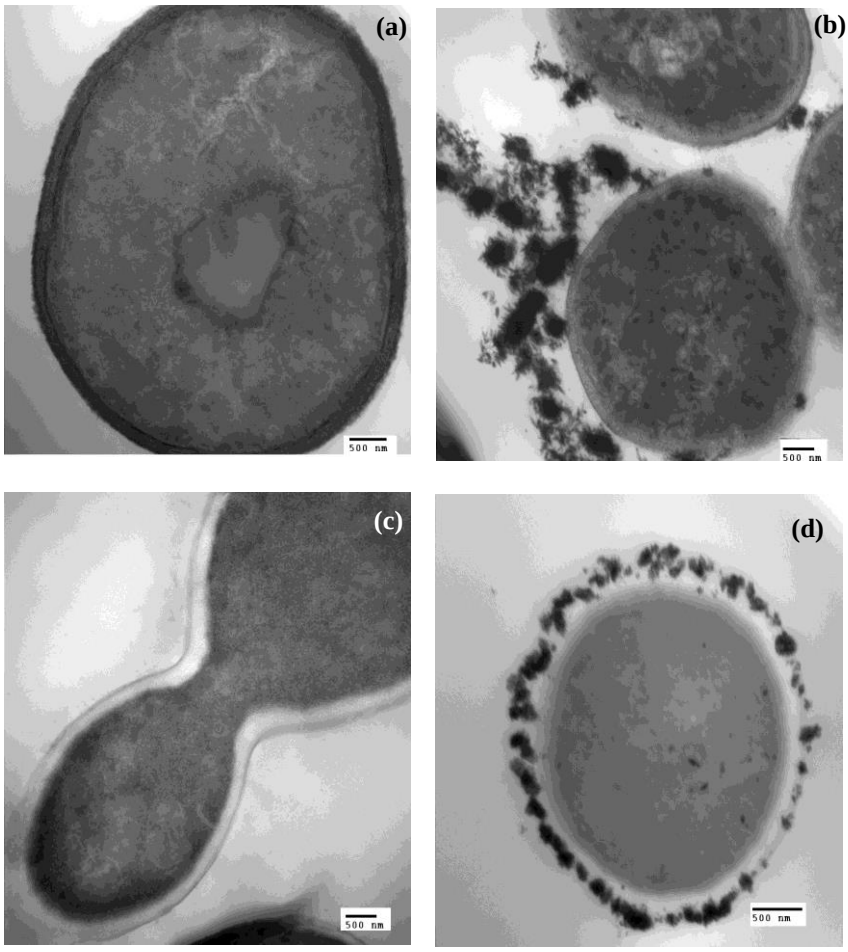

Fig. 6. TEM of *S. cerevisiae* AUMC 3875*:* (a) Native dead cells, (b) After lead(II) biosorption, (c) Native live cells, (d) After lead(II) biosorption (100kv).

FIIR spectroscopy results

The yeast wall composition was reported by Brady *et al.* (1994) to contain a large number of complex organic compounds and their polymers like glucan (28.8% w/w), mannan (31%), proteins (13%), lipids (8.1%), chitin and chitosan (2%) and inorganic ions such as Ca^{2+}, Mg^{2+}… etc (3%), the FTIR is highly complex. In spite of its high complex nature, the spectra contain information of yeast components which are represented by specific peaks in the fingerprinting regions: the region between 790 and 1180cm^{-1} corresponding to the sugar component of the cells; between 1200 and 1290cm^{-1} corresponding to the nucleic acids; between 1400 and 1700cm^{-1} corresponding to the protein component ; between 2500 and 3800cm^{-1} corresponding to -OH and -NH groups and hydrogen bonding; finally peaks at 2900cm^{-1} may be because of chitin (Brugnerotto *et al.,* 2001; Galichet *et al.,* 2001 and Padmavathy *et al.,* 2003). The peaks at 810, 586 and 537cm^{-1} may be traced to stretching and bending modes, respectively, of the phosphate moiety (Nakamoto, 1963).

The infrared spectra of the control and lead(II)-loaded samples in the case of live and dead biomass are shown in Fig. 7 (a,b,c and d) and their bands assignments are listed in Table 2. The infrared spectra of the dead and live biomass are compared with those of the free dead and live biomass in order to determine the sites of donation that may be involved in the interaction. There are some guide peaks in the spectra of the control which are of good help for achieving this goal. The position or the intensities of these peaks are expected to change upon of the interaction of biomass with lead(II), also new peaks are found in the spectra of lead(II)-loaded samples that gave an indication or chelating between oxygen- or nitrogen- sulphur- or phosphorus- containing ligands of biomass with metal ions.

