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Full Length Research Paper

Biosorption of zinc and cadmium by *Klebsiella pneumonia* KM609983 isolated from Sohag, Egypt

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Biosorption experiments for zinc (Zn^{+2}) and cadmium (Cd^{+2}) were investigated in this study using living and lyophelized biomass of a bacterial strain isolated from waste water of Rawafeih Al-Kusairdrainat, Sohag governorate in Egypt. It was identified as *Klebsiella pneumonia* KM609983. The experimental adsorption data were fitted towards the models postulated by Langmuir and Freundlich isotherms equations. The maximum biosorption capacities for zinc and cadmium obtained by using lyophelized and living cells were 243.9 and 227.3mg/g, respectively, indicating higher efficiency of lyophilized cells to heavy metals compared to living cells.

Keywords: Biosorption-*Klebsiella pneumonia* KM609983-Zinc -Cadmium -Sohag -Egypt

INTRODUCTION

Heavy metal pollution in wastewater has been always a serious environmental problem, because heavy metals are not biodegradable and can be accumulated in living tissues (Tsekova et al, 2010). Cadmium is one such heavy metal responsible for polluting the ecosystem. Cadmium is effectively bound by high molecular protein such as albumin and non-protein sulfhydryl group in the human body (Doshi, et al, 2007).This is accumulated in the kidneys and liver. Excess cadmium in the organisms can damage DNA sequencing and may cause genetic changes and cancer (Carmichael, 1994).Zinc is an essential trace element, but exposure to high doses has toxic effects . Reports indicate that accumulation of intracellular zinc results in respiratory disorders and may also induce cell death by inhibition of the energy metabolism (Plum et al, 2010). Conventional physiochemical methods for metals remediation include precipitation, coagulation, ionic exchange, inverse osmosis and adsorption. Although these are efficient processes, they have disadvantages when used in industrial waste conformed by diluted metallic solutions. Furthermore, these processes are expensive in terms of energy and chemical products consumption (Velquez and Dussan, 2009).Bioremediation of toxic metals by bacterial biosorptionas an alternative technology to chemical speciation for the metal removal of industrial and mining waste has received much attention recently (Veglio and Beolchini, 1997). The biosorption is ability of biomass, whether microbial or plant, to bind metals from aqueous environments on cells of biomass .Biosorption is passive and is independent on metabolism compared with complex process of bioaccumulation, where metals can be carried out using inactivated or dead biomass (Babak et al 2012;Ahluwalia and Goyal 2007; Volesky, 2007).The outer membrane of a Gram-negative bacterium is composed of lipopolysaccharides (LPS), phospholipids, and lipoproteins which plays a very important role in the survival of the

bacterium under environmental pressure, it prevents the whole bacterium from heavy metals (Suriya, et al, 2013).Equilibrium studies that give the capacity of the adsorbent and the equilibrium relationships between adsorbent and adsorbate are described by adsorption isotherms which is usually the ratio between the quantity adsorbed and the remaining in solution at fixed temperature at equilibrium.Freundlich and Langmuir isotherms are the earliest and simplest known relationships describing the adsorption equation (Hussein et al, 2004).The objective of the present study was to assess and to compare between the biosorption capacities of Zn^{2+} and Cd^{2+} by non- treated (living)and treated (lyophilized)biomass of *Klebsiella pneumonia* KM609983.

MATERIALS AND METHODS

Isolation and identification of the microorganism

The bacterial strain used in the present study was isolated from waste water of Rawafeih Al-Kusairdrain at, Sohag governorate in Egypt. It was isolated on nutrient agar. The isolate was purified and identified according to Bergey's manual 2005, then it was further identified using 16S rRNA gene sequencing. The tolerance to heavy metals was determined by the agar dilution method on tris minimal medium (Mergeay, 1995).

DNA extraction, sequencing, and phylogenetic analysis

Bacterial genomic DNA sample was extracted using aInsta Genetm Matrix (BIORAD).The primers 27F 5'(AGA GTT TGATCM TGG CTC AG)3' and 1492R 5' (TACGGY TAC CTT GTT ACG ACT T)3' were used for the PCR.The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 $\mu\ell$ reaction mixture by using a EF-Taq (Sol Gent, Korea) as follows :activation of Taq polymerase at 95 °C for 2minutes, 35cycles at 95 °C for 1minute, 55°C, and 72 °C for 1minutes each were performed, finishing with a 10-minute step at 72 °C.

The amplification product was purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA).Sequencing reaction was performed using a PRISM Big Dye Terminatorv3.1 Cycle sequencing Kit).The DNA sample containing the extension product was added to Hi-Di form amide (Applied Biosystems, Foster City), CA. The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer, primers 518F 5' (CCA GCA GCC GCG GTAATA CG)3' and 800R 5' (TAC CAG GGT ATCTAA TCC)3' were used for sequencing.Sequence was submitted to the National Center of Biotechnology and Information (NCBI)for similarity search through Blast. Selected sequences of other microorganisms with greatest similarity to the 16S rRNA sequences of the bacterial isolates were extracted and aligned using CLUSTAL W1.81 (Multiple Sequence Alignment) and N.J.plot generating a phylogenetic tree. The 16SrRNA gene sequence of the isolate was deposited in the DDBJ/EMBL/Gen Bank with accession number of *Klebsiella pneumonia* KM609983. Applied Bio systems, Foster City, CA.

Preparation of *Klebsiella pneumonia* KM609983 biomass for biosorption

Bacteria were cultured in nutrient broth medium for 24 h at 37°C, with shaking at 150 rpm, then harvested by centrifugation for 10 min at 10,000 rpm using (Eppendorf centrifuge, PLC-012), the suspension was rinsed three times with sterile distilled water, then freeze dried using (Labconco 94814) bench-top lyophilizer (Aksu and DÖnmez, 2001;Puranik and Paknikar1999).

Infrared analysis

Raw samples and biomass loaded with 40ppm Cd^{2+} and Zn^{2+} were analyzed by an Infrared spectrophotometer IR (Model 470 Shimadzu) corporation adopting KBr disk technique, which was performed to give a qualitative and preliminary characterization of the main chemical groups present on the cell wall that are responsible for heavy metal biosorption (Selatnia et al., 2004).

Effect of pH on biosorption

The impact of the solution pH on the metal biosorption was investigated in the biomass of *Klebsiella pneumonia* KM609983 and conditioned to different pH environments (ranging between 2.0 and 8.0) containing 20 ml of metal solution. The pH adjustment was done with the addition of either0.1M NaOH or 0.1M HCl.Sodium nitrate (0.1M) was used as a supporting electrolyte for all experiments. The method was carried out according to Seki et al (1998).

Effect of time on biosorption

Experiments for determining the kinetics of the process were carried out using 40 mg l^{-1} from the initial metal concentrations of Zn^{2+} and Cd^{2+} ions, in 20 ml of metal solution.

Adsorption experiments

The Cd^{2+} and Zn^{2+} ions biosorption isotherms were obtained at pH 7.0 .The sorption experiments were carried out using 20 mg of the untreated (living) cells or lyophilized cells in conjunction with concentrations of Cd^{2+} starting from 0 to 160 ppm and 20 ml of 0.1M $NaNO_3$ as supporting electrolyte solution with shaking at 200 rpm for 30 min to attain equilibrium. Experiments were conducted at room

Table 1. Morphological, physiological and biochemical characteristics of *Klebsiella pneumonia*

Feature	±
Gram staining	-
Motility	-
Shape	Rod-shape
Spore forming	Non- Spore forming
D-glucose acid/gas	+
D-mannitol fermentation	+
Sucrose fermentation	+
Lactose fermentation	+
D-sorbitol fermentation	+
V-P reaction	+
Catalase test	+
Oxidase test	-
Urease test	+
Citrate test	+
Oxidation-fermentation (O-F)	+

temperature (30°C).Then the samples were centrifuged at 10,000 rpm for 5 min and the heavy metal concentration in supernatants was measured by Atomic absorption spectrophotometric (AAS)Model 210 VGP Buck Scientific.

Data evaluations

The biosorption equilibrium isotherms were obtained by the Freundlich model (Eq. 1) and the Langmuir model (Eq. 2), respectively (Volesky 1990).

$q = K_f C_e^{1\backslash n}$ (1)

where K_f and n are the distribution coefficient and a correction factor, respectively. By plotting the linear form of Eq. (1), $\log q = 1/n \log C_e + \log K_f$, the slope is the value of $1/n$ and the intercept is equal to $\log K_f$.

$q= kC_eb/(1+kC_e)$ (2)

where k is a constant related to the adsorption capacity and b is the maximum metalad sorption, q_{max}. Rear ranging to a linear form, Eq. (2) becomes $C_e/q= 1/kb+ C_e/b$. Plotting C_e/q vs. C_e, the slope is $1/b$ and the intercept is $1/kb$.

RESULTS AND DISCUSSION

Identification of the microorganism

The isolate no.92 was able to resist Zn^{2+} and Cd^{2+} up to 300 and 400 ppm, respectively **.**Shamim and Rehman (2012**)** reported at *Klebsiella pneumonia*s rain CBL**-**1 which was isolated from heavy metal industrial waste water was able to tolerate Cd^{2+} and Zn^{2+} at up to 1500 and 700 mgl^{-1}, respectively. Isolate was identified as *Klebsiella pneumonia* 92 based on the basis of morphological, physicochemical and physiological characterization (Table1)**.** Then, it was further identified using 16S rRNA gene sequencing. Isolate 92 indicated greatest similarity to members of the *Klebsiella* sp. group. The 16S rRNA gene sequences of the isolate were deposited in the DDBJ /EMBL /Gen Bank nucleotide sequence databases with the accession number: KM609983 *(Klebsiella pneumonia* KM609983)**.**

Phylogenetic analyses

The partial LSU (large subunit unit) rDNA sequence of *Klebsiella pneumoniae* aligned with representatives of K. pneumonia strains along with representatives of other related genera with *Wiggles worthiaglossinidia* was used as out group. In total, the LSU rDNA dataset included 18 taxa and consisted of 1117 characters, of which 932 were constant, 108 were variable but parsimony-uninformative, and 77 were parsimony informative. The three most parsimonious trees produced using heuristic search parameters were of equal length with 296 steps, and have a CI of 0.7399, an RI (retention index) of 0.7308, and a Rescaled CI (consistency index) of 0.5407. The maximum likelihood analysis produced one tree which is shown in Figure 1. The maximum parsimony, neighbor joining and likelihood analyses produced trees with similar topologies (data not shown).

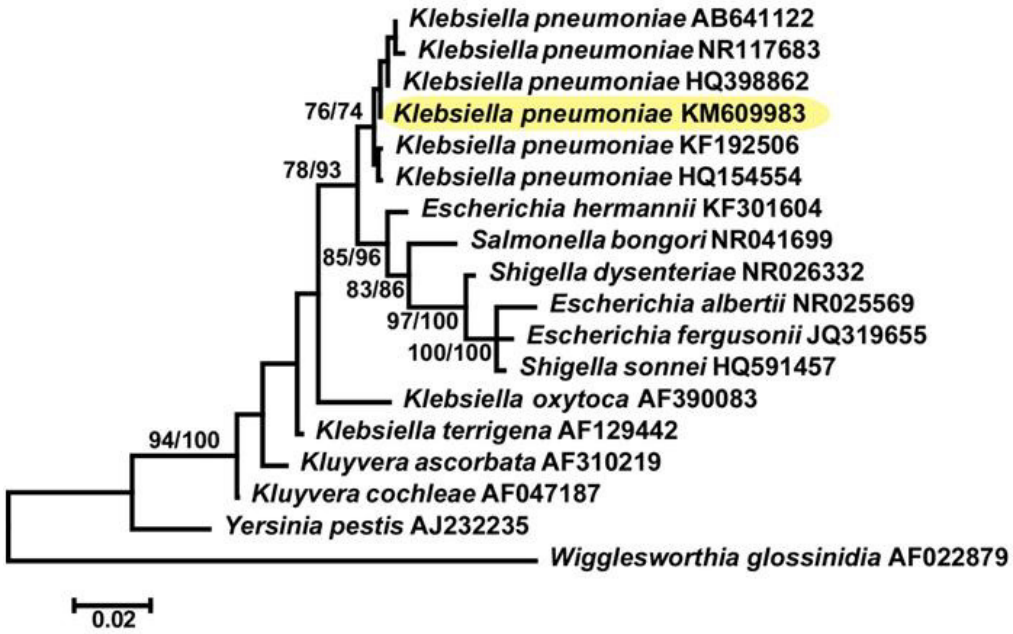

Figure 1. Phylogenetic relationships of *Klebsiellapneumoniae* and five strains of the same species retrived from Gen Bank and related species and genera in the family Entrobacyeriaceae on the nucleotide sequences of LSU rDNA.. The strain used in the present study is highlighted. The maximum likelihood tree (ML) was constructed in MEGA6. The numbers indicate pp values ≥ 95 % (in bold), MP, ML and MP bootstrap values ≥ 70.

Infrared analysis

The organic functional groups and their corresponding wave numbers were identified in the lyophilized cells of *Klebsiella pneumonia* KM609983 biomass. Figure 2 (a, b and c) represents the FTIR spectra of unloaded and metal loaded biomassin the range of 500-4000cm^{-1}which were taken to confirm the presence of functional groups that are usually responsible for the biosorption process.

In figure (2a), one can recognize the characteristic absorption broad bands of hydroxyl and amine groups assigned at 3500 and 3250cm^{-1}, respectively, where alkyl chains were recognized at 2931.4cm^{-1}. The absorbance at 1654.2 and cm^{-1} is related to the C=O of amide; COO^{-} of the carboxylate groups appeared at 1547.1 cm^{-1}. At 1395.7 cm^{-1}, a band related to methyl group was detected. Moreover, the band located at 1063.9 $cm^{-1}$was attributed to the organic phosphate groups and the wave number that appeared at 545.0 cm^{-1} was attributed to the S–S stretching of disulfide groups. These findings are in agreement with studies developed by Volesky, (2007) and Pavan et al., (2008) who concluded that the main functional groups responsible for a biosorption process are the hydroxyl, carbonyl, carboxyl, sulfonate, amide, imidazole, phosphate and phosphodiester groups, some of them present on *Klebsiella pneumonia* KM609983.

The IR spectra of $Cd^{2+}$and Zn^{2-+}loaded biomass are shown in Figure 2 (b, c,) , comparing the native biomass with that found in the case of metal-loaded, one can reveal the following:

- Bands assigned at 3421.2 and 3372.0$cm^{-1}$are characterizing the presence of NH group only instead of NH and OH, indicates the interaction of metal ions with OH -groups on the surface of the biomass.
- The appearance of a new band being located at 2356.3cm^{-1}, was attributed to the loading of Cd^{2+}.This band may be explained by a resonance between C=N -and C=C caused by the interference of Cd^{2+}.
- Loading of Zn^{2+} resulted in the appearance of two new bands assigned at 1326.2 cm^{-} and 1149.7 cm^{-1} indicating an interference of Zn^{2+} with the surface of biomass .

These changes of the spectra clearly show the complexation/coordination of the metal ions during the biosorption