

Cadmium Biosorption from Wastewater by *Yarrowia lipolytica* AUMC 9256

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THE PRESENT study aimed to assess the biosorption capacity of live, autoclaved and dried biomass of *Yarrowia lipolytica* AUMC 9256 for cadmium (Cd(II)). Minimum inhibitory concentration 500mg/L proved great tolerance of *Y. lipolytica* AUMC 9256 to Cd(II). The time course growth in the presence of different concentrations of Cd(II) was studied. For live, autoclaved and dried biomass, maximum uptake capacities accomplished at pH 5.0, initial metal ion concentration 400mg/L, biomass dosage 1g/L, and contact time 20min for autoclaved and dried biomass and 240min for live cells. The potential of *Y. lipolytica* to produce cadmium nanoparticles was determined by UV-Visible spectroscopy measurements and transmission electron microscopy examinations. Moreover, to identify the possible mechanisms of Cd(II) uptake, TEM examinations, Fourier Transform Infrared spectroscopy, Energy dispersive X-ray microanalysis, and X-ray powder diffraction analyses were carried out. The potential implementation of dried *Y. lipolytica* biosorbent for heavy metal removal from different water samples was successfully accomplished using the multistage microcolumn technique. Accordingly, *Y. lipolytica* AUMC 9256 can be considered as a very promising potential to bioremediate Cd(II) ions and biosynthesis of Cd NPs.

Keywords: *Yarrowia lipolytica* AUMC 9256, Cadmium, Biosorption, Mechanism, Nanoparticles.

Introduction

Heavy metals constitute dangerous environmental pollutants due to their high toxicity and tendency to bioaccumulate in the food chain. Cd(II) is nonessential and non-biodegradable and listed as a human carcinogen heavy metal with the half-life of more than 20 years (Vinodini et al., 2015). Moreover, the serious form of Cd(II) toxicity in humans is "Itai-itai" bone softening and fractures in humans (Wang & Du, 2013). Cd(II) released to the environment in numerous ways: Firstly, natural activities (e.g., volcanic explosions and geological weathering); secondly, human being activities like mining, electroplating, smoking, smelting, steel industries, incineration of municipal waste (especially nickel-cadmium (Ni-Cd) rechargeable batteries and plastics), and production of solar cells, pigments, and phosphate fertilizers and thirdly, remobilization of historical sources such as the contamination of water courses by drainage water from mines of metals (WHO, 2010). According to the findings of the British Geological Survey, the total worldwide

production of Cd(II) in 2015 was approximately 24,900 metric tons (Brown et al., 2017).

Traditional physicochemical methods for the removal of Cd(II) are usually expensive, time wasteful, useless at low metal concentrations of 1-100mg/L and generate secondary waste that adds to the environmental problems (Joshi, 2017). Therefore, the bioremoval of heavy metals by biosorption or bioaccumulation processes is a potentially attractive technique because of decreasing the heavy metal concentration in solution from ppm to ppb level, time and environmentally friendly strategy, and reusability of biomass (Abbas et al., 2014). In the conception of biosorption, many physicochemical processes may be involved, such as adsorption, absorption, ion exchange, coordination, surface complexation, chelation, microprecipitation and crystallization (Joshi, 2017).

Because of the wide applicability of *Yarrowia lipolytica* (e.g., enzyme production, bioremediation, and biotransformation), a large

quantity of biomass has been produced (Bankar et al., 2012, 2018; Bendigiri et al., 2018 and Katre et al., 2018). Moreover, it has inbuilt mechanisms for detoxifying heavy metals (Zinjarde et al., 2014). Accordingly, the reductive mechanism and formation of nanoparticles are among these mechanisms (Seshadri et al., 2011 and Apte et al., 2013).

For the best of my knowledge, few publications on the utilization of *Y. lipolytica* biomass in the removal of Cd(II) ions and synthesis of Cd NPs are found.

The primary goal of this study was to: (i) Study the growth pattern of *Y. lipolytica* AUMC 9256 in the presence of different concentrations of Cd(II), (ii) Determine the biosorption potential of live, autoclaved and dried pellets of *Y. lipolytica* AUMC 9256 for Cd(II) under different parameters, (iii) Characterize the mycosynthesized Cd NPs and (vi) Determine the possible mechanisms of Cd(II) uptake by *Y. lipolytica* AUMC 9256.

Materials and Methods

Preparation of Yarrowia lipolytica AUMC 9256 biomass

Y. lipolytica AUMC 9256 was kindly provided from Assuit University Mycological Centre was preserved on YPD agar slants containing (g/L): Glucose, 20; yeast extract, 10; peptone, 20 and agar, 15. Slants were inoculated and incubated at 30°C for 48h and stored ordinarily at 4°C. Live, autoclaved and dried biomasses were prepared according to Huang et al. (2013). Accordingly, the yeast strain was cultured in 500ml Erlenmeyer flasks containing 100ml of yeast-extract-peptone (YPD) broth medium for 48h at 30°C and 120rpm. After the centrifugation at 6000rpm for 10min at 4°C, the yeast pellets were rinsed thrice with deionized water. To ensure equal weights in three biomasses, the dry weights were determined after drying at 60°C till constant weight (Suh et al., 1998).

Preparation of metal solution

Cd(II) solution at the concentration of 2000mg/L was prepared by dissolving CdSO₄.2H₂O in deionized water. Series of metal ion concentrations were accomplished by subsequent diluting the solutions with deionized water.

Determination of minimum inhibitory concentration (MIC)

A hundred microliters of yeast suspension (1×10⁶ CFU/ml) was inoculated onto YPD plates supplied with the sterilized CdSO₄.2H₂O solution to get the final concentration of 0 to 700mg/L. MIC was noted when the strain fails to grow on plates even after 7 days of incubation.

Effect of different concentrations of Cd(II)

Aliquots were obtained from the log growth phase and were inoculated in 250 Erlenmeyer flasks containing 50ml YNB-glucose broth medium supplemented with cadmium sulfate solution in different concentrations ranging from 0 to 280mg/L. Initial pH was achieved to 5.5 with 0.1N HCl and 0.1N NaOH. YNB-glucose contained (in g/L): Glucose, 20 and yeast nitrogen base without amino acids, 6.7. YNB-glucose was used to prevent the interaction of cadmium sulfate with ingredients of the media (Paš et al., 2004). Positive and negative controls were considered. Samples were withdrawn every 8h and observed as turbidity by optical density at 600nm. All the experiments were carried out in triplicates and for all graphical illustrations, standard error bars were plotted.

To evaluate the removal efficiency (R_e%), *Y. lipolytica* AUMC 9256 growth suspension was centrifuged and the supernatant was tested for the remaining Cd(II) by an atomic absorption spectrophotometer ((Model Unicam 969, Centric Laboratory of Agriculture Faculty, Zagazig University). R_e% was estimated according to the following equation:

$$R_e (\%) = \frac{C_i - C_e}{C_i} \times 100 \quad (\text{Huang et al., 2013})$$

where C_i and C_e are the initial and final Cd(II) concentrations (mg/L), respectively.

Elemental K(I) and Ca(II) analysis

Analysis of K(I) and Ca(II) contents of the supernatant of metal-unloaded and Cd(II)-loaded biomass sorption solutions was carried out by atomic absorption spectrophotometer (Bairagi et al., 2011).

Cellular distribution of Cd(II)

The localization of bioaccumulated Cd(II) was established according to Huang et al. (2013). Accordingly, the Cd(II)-loaded pellets were vortexed in deionized water for 10min.

After centrifugation, Cd(II) in this supernatant (represent the loosely bounded Cd(II) to the cell surface) was determined, then the pellets were agitated with 100mM EDTA (tetrasodium salt dehydrate) for 30min to remove Cd(II) that tightly bounded to cell surface. The cell pellets were subjected to HNO₃ digestion in a microwave oven to evaluate the extent of intracellular Cd(II) (Huang et al. 2013).

Batch biosorption experiments

The batch biosorption experiments were conducted in 500ml Erlenmeyer flasks with 100ml of metal solution at desirable pH (2-6), initial metal ion concentration (50-500mg/L), biosorbent mass (1-5g/L) and contact time (10min- 24h). Initial pH of metal solutions was adjusted to the values 2, 3, 4, 5 and 6 with 1N NaOH and 0.1N HCl before mixing biomass. The biomass dosage (with respect to cell dry weight) was subsequently added at 30°C and 120rpm. After centrifugation, residual Cd(II) ion concentration in the supernatant was measured by using an atomic absorption spectrophotometer. All experiments were done in triplicates. The biosorption capacity (q_{eq}) was calculated from the following equation:

$$q_{eq} = C_i - C_{eq} / m \times V \quad (\text{Bairagi et al., 2011})$$

where q_{eq} is metal uptake capacity (mg of metal/g of biosorbent), C_i and C_{eq} are the initial and equilibrium Cd(II) concentration (mg/L), respectively, m is the biosorbent mass (g dry cell/L) and V is the volume of liquid sample.

Effect of pH on desorption of Cd(II)

Live, autoclaved and dried Cd(II)-loaded pellets obtained from earlier biosorption experiments were washed, resuspended in deionized H₂O and pH value were then adjusted to 1.0 -7.0 with 0.1N HCl and 0.1N NaOH. After 24h gentle agitation, the pellets were harvested by centrifugation (6000rpm, 20min) and the metal ions released into the supernatant were detected with atomic absorption spectrophotometer (Lu et al., 2006). The desorption efficiency (%) was calculated with the following equation:

$$\text{Desorption efficiency (\%)} = \frac{D_r}{D_a} \times 100 \quad (\text{Li et al., 2010})$$

where D_r is the amount of Cd(II) released in the supernatant (mg) and D_a depicts the amount of Cd(II) initially adsorbed on the biosorbent (mg).

Characterization of Cd NPs synthesized by Y. lipolytica AUMC 9256

Y. lipolytica AUMC 9256 biomass (1g/L with respect to cell dry weight) were suspended separately in 1000ml of 400mg/L concentration of Cd(II) under optimum conditions (pH 5.0 and 1g biomass/L). Following centrifugation, the supernatants were then refined through 0.22µm filter to get them cell-free supernatant (CFS). The mycosynthesized Cd NPs were collected by ultracentrifugation, washed thrice and redispersed in deionized water. They were subjected to UV-Visible spectroscopy measurements (T80 UV-Vis spectrophotometer, Germany) and transmission electron microscopy (TEM) investigations (a JEOL TEM -1400 electron microscope) to characterize Cd NPs. The pellets were washed thrice with a generous amount of deionized water to remove any remains of media.

Mechanism of Cd(II) uptake

A portion of the obtained pellets before and after Cd(II) uptake was examined by TEM (JEOL TEM -1400 electron microscope at Regional Center of Mycology and Biotechnology, Cairo, Egypt) to realize the cellular localization of accumulated metal ions and the uptake mechanism. To recognize the functional groups included in the uptake (Iqbal et al., 2009), FTIR of metal-free, live, autoclaved and dried Cd(II)-loaded cells were recorded over the region 400-4000cm⁻¹ with Perkin-Elmer FTIR 1650 spectrophotometer (Center of Microanalysis, Cairo University, Cairo, Egypt). The biomass was examined in KBr discs containing 3% (w/w) of finely ground powder of each sample. The elemental analysis of metal-free and Cd(II)-loaded pellets was carried out by X-ray microanalyzer (EDX) (Model Oxford 6587 INCA x-sight) attached to JEOL JSM-5500 LV scanning electron microscope at Regional Center of Mycology and Biotechnology, Cairo, Egypt. XRD patterns of powder biomass were recorded in a Broker D8 Advanced target Cuka powder diffractometer ($\lambda = 1.5418 \text{ \AA}$) over the area of 0-80(2θ) (Central Metallurgical & Development Institute, Helwan, Egypt).

Application of dried Y. lipolytica AUMC 9256 biosorbent to environmental samples

Temperature, pH and dissolved Cd(II) (mg/L) of wastewater (of a ceramic factory in Industrial area of Tenth of Ramadan, Sharkia, Egypt) were

measured. Adsorptive removal of heavy metals from water samples was performed by passing 1L solution through a microcolumn with length, 10cm, and internal diameter, 0.5cm packed with 1g of dried *Y. lipolytica* biomass according to Mahmoud et al. (2017).

Results

Cd(II) tolerance by Y. lipolytica AUMC 9256

The strain was tolerant to Cd(II) with the MIC of 500mg/L. No changes in the color of the colonies were seen.

Growth pattern of Y. lipolytica AUMC 9256 and Cd(II) toxicity

The growth of *Y. lipolytica* AUMC 9256

in the broth supplemented with 20mg/L Cd(II) was relatively similar to that of the control (Fig. 1). Meanwhile at 280mg Cd(II)/L, a complete inhibition of the yeast growth was observed.

Figure (2) showed that at low Cd(II) concentrations (20 and 50mg/L), values of $R_e\%$ were found to be 55.5 and 50.6%, respectively. A gradual increase in Cd(II) concentration was accompanied by a gradual drop in $R_e\%$ and reached its minimum value (17.7%) at 250mg Cd(II)/L.

The release of K(I) and Ca(II)

The amounts of K(I) and Ca(II) increased with an increase in Cd(II) concentration (up to 250mg/L) (Table 1).

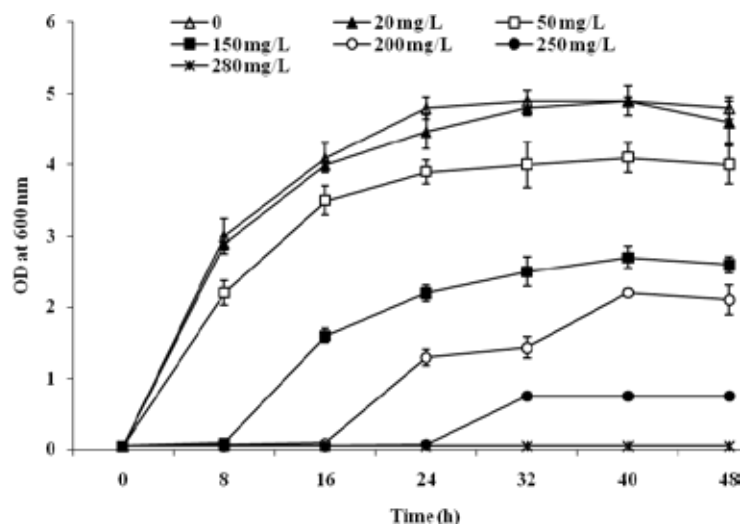


Fig. 1. Growth pattern of *Y. lipolytica* AUMC 9256 in the absence and presence of different initial concentrations of Cd(II) at 30 °C, 150 rpm and initial pH 5.5

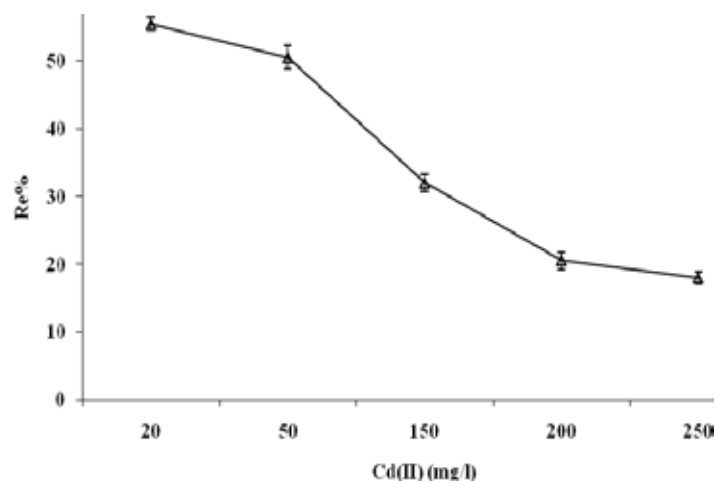


Fig. 2 Removal efficiency ($R_e\%$) of *Y. lipolytica* AUMC 9256 at different initial concentrations of Cd(II) in liquid media.

TABLE 1. Release of K(I) and Ca(II) ions as a result of Cd(II) uptake by *Y. lipolytica* AUMC 9256.

Concentration of Cd(II)	Release of Ca(II) (mg/l)	Release of K(I) (mg/L)
0	0.035	0.057
20	0.073	0.099
50	0.551	.192
150	0.753	0.253
200	0.834	0.401
250	0.901	0.580

Cellular distribution of bioaccumulated Cd(II)

With an increment in Cd(II) concentrations from 20mg/L to 250mg/L, EDTA washable fraction (tightly cell surface bounded Cd(II)) declined from 24.3 to 8.0% while EDTA non washable fraction (intracellular bounded Cd(II)) raised from 72.5 to 85.0% (Fig. 3).

*Biosorption of Cd(II)**Effect of initial pH*

The uptake of Cd(II) by live, autoclaved and dried biomass of *Y. lipolytica* AUMC 9256 has been found to increase with an increase in pH and reach its upper value at pH 5.0 (351.83, 389.11, and 445.53mg/g for live, autoclaved and dried biomass, respectively) (Fig. 4). The following biosorption experiments were conducted at pH 5.0. The Cd(II) biosorption capacity of dried cells was higher than that of autoclaved and live cells.

Effect of initial metal ion concentration

Figure 5 demonstrated that the uptake capacity was increased from 56.67 to 340.00 (mg/g) (live),

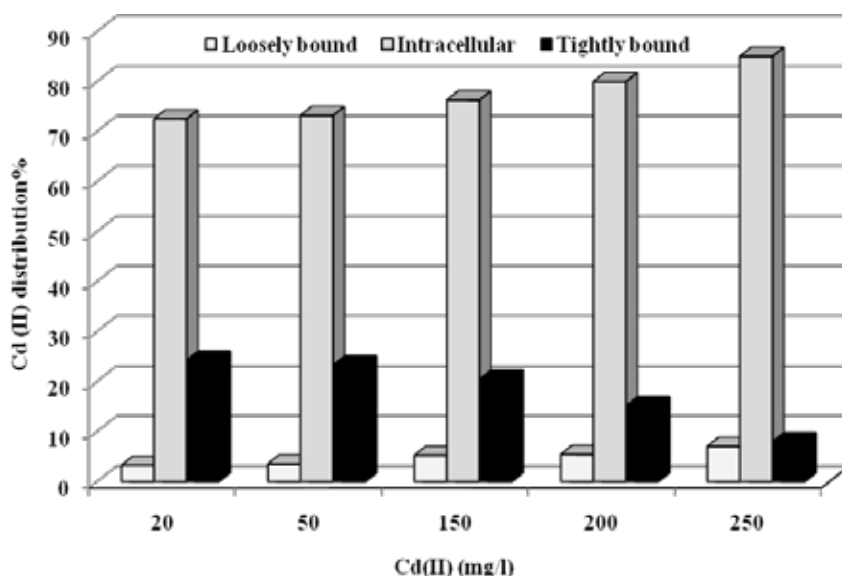
from 65.74 to 400.43 (mg/g) (autoclaved) and from 73.37 to 491.24 (mg/g) (dried) with a rise in Cd(II) concentration from 50 to 400mg/L.

Effect of biosorbent dose

An increase in the biomass concentration from 1.0 to 3.0g/L led to a decline in the uptake capacity from 330 to 90.9mg/g (live), from 400 to 140.1mg/g (autoclaved) and from 490 to 170.9mg/g (dried) (Fig. 6). This decline in the uptake capacity becomes less with the further rise in biomass concentration from 4.0 to 5.0g/L.

Effect of contact time

The rate of Cd(II) biosorption by dried cells and autoclaved cells was rapid and reached the upper value within 20min. After that no further increment in adsorption capacity was noticed, meaning that the metal adsorption reached its equilibrium level. In contrast, the rate of biosorption by live cells was slow and reached its maximum value after 120min (Fig. 7).

**Fig. 3 . Cd(II) distribution percentage in different cellular compartments of *Y. lipolytica* AUMC 9256**

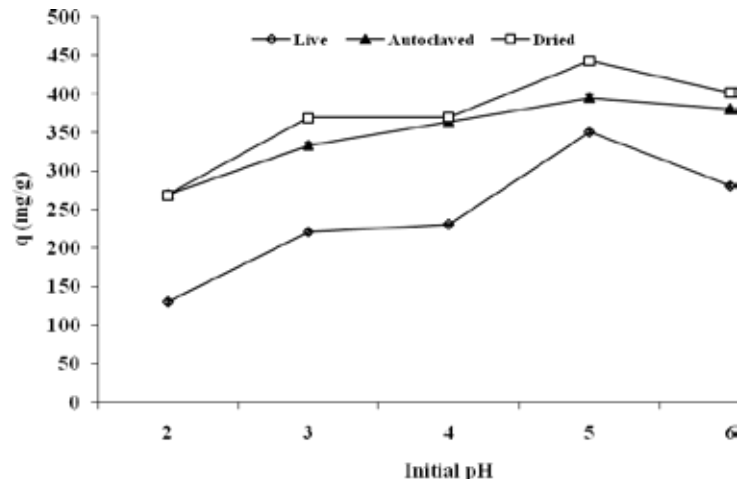


Fig. 4. Effect of initial pH on Cd(II) uptake capacity of live, autoclaved and dried biomass of *Y. lipolytica* AUMC 9256 [Biosorption conditions: Initial metal ion concentration (C_i)= 400mg/L; biosorbent dose (m)= 1g/L; contact time (t)= 20min (for autoclaved and dried biomass) and 2h (for live biomass)].

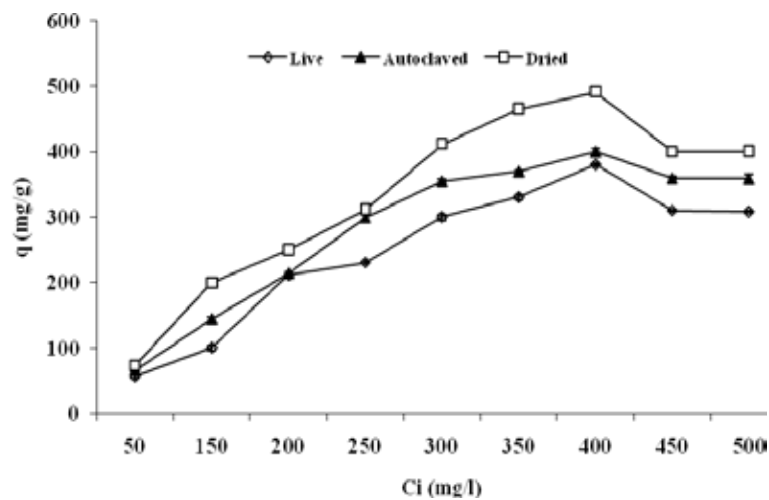


Fig. 5. Effect of initial metal ion concentration (C_i) of Cd(II) on uptake capacity of live, autoclaved and dried biomass of *Y. lipolytica* AUMC 9256 [Biosorption conditions: Initial pH= 5.0; m = 1g/L; t = 20min (for autoclaved and dried biomass) and 2h (for live biomass)].

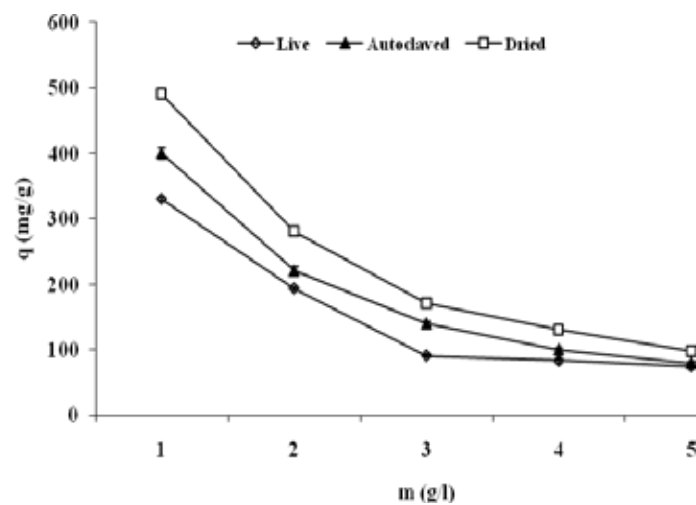


Fig. 6. Effect of biosorbent dose (m) on Cd(II) uptake capacity of live, autoclaved and dried biomass of *Y. lipolytica* AUMC 9256 [Biosorption conditions: Initial pH= 5.0; m = 1g/L; t = 20min (for autoclaved and dried biomass) and 2h (for live biomass)].

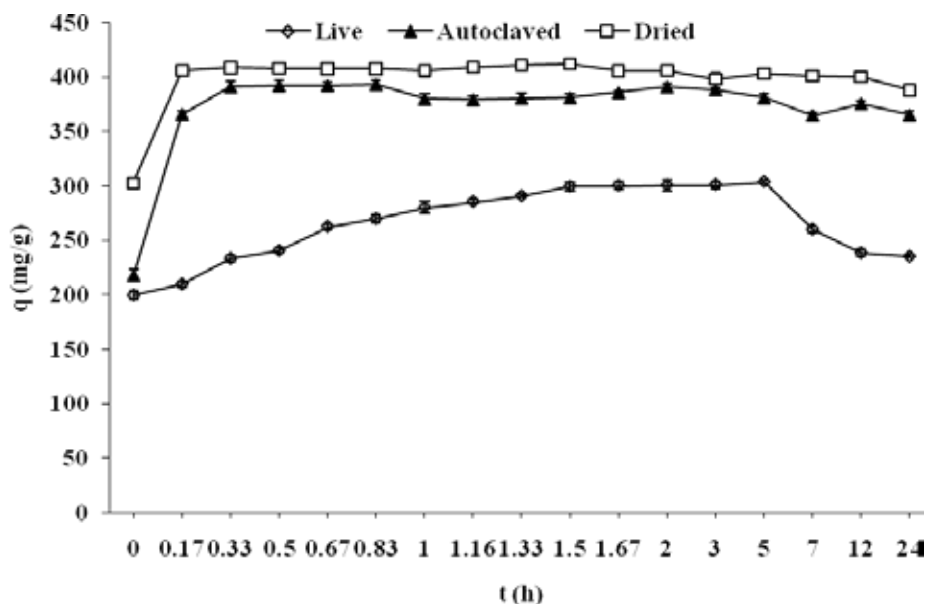


Fig. 7. Effect of contact time (t) on Cd(II) uptake capacity of live, autoclaved and dried biomass of *Y. lipolytica* AUMC 9256 [Biosorption conditions: Initial pH= 5.0; C_i = 400 mg/L; m = 1g/L].

Desorption efficiency

As shown in Fig. 8, the desorption efficiency decreased with increasing pH value. The highest desorption efficiency was found to be 60.1%, 51.4% and 31.2% at pH 1.0 for autoclaved, dried and live biomass, respectively.

supernatants (CFS) were recorded at λ_{max} 378, 418, and 414nm for living, autoclaved and dried biomass, respectively (Fig. 9 a-c).

Characterization of Cd NPs synthesized by Y. lipolytica AUMC 9256

The UV-Visible spectra of cell free

TEM investigation of CFS of Cd(II)-loaded biomass revealed the formation of spherical Cd NPs with sizes ranging from 34 to 87nm (live) and aggregates of irregular Cd NPs (autoclaved and dried) were observed (Fig. 10 a-c, respectively).

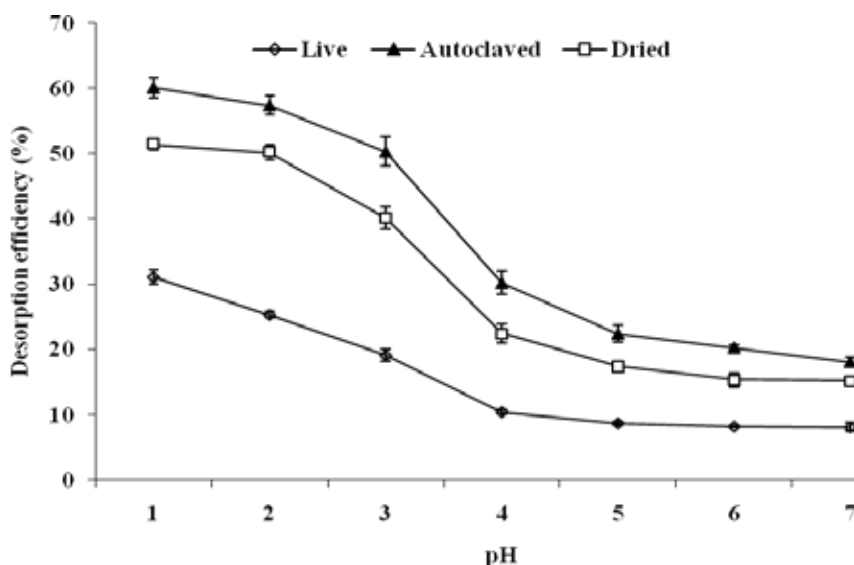


Fig. 8. Effect of pH on desorption efficiency (%) of Cd(II) of live, autoclaved and dried biomass of *Y. lipolytica* AUMC 9256.

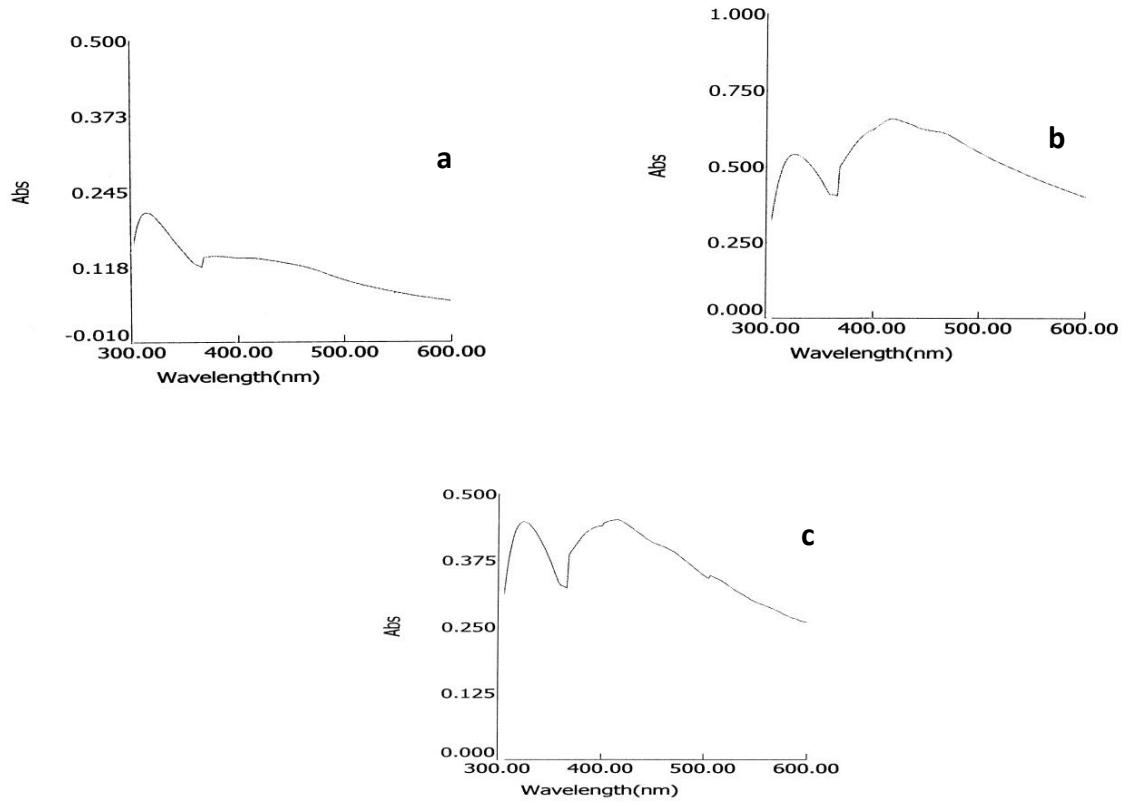


Fig. 9 . UV-visible spectra of cell free supernatant (CFS) of *Y. lipolytica* AUMC 9256 (a) Live, (b) Autoclaved and (c) Dried biomass after Cd(II) uptake.

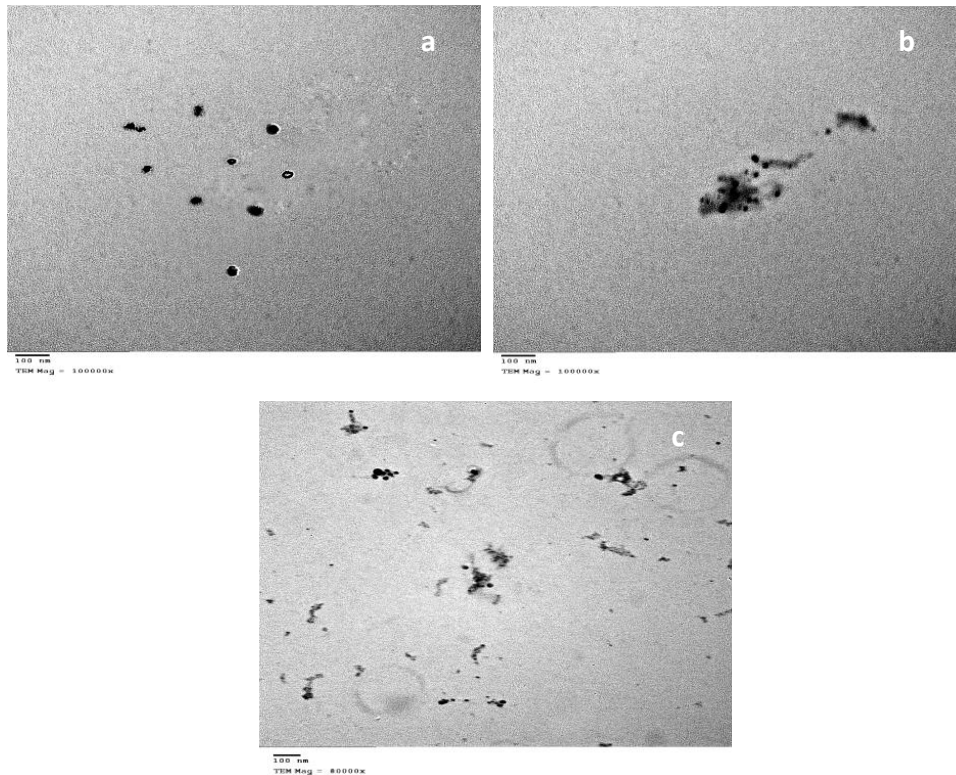


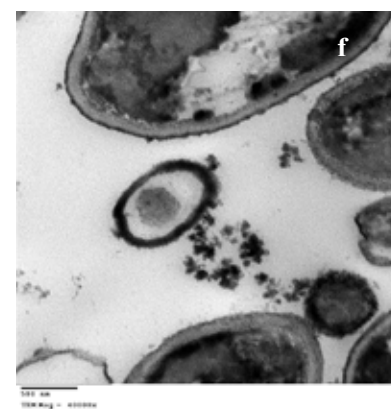
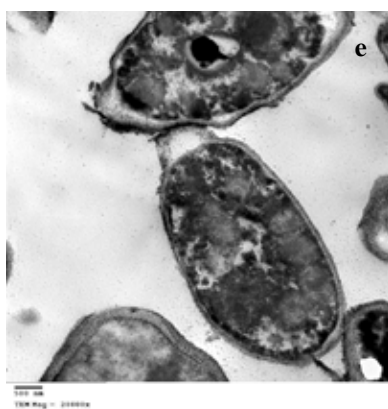
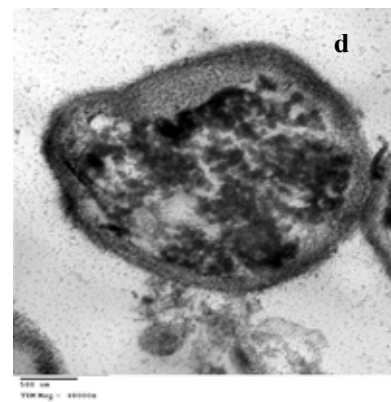
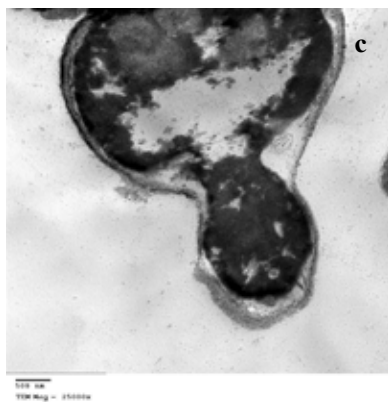
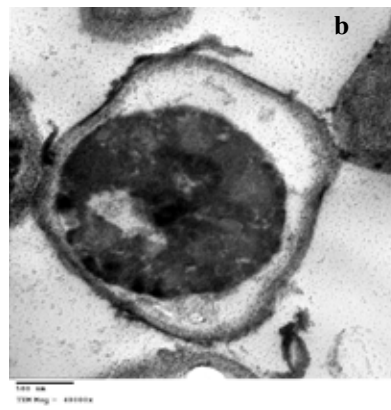
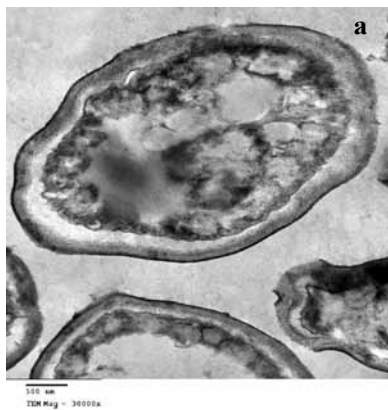
Fig. 10. TEM micrographs of Cd NPs synthesized by (a) Live biomass, (b) Autoclaved biomass and (c) Dried biomass of *Y. lipolytica* AUMC 9256.

Mechanism of Cd(II) removal

Transmission electron microscopy (TEM)

TEM investigations of native live cells showed a regular smooth cell wall, plasma membrane and clear cytoplasm (Fig. 11 a). In Cd(II) loaded-live cells, the blackening and stripping of the cell wall and wide irregular periplasm were observed. Electron-dense deposits were evident within cell wall microfibrils, periplasm and stained the cellular contents (Fig. 11 b, c). Internal cellular damage

with extrusion of some internal contents (Fig. 11 d) can be observed. Vacuolar sequestration to decrease the cytosolic Cd(II) concentration can be also observed (Fig. 11 e). From TEM micrographs of Cd(II)-loaded autoclaved biomass (Fig. 11 f, g) and Cd(II)-loaded dried biomass (Fig. 11 i, j), it can be noticed that extracellular precipitation and capture of Cd(II) within the cell wall microfibrils, respectively, were more apparent than other mechanisms.



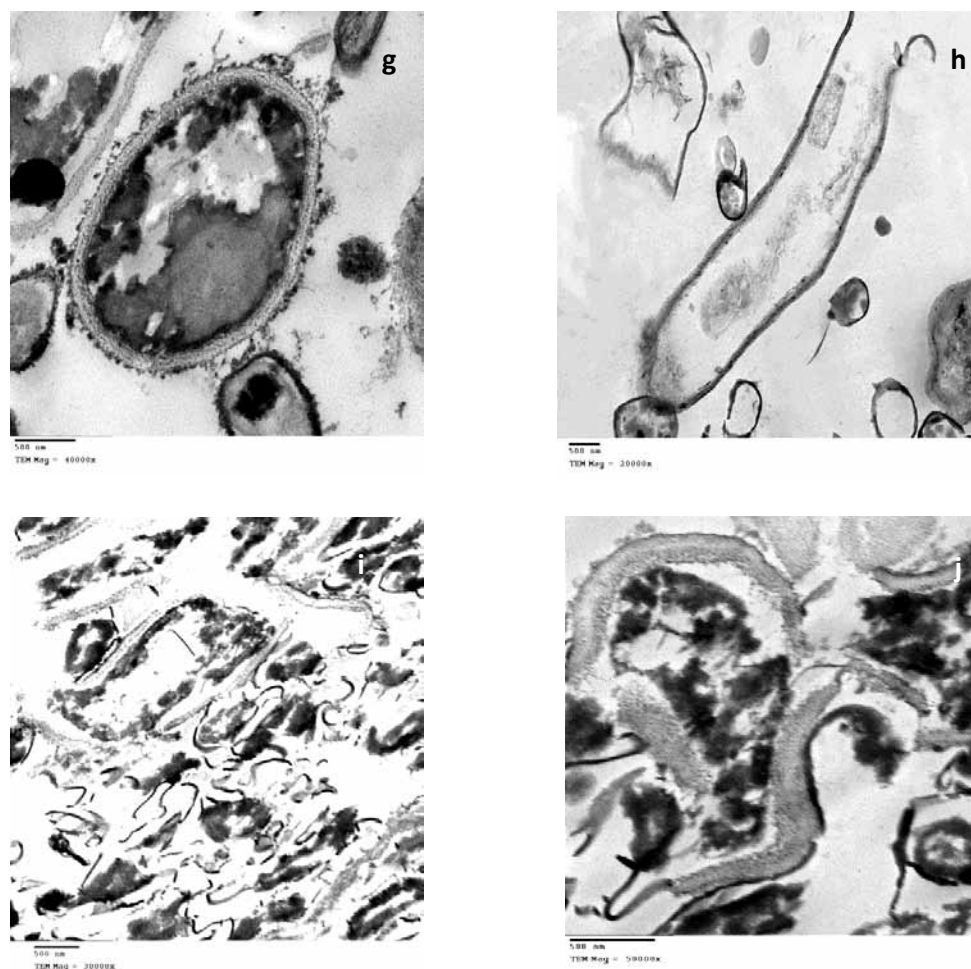


Fig. 11. TEM micrographs of *Y. lipolytica* AUMC 9256 biomass (a) Native live biomass, (b-e) Cd(II)-loaded live, (f and g) Cd(II)-loaded autoclaved, (h) Native dried, (i and j) and Cd(II)- loaded dried biomass.

FTIR analysis

FTIR spectra of native and Cd(II)-loaded biomass were shown in Fig. 12 a-d. The marked shift at 3421cm^{-1} (live, autoclaved and dried (Fig. 12 b-d, respectively) and the appearance of new peaks at 3743cm^{-1} (autoclaved) and 3742cm^{-1} (dried), suggesting the interaction of alcoholic OH groups and NH_2^- asymmetric stretching mode of primary amines with Cd(II) removal. A slight shift at 2927cm^{-1} , appearance of new peak at wave number 2859cm^{-1} (autoclaved) and new shoulder at 2870cm^{-1} (dried) can be assigned to alkyl C-H stretching and O-H of carboxylic acids and their derivatives and asymmetric and symmetric stretching vibrations of $-\text{CH}_2$ of acyl chains. The shift at the wave number 2362cm^{-1} (dried) can be attributed to NH and P-H stretching. The complete disappearance of bands at 2138cm^{-1} indicated the role of symmetric P-O-H stretching and $\text{C}\equiv\text{C}$ stretching. The shift

at wave number 1649cm^{-1} assigned to the role played by alkene $\text{C}=\text{C}$ stretch and $-\text{CO}$ stretching mode conjugated to NH^- deformation mode and amide I in Cd(II) biosorption. A change in wave number 1543cm^{-1} is due to the presence of amide II band (mainly N-H vibrations of peptide bonds in different protein conformations), aromatic $\text{C}=\text{C}$, C-H bending or $\text{N}=\text{O}$ stretching. A marked shift at 1404cm^{-1} was more evident in dried ($\Delta 44\text{cm}^{-1}$) and autoclaved ($\Delta 48\text{cm}^{-1}$) biomass than in live biomass ($\Delta 25\text{cm}^{-1}$) and specified the role played by C-O-H in-plane bending of carboxylic acids, C-N stretching of amide III band and $-\text{CH}_2$ & CH_3 deformation. The small shift (live), and appearance of new shoulder (autoclaved and dried) at wave number 1245cm^{-1} assigned to PO_2^{2-} in DNA and RNA and phospholipids, C-N stretching of amines, and C-O stretching. The appearance of new shoulders at 1327cm^{-1} and 1372cm^{-1} (live) was suggestive of the involvement of sulfonyl,

sulfonamide groups and amide III in Cd(II) uptake. The marked shift at 1074cm^{-1} in Cd(II)-loaded dried biomass ($\Delta 39\text{cm}^{-1}$) may be due to the interaction of β (1 \rightarrow 3) glucan, carboxylic groups, S=O stretching, and PH_2 deformation in Cd(II) uptake. The role of phosphorus, P=S stretching, S-OR esters, C-S stretching and C-Cl stretching in Cd(II) biosorption was very prominent in either complete absence of bands at 887cm^{-1} or very marked shift at 607cm^{-1} ($\Delta 59\text{cm}^{-1}$) (autoclaved).

The appearance of five new shoulders at 957, 630, 521, 466 and 435cm^{-1} (dried) and at 565cm^{-1} (autoclaved) indicated the role of C-S stretching and P=S stretching, M-O, O-M-O (M=metal ion) and ring deformation in the process. It is known that Cd(II) belongs to the so-called Pearson's soft acids, which have a susceptibility to bind with soft polarized nitrogen and sulfur-containing ligands (Javanbakht et al., 2014).

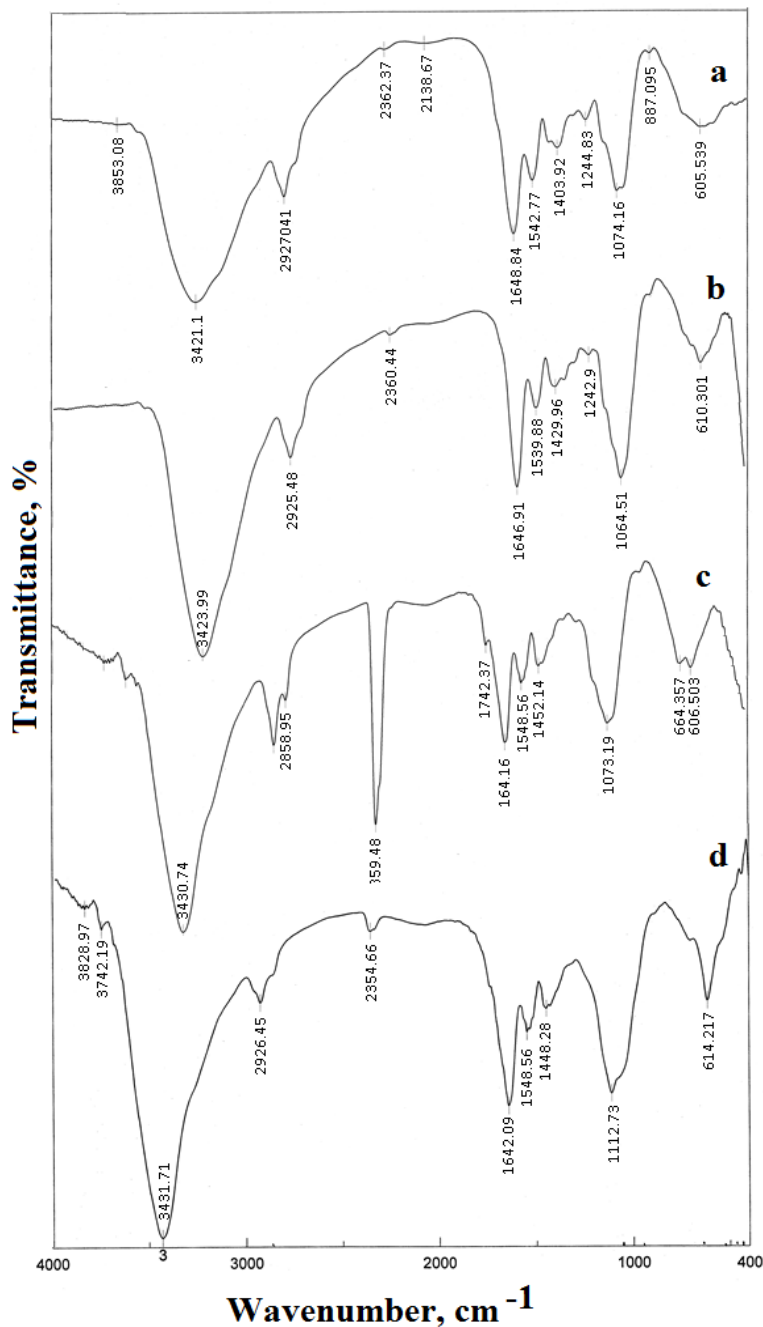


Fig. 12. FTIR spectra of *Y. lipolytica* AUMC 9256 biomass (a) Native biomass, (b) Cd(II)-loaded live, (c) Cd(II)-loaded autoclaved and (d) Cd(II)-loaded dried biomass.

Energy dispersive X-ray (EDX) microanalysis

EDX spectrum of the native biomass of *Y. lipolytica* AUMC 9256 showed peaks for potassium, phosphorus, sulfur, and zinc with element % 41.69, 33.84, 18.7 and 5.76, respectively (Fig. 13 a). Potassium ions are an important constituent of the membrane and the cell wall. EDX spectra of Cd(II)-loaded live, autoclaved, and dried biomass (Fig. 13 b-d,

respectively) clarified distinct peaks for Cd(II), phosphorus and sulfur with element % 66.40, 14.43, 15.81, 79.06, 1.05, 19.89 and 77.69, 2.45, 19.76, respectively. The atomic% of sulfur was increased from 16.85 % (control) to 27.27% (live), 45.69% (dried), and 41.65% (autoclaved). There was a sharp decrease in atomic % of phosphorus (from 33.84% (control) to 24.13% (live), 10.76% (dried) and 2.50% (autoclaved)).

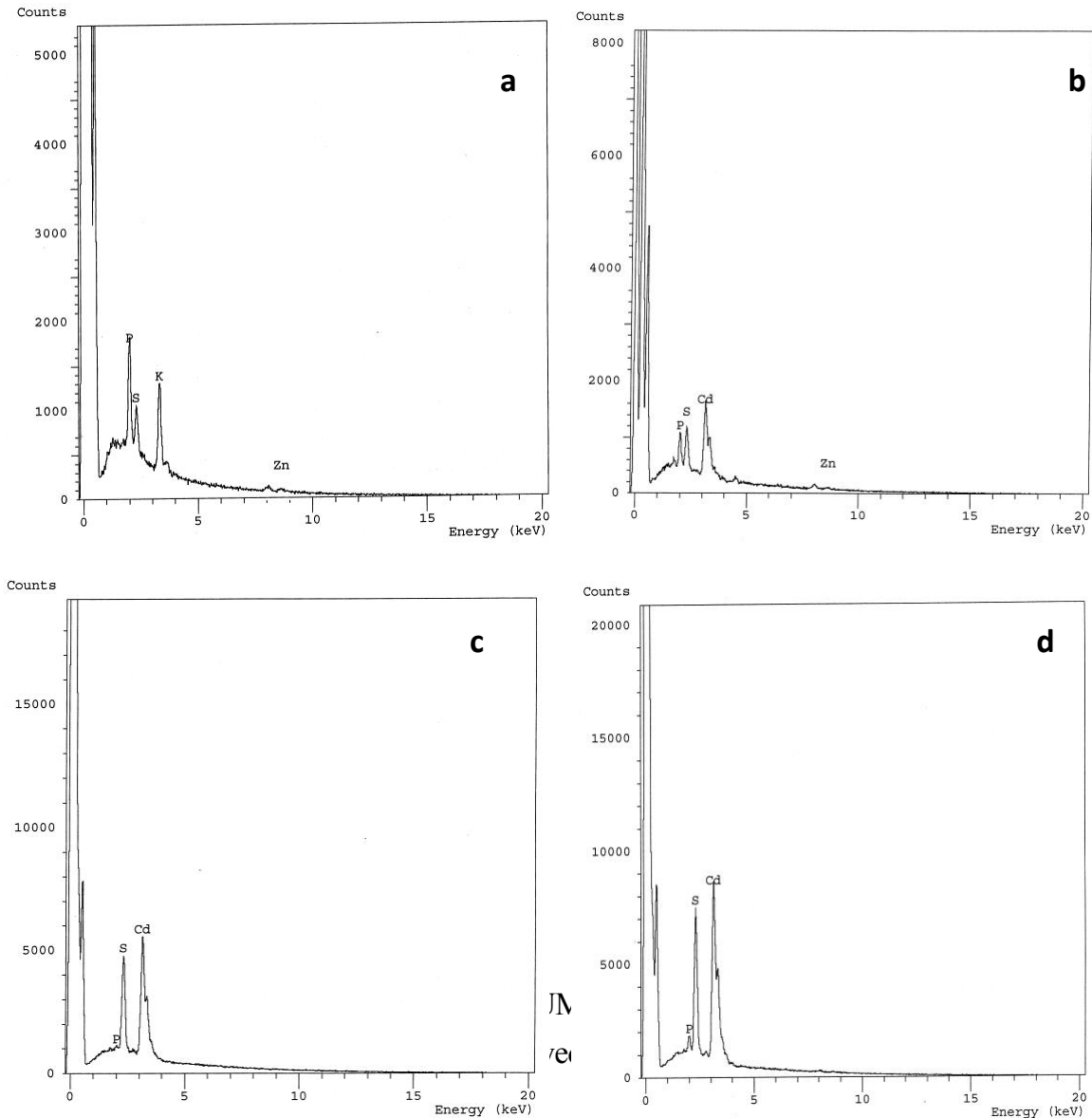


Fig. 13 EDX analysis of *Y. lipolytica* AUMC 9256 biomass (a) Native biomass, (b) Cd(II)- loaded live (c), Cd(II)- loaded autoclaved and (d) Cd(II)-loaded dried biomass.

X-ray diffraction (XRD) analysis

XRD is primarily used to distinguish between amorphous and crystalline materials (Fig. 13 a-d). The control biomass was amorphous (Fig. 14 a). Conversely, XRD spectra of loaded biomass showed distinct reproducible patterns (especially in live-loaded biomass) typical for the presence of crystalline materials. XRD pattern of loaded live biomass (Fig. 14 b) showed 6 peaks at 2θ : 20.70, 21.87, 22.49, 23.19, 29.58 and 32.69 $^{\circ}$ and corresponding to respective d-spacing 4.29, 4.06, 3.95, 3.83, 3.02 and 2.74 \AA (characteristic lines at 10.9, 11.3, 12.6, 9.97, 10.3 and 8.85 \AA) and crystal size was varying from 3 to 28.2nm. The crystal size was determined by Topas 2, Broker. XRD patterns of Cd(II)- loaded autoclaved cells (Fig. 14 c) and dried cells (Fig. 13 d) showed peaks at 2θ : 17.98, 19.22 and 24.83 and 14.40 $^{\circ}$, respectively. From TEM and XRD analyses, the nanoparticles formed by live pellets of *Yarrowia*

lipolytica were more crystalline in nature. In this connection, Velmurugan et al. (2010) reported that that dead biomass of *Fusarium* spp. was not significantly involved in the production of zinc nanocrystals.

Adsorptive removal of Cd(II) by dried *Y. lipolytica* AUMC 9256 biosorbent from environmental samples.

pH value and temperature of ceramic wastewater were 8.3 and 35 $^{\circ}$ C, respectively. The extraction percentage of Cd(II) was identified in the range 32.7% (first run) to 60.23% (second run) to 79.0% (third run). Therefore, the results from this study provide good evidence for the potential applications of dried *Y. lipolytica* AUMC 9256 biosorbent for removal of Cd(II) ions from real water samples.

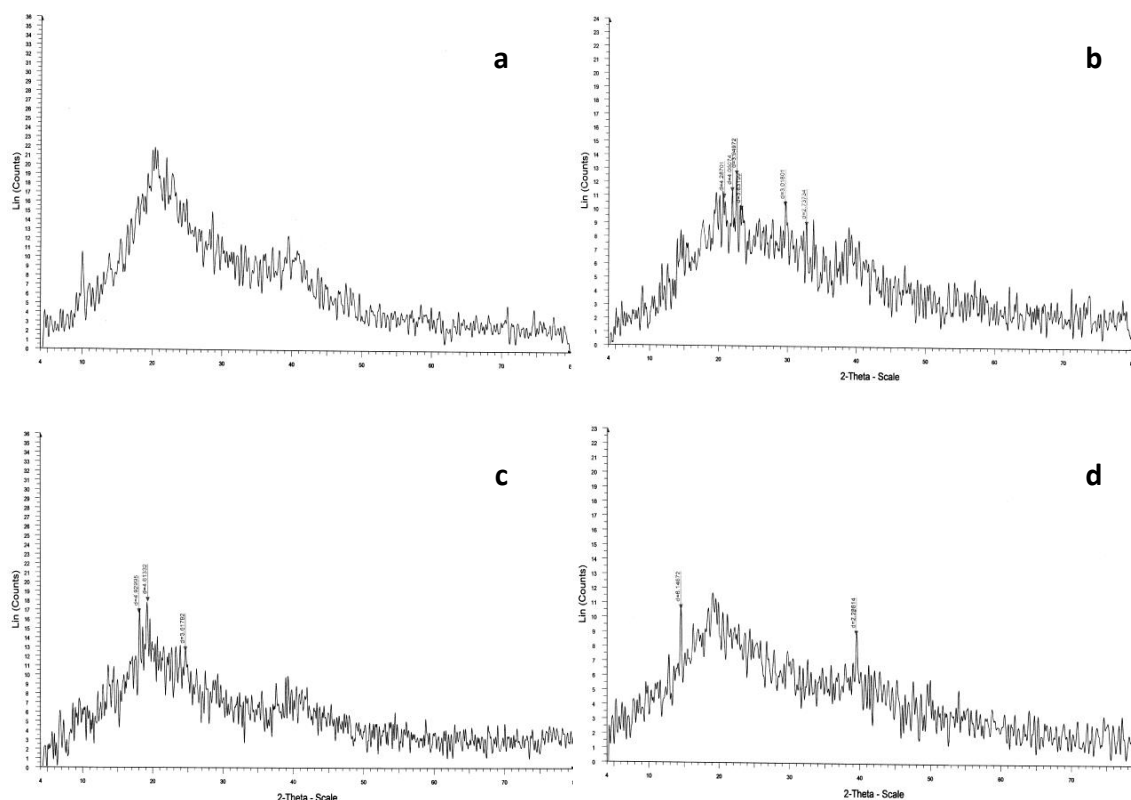


Fig. 14. XRD analysis of *Y. lipolytica* AUMC 9256 biomass (a) Native biomass, (b) Cd(II)- loaded live, (c) Cd(II)- loaded autoclaved and (d) Cd(II)- loaded dried biomass.

Discussion

The tolerance of *Y. lipolytica* AUMC 9256 to Cd(II) (500 mg Cd(II)/l) was higher than that was recorded by Bankar et al. (2012) (367mg/L). This value is 100 000 fold of the maximum adequate limit allowed in drinking water (0.005mg/L). Owing to different conditions of availability, complexation, and diffusion of Cd(II) ions, the higher MIC was reported in solid media in comparison to broth media (280 mg Cd(II)/l). In this regard, Kumar et al. (2012) revealed that microbe-metal interaction, such as removal, and precipitation are strongly affected by growth media composition. However, the range of metal concentrations tolerated in solid and liquid media yielded information on the potentiality of adsorption and complexation of Cd(II). In general, extracellular sequestration (avoidance of metal entry) and intracellular physical sequestration either by extruding metal ions out of the cell or by vacuolation have been proposed for heavy metal tolerance in fungi (Zinjarde et al., 2014).

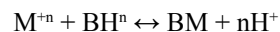
Pasternakiewicz (2006) stated that a low concentration of Cd(II) may enhance the synthesis of intracellular proteins that bind to heavy metal to form chelates. The lower toleration to Cd(II) in broth may be due to the greater close contact between the metal ions and the cells and the absence of chelating effect of agar (Ruta et al., 2010). Cd(II) is similar to Ca(II) and Zn(II) in terms of size and charge and can, therefore, substitute them from the metalloproteins (Zhou et al., 2015). Moreover, it has a high affinity for thiol groups and hence opposes with protein function by binding to cysteine residues (Helbig et al., 2008).

An increase in Cd(II) concentration led to a decrease in the biomass production and thereafter a rise in the residual Cd(II). The results of this study are in the line with the conclusions of Nongmaithem et al. (2016) on *Trichoderma* isolates and Cd(II). Monovalent cations (H^+ , Na^+/K^+) were stated to be associated with the ion exchange process due to a weak attachment with biomass as compared to divalent cations (Verma et al., 2008).

An increase in the intracellular bounded Cd(II) from 72.5 to 85% confirmed that *Y. lipolytica* AUMC 9256 is highly resistant to Cd(II). Machalová et al. (2015) affirmed that *Bacillus*

luteus exhibited higher intracellular Cd(II).

pH is an important parameter which influences cell surface binding sites and metal speciation in solution. The reaction of metal ions in solution with the sorbent may be described as:



where M: States the metal, n: Its charge, and B: The active binding sites of the adsorbent (Iqbal et al., 2009). The uptake of Cd(II) by *Y. lipolytica* AUMC 9256 reached its optimum value at pH 5.0 where the metal-binding sites were more deprotonated with a subsequent increase in the attraction between these sites and the positively charged Cd(II) ions (Wierzba, 2017). Above pH 5.0 a decrease in biosorption capacity might be due to the formation of hydroxylated complexes of Cd(II) ions ($Cd(OH)_2(aq)$) and their competition for the active binding sites (Vimala & Das, 2009). On the other hand, low pH (2.0) had a more negative correlation with Cd(II) biosorption capacity for live cells. This may be due to protein denaturation, the dominance of higher hydrated species ($Cd^{2+}(aq)$) with low mobility, and Columbic repulsion between Cd(II) ions and H^+ or H_3O^+ ions present in the solution (Bairagi et al., 2011). The Cd(II) biosorption capacity of dried cells was higher than that of autoclaved and live cells. It seemed that the mechanical disruption by dry thermal method increased the number of binding sites on the cell surfaces more than the wet one (Soleimani et al., 2015). At low Cd(II) concentration, the proportion of solute moles to the available surface area was low; subsequently, the sorption becomes distinct from the initial metal concentration (Binupriya et al., 2007). On the other hand, high metal ion concentration is known to provide a driving force in overcoming the mass transfer resistance and increasing number of collisions between the biomass and the Cd(II) ions (Shinde et al., 2012). At higher concentrations (450 and 500mg/L), the saturation of available sites on cell surfaces may limit the further adsorption of Cd(II) ions (Binupriya et al., 2007). Farhan & Khadom (2015) suggested that higher sorption at lower biomass concentration might be due to increased metal to biosorbent ratio. In contrast, the decrease in the biosorption capacity at high biomass concentrations may be due to the presence of metal binding sites on the adsorbent more than the available metal ions and/or a shell effect that protecting the binding

sites from being occupied by metal ions (Aryal et al., 2012). The equilibrium may be due to the saturation of active sites and/or the repulsive forces between the solute molecules on the biomass surface as the contact time increased (Wongjunda & Saueprasearsit, 2010). Metal ion uptake by yeasts is known to involve an initial rapid phase (passive uptake) through which physical adsorption or ion exchange at the surface of biomass has been occurred, followed by much slower phase through which other mechanisms such as microprecipitation and complexation have taken place (Goyal et al., 2003).

Ideally, a typical biosorbent should possess characteristics like availability, non-toxicity, high metal binding capability, large-scale usability, and recovery/re-usability (Wang & Chen, 2009). The binding strength of metal ions with autoclaved and dried cells seemed to be weaker than that of living cells. The desorption process differs with the type of biomass and pH value (Huang et al., 2013).

Yarrowia lipolytica was reportedly capable to efficiently remove metals from the metals-rich system due to activities of great reducing agents (Rao et al., 2013). Values of λ_{\max} are consistent with earlier reports on Cd NPs synthesized by microorganisms (Pawar et al., 2012) and by chemical method (Dumbrava et al., 2010). CFS contained the extracellular biomolecules was possibly being used as reducing and capping agents (Soleimani et al., 2015). The mechanism formation of Cd NPs by the dead biomass of yeast *Yarrowia lipolytica* is not fully understood at the moment. Some organic functional groups of cell walls were included in the reduction of metal ions by dead biomass (Lin et al., 2001; Pawar et al., 2012 and Salvadori et al., 2013).

In order to give a better understanding of the biosorption mechanism, TEM, FTIR, EDX and XRD investigations were performed. Blackening and appearance of electron-dense deposits within cell wall microfibrils indicated the association of complexation and sequestration of metal ions within cell wall layers as an avoidance mechanism. Internal cellular damage with extrusion of some internal contents may result from an alteration in membrane permeability. Once inside the cell, Cd(II) can be detoxified through the precipitation in electron-dense bodies, such as polyphosphate granules or through conjugation with intracellular

molecules (Siddiquee et al., 2015). *S. pombe* deposits Cd(II) in the form of vacuolar high molecular mass phytochelatin-Cd(II) aggregates with CdS nanocrystals (Mendoza-Coastal et al., 2005). *Y. lipolytica* has the ability to produce biosurfactants and extracellular polymeric substances which are able to demobilize bioavailable metal ions (Javanbakht et al., 2014). The cell wall of *Y. lipolytica* is made of 70% neutral carbohydrates, 15% proteins, 7% amino sugars, 5% lipids and 0.8% phosphorus. Mannan, chitin, and glucan are the main cell wall polymers of *Y. lipolytica* (Vega & Dominguez, 1986). Dried pellets incurred more changes ($\Delta 151\text{cm}^{-1}$) followed by autoclaved ($\Delta 141\text{cm}^{-1}$) and live ones ($\Delta 55\text{cm}^{-1}$) during the Cd(II) modification. The protein region ($1400\text{-}1700\text{cm}^{-1}$) involved similarly in Cd(II) uptake by dried and autoclaved biomass ($\Delta 56\text{cm}^{-1}$ and 62cm^{-1} , respectively) and to a lesser extent by live biomass ($\Delta 32\text{cm}^{-1}$). Yeast cell proteins were released during the autoclaving process and bound on the cell surface (Salvadori et al., 2013). On the other hand, the role played by the sugar region ($790\text{-}1180\text{cm}^{-1}$), including β (1 \rightarrow 3) glucan at 1074cm^{-1} was very obvious in dried biomass ($\Delta 38\text{cm}^{-1}$) more than in live ($\Delta 10\text{cm}^{-1}$) and autoclaved biomass (1cm^{-1}). Chitin region (around 2900cm^{-1}) and nucleic acid region ($1200\text{-}1290\text{cm}^{-1}$) played a limited role in Cd(II) modification in the studied biomass. Sulfur and phosphorus-containing functional groups played a noticeable role in Cd(II) uptake by autoclaved and dried biomass. EDX revealed that pores present on the biomass surface facilitate sorption of metal ions (Ahmad et al., 2018). From EDX microanalysis, the appearance of the specific signature peak for Cd(II) confirmed the complexation of metal ions on the yeast cell surfaces. Disappearance of K(I) signals may be due to the association of ion exchange mechanism in Cd(II) uptake. Michalak & Chojnacka (2010) mentioned that ion exchange is a substantial mechanism for biosorption of metal ions. However, the definite mechanisms may range from physical binding (i.e., electrostatic or London-Van der Waals forces) to chemical bonding (i.e., ionic and covalent) (Li et al., 2009). In terms of metal/metalloid detoxification, sulfur overproduction seemed to be worthwhile in yeast (Pócsi, 2011). Accordingly, the atomic % of sulfur increased. A sharp decrease in atomic % of phosphorus may be due to the complexation of Cd(II) with phosphate groups to the biomass surface (Siddiquee et al., 2015). XRD spectra for Cu(II)-loaded biomass established its crystalline

nature. Velmurugan et al. (2010) found that accurate triangle zinc nanocrystals were produced by live biomass of *Fusarium* sp., while highly irregular crystals were produced by autoclaved and dried biomass. The crystalline formation following a long period of metal accumulation could be due to the complexation of metal ions and metal precipitation in a crystalline state (Lopez et al., 2000).

The treatment of potentially toxic elements in wastewater is a challenge from a commercial and environmental point of view (Bilal et al., 2018). Biosorption of Cd(II) using *Y. lipolytica* AUMC 9256 is a safe, useful, and eco-friendly solution to wastewater treatment. According to the extraction percentage of Cd(II) which ranged from 32.7% to 79.0%, the results of this study present good indication for the potential utilization of dried *Y. lipolytica* AUMC 9256 biosorbent for removal of Cd(II) ions from real water samples.

Conclusion

The *Yarrowia lipolytica* AUMC 9256 showed the ability to synthesize cadmium nanoparticles during the biosorption of cadmium by live, autoclaved and dried biomass. Cadmium nanoparticles synthesized by live biomass were spherical in shape with size ranged from 34 to 87 nm. From TEM investigations the occurrence of extracellular precipitation, vacuolar sequestration and capture of Cd(II) within the cell wall microfibrils can be noticed. EDX analysis further showed that the ion exchange mechanism was very important in the biosorption of Cd(II). The role played by the sugar region (790-1180 cm^{-1}), including β (1 \rightarrow 3) glucan at 1074 cm^{-1} was more obvious in dried biomass (Δ 38 cm^{-1}) than in live (Δ 10 cm^{-1}) and autoclaved biomass (Δ 1 cm^{-1}). The results declared that the dried *Y. lipolytica* AUMC 9256 can be utilized successfully as a biosorbent for removal of Cd(II) ions from real wastewater samples and synthesis of cadmium nanoparticles.

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الإمتصاص البيولوجي للكاديوم من المياه الصرف الصحي وتخليق جزيئات الكاديوم النانوية بواسطة يارويا ليبوليتيكا

منال توفيق السيد

قسم النبات – كلية العلوم – جامعة الزقازيق – الزقازيق – مصر.

هدفت الدراسة الحالية إلى تقييم قدرة الإمتصاص الحيوي للكتلة الحيوية للخميرة يارويا ليبوليتيكا للكاديوم. أثبت الحد الأدنى من تركيز المثبطة 500 ملغم كاديوم/ لتر تحمّل كبير من يارويا ليبوليتيكا للكاديوم. تمت دراسة النمو في وجود تراكيز مختلفة من الكاديوم. بالنسبة للكتلة الحيوية الحية والمعقمة والمجففة أقصى قدرة امتصاص تم تحقيقها عند الرقم الهيدروجيني 5.0، تركيز أولي لعنصر الكاديوم 400 ميلي غرام لكل لتر، جرة الكتلة الحيوية 1 جم/لتر، و وقت التلامس 20 دقيقة للكتلة الحيوية المعقمة والمجففة و 240 دقيقة للخلايا الحية. تم تحديد قدرة يارويا ليبوليتيكا لإنتاج الجسيمات النانوية الكاديوم بواسطة قياسات التحليل الطيفي للأشعة فوق البنفسجية المرئية واختبارات فحص المجهر الإلكتروني. وعلاوة على ذلك، لتحديد الآليات الممكنة لأمتصاص عنصر الكاديوم، تم إجراء فحوص بواسطة المجهر الإلكتروني النافذ، و التحليل الطيفي بالأشعة تحت الحمراء، و التحليل الدقيق للأشعة السينية المشتتة للطاقة، و تحليلات حيود الأشعة السينية. تم استخدام الكتلة الحيوية المجففة ليارويا ليبوليتيكا لإزالة الكاديوم من عينات المياه المختلفة بنجاح باستخدام تقنية متعددة المراحل.