The marked shift to a lower wave number at 3415 cm^{-1} for dead cells and at 3413cm^{-1} for live cells may be because of the interaction of -NH and -OH groups with lead(II). A small Shift of bands at 2928 cm^{-1} for dead and live cells was indicative of the interaction between –NH group of chitin present in yeast cell wall with lead(II). An increase in the wave number from 1647 to 1652 cm^{-1} for dead cells and a slight decrease in the wave number from 1644 to 1641cm^{-1} indicated the involvement of C=0 of amide groups in lead(II) uptake. The decrease in the wave number from 1550 to 1543 cm^{-1} in live cells only owed to the presence of protein amide II band (mainly C-N and N-H vibrations of the peptide bond in different protein conformations). Shifting of band at 1453 cm^{-1} in dead cells may be because of the involvement of various CH2/CH3 bending vibrations in lipids and proteins in lead(II) biosorption. In live cells, a peak shift to lower wave number (1409-1402 cm^{-1}) indicated that $C(CH_3)_2$ stretching mainly in proteins could be play a role in lead(II) biosorption. Still in protein region , peak shift in dead cells from 1396 to 1392 cm^{-1} pointed out that C=O of symmetric stretching in proteins was important in lead(II) biosorption. Only, in dead cells a peak shift from 1238 to 1235 cm^{-1} may be because of the involvement of PO^{-}_2 in DNA and RNA and phospholipids in lead(II) biosorption. In live cells and dead cells, a large peak shifts to lower wave

number were observed in sugar; 1062 to 1052cm^{-1} (dead cells) and 1072 to 1062cm^{-1} (live cells) indicated that both β(1 → 3) glucan (the major structural component of the cell wall (30-45% of the wall mass) and sulphur-oxygen compounds (sulfoxides, S=0) interact with lead(II). The disappearance of band at 925 cm^{-1} ,appearance of a new peak at 810cm^{-1} were due to P=S stretching . An appearance of new shoulder in FTIR of dead cells at 875 cm^{-1} after lead(II) uptake indicated the intervention of phosphorus and P=S stretching in the process . The disappearance of a peak at 586cm^{-1} and appearance of new peak at 537cm^{-1} (dead cells) and a small peak shifts 537 to 535 cm^{-1} (live cells) are indicative of C-S stretching, Pb-O and ring deformation in dead cells.

TABLE 2. Infrared frequencies (cm^{-1}) and tentative assignments for (A) Native dead cells (B) Dead treated cells (C) Native live cells (D) Live treated cells.

A	B	Shift	C	D	Shift	Assignment
3415w	3387vw1	28	3413w	3407w	6	ν (O-H)
2928vs	2927vs	1	2928w	2926m	2	ν (N-H)
2361m	2363w	2	2361m	2360s	1	
1647s	1652vs	5	1644v	1641s	3	ν (C=O) of amide groups
1536s	1536s	-	1550s	1543s	7	Amide II: N-H and C-N vibrations of the peptide bond in different protein conformations.
1453s	1451s	2				VariousCH_2/CH_3 bending vibrations in lipids and proteins.
			1409s	1402m	7	$C(CH_3)_2$ stretching in proteins.
1396m	1392m	4				C=O of COO^- symmetric stretching in proteins
1238s	1235s	3	1239m	1239m	-	PO_2^{2-} in DNA and RNA and phospholipids
1062m	1052s	10	1072m	1062m	10	β(1 → 3) glucan and S=O
925w	-	-	-	-	-	ν P=S
-	875sh					Mannans, phosphorus and ν P=S
-	810m					
586br	-		-	-	-	ν (Pb-O) + ring deformation, and ν C-S
-	537m		537br	535br	2	

s= strong; w= weak; sh= shoulder; v= very; br= broad; ν, stretching.

Metal ions adsorbed first to the cell surface by interaction with metal-functional groups such as carboxyl, phosphate, hydroxyl, amino, sulphur, sulphide, thiol, etc., and then penetrate the cell membrane and enter into the cells (Wang & Chen, 2006). Complexation, ion change adsorption in organic microprecpitation, oxidation and / or reduction have been proposed to explain the metal uptake process (Liu *et al.*, 2002), FTIR spectra of *S. cerevisiae* revealed that cell walls were the major sorption sites of uranium, and – O – H, C = O and PO^{2-} contributed to the major binding groups (Lin *et al.*, 2010). Limin *et al.* (2009) mentioned that COOH, C= O, C- O and N-H are the main active binding sites for the process of lead(II) adsorption by *S. cerevisaie.* The increase of nickel removal by dead cells of *S. cerevisiae* could likely be attributed to the exposition of further metal-binding sites present inside the cells (Machado *et al.*, 2008).

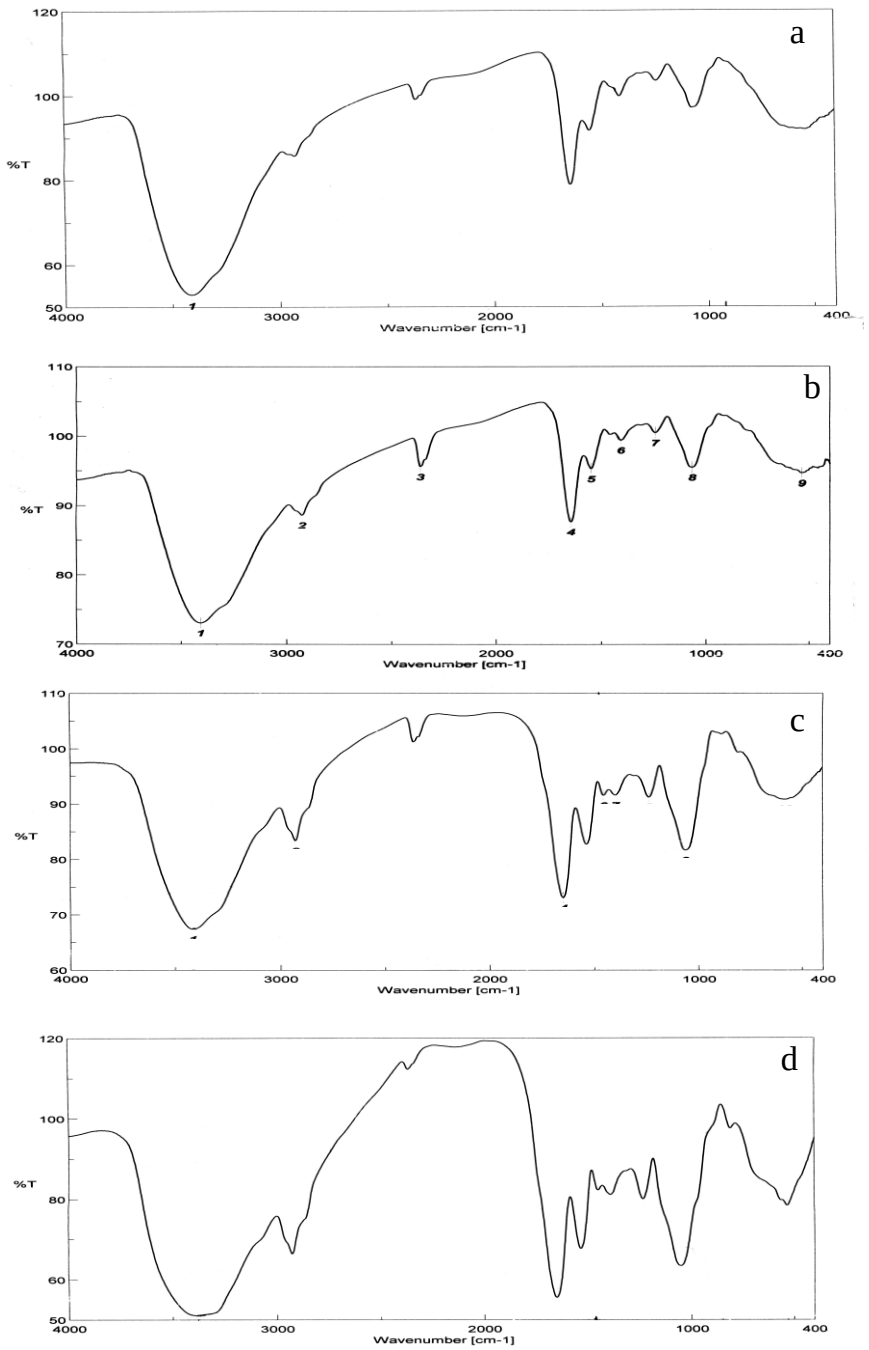

Fig. 7. FTIR spectrum of *S. cerevisiae* biomass (a) Native live, (b) Lead-loaded live, (c) Native dead, (d) Lead-loaded dead.

X- ray diffraction (XRD) analysis

To elucidate the chemical nature of yeast cell bound lead, test biomass was subjected to X- ray diffraction analysis before (control) and after lead sequestration (Fig. 8 a, b, c and d). In contrast to untreated control biomass, which is expectedly amorphous (Fig. 8 a), XRD spectrum for live and dead biomass loaded with lead showed distinct reproducible patterns typical for the presence of crystallized materials. Following lead accumulation, the XRD pattern of live yeast biomass (Fig. 8 b) showed 8 distinct peaks at 2θ; 20.70, 21.67, 26.53, 27.54, 30.30, 31.31, 41.19 and 44.08A^o and corresponding to respective d-spacing 4.29, 3.36, 2.95 and 2.19A^o. Based on spacing values, these peaks are attributed to the presence of crystalline lead sulphate compounds ($Pb_3O_2SO_4$ and Pb_2OSO_4) (Characteristic lines at 41.4, 52.1, 42.6, 41.7, 135, 47.8, 15.1 and 27.4 A^o). Lead loaded dead biomass (Fig. 8 c) showed 5 distinct peaks at 2θ; 21.40, 26.15, 27.71, 30.09 and 43.82 and corresponding to d-spacing 4.15, 3.40, 3.22, 2.97 and 2.06 A^o. Based on spacing values these peaks are attributed to the presence of crystalline lead sulphate compounds; $Pb(SO_4)$ and Pb_2OS_4 (characteristic lines at 29.8, 32, 30.2, 74.1 and 23.1A^o). FTIR spectroscopic analysis of the tested biomass also indicated involvement of cellular sulfur – oxygen compounds in lead binding as described above.

Energy dispersive X- Ray microanalysis (EDAX) was employed to estimate the elemental content of the biomass sample. X-ray peaks showed a broadening in the peak Full Wave Height Maximum (FWHM) which confirmed and proprtionaled with the size of pellets of the $PbSo_4$ compounds. Also the height of the peak proportional with the weight percent of the compound or its ingredients Pb, S and O. Figure 9 (a and b) represented EDAX spectra and concentrations of semi quantified results of lead – loaded live and dead biomass. This bulk technique gives an elemental ratio of the population as a whole and analyzing the whole pellet following metal exposure. EDAX spectrum of lead- loaded live biomass (Fig. 9 a) showed distinct peaks for: oxygen, sulfur and lead with element % 63.8, 1.48 and 34.72, respectively. Also, EDAX spectra of lead – loaded dead biomass (Fig. 9 b) showed distinct peaks for oxygen, sulfur and lead with element % 30.87, 1.85 and 67.48, respectively. EDAX show an excellent agreement with the corresponding XRD analysis which confirmed the presence of $Pb_3O_2SO_4$ and $PbOSO_4$ on live biomass and $Pb(SO_4)$ and Pb_2OSO_4 on dead biomass.

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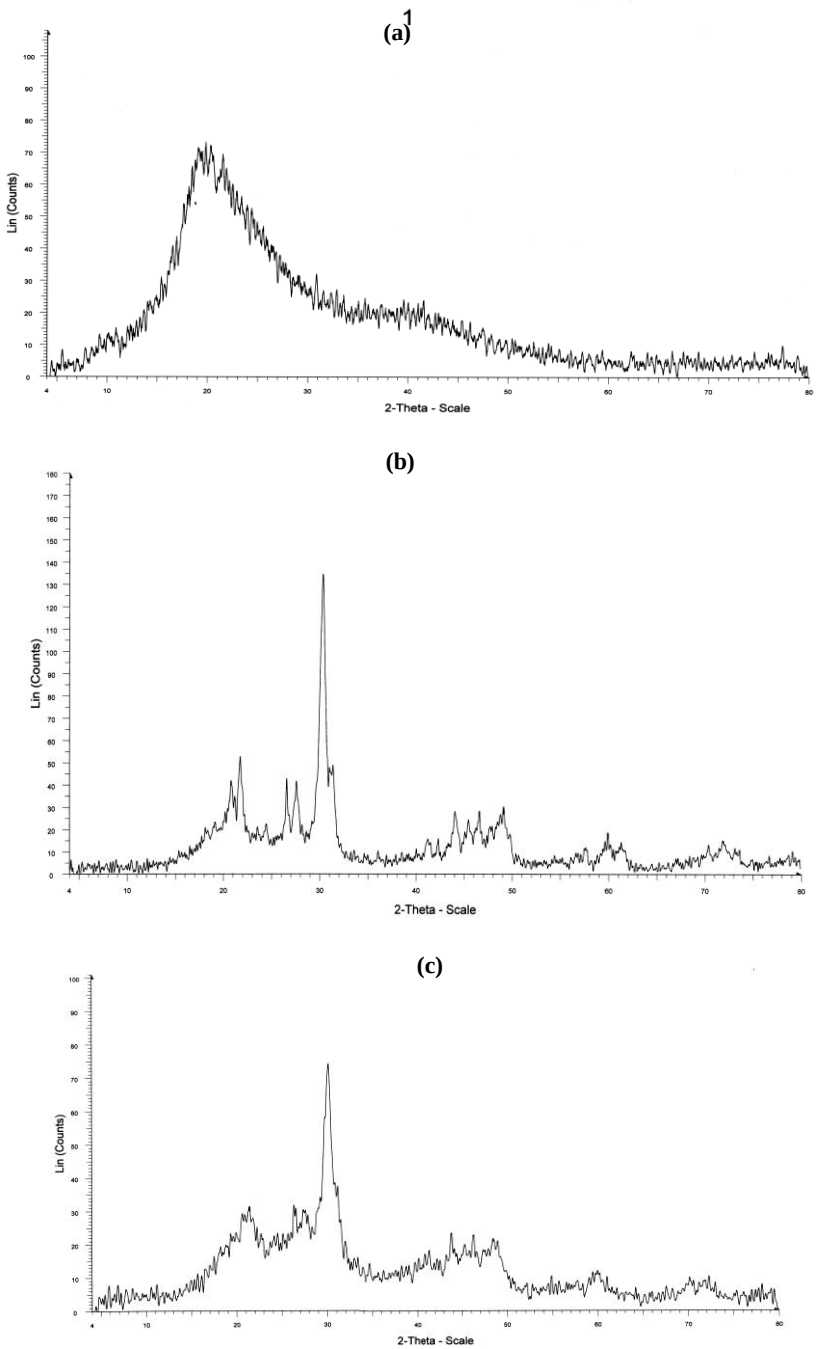

Fig. 8. XRD analysis of *S. cerevisiae* biomass (a) Native (b) Lead-loaded live, (c) Lead-loaded dead.

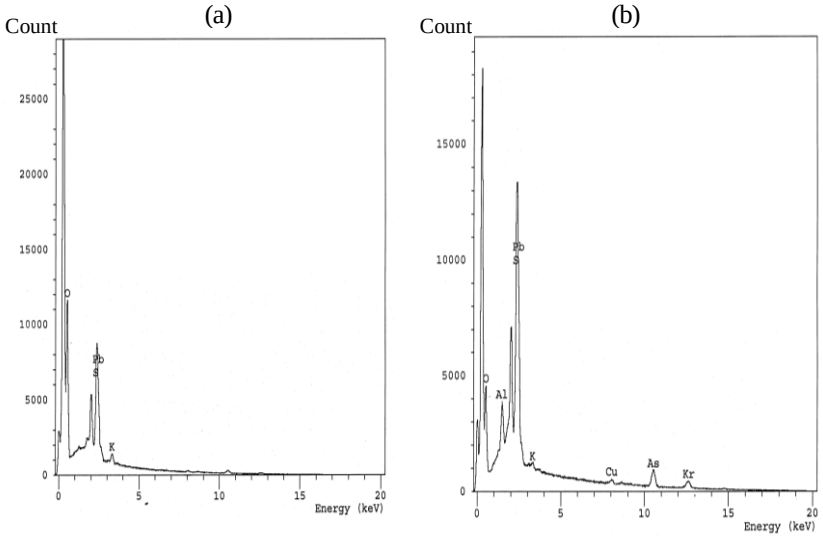

Fig. 9. EDAX analysis of *S. cerevisiae* biomass (a) Lead-loaded live, (b) Lead-loaded dead.

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ازالة عنصر الرصاص بإستخدام فطرو *سكارواميسس سيرفيسى*

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قسم النبات- كلية العلوم- جامعة الزقازيق .

تم دراسة ازالة عنصر الرصاص من محلول صناعى بإستخدام خلايا حيه وخلايا ميته من فطرة *سكارواميسس سيرفيسى*. اقل درجة تثبيط كانت 600ملليجرام/لتر. تم دراسة تأثير العوامل مثل تركيز ايون الهيدروجين وتركيز عنصر الرصاص وتركيز البيوماس والوقت على قدرة الفطره على امتصاص العنصر. تم استخدام FTIR, XRD,SEM, TEM لمعرفة آلية عملية الإمتصاص.تم تطبيق هذه القدره عمليا على مياه صرف مصنع بطاريات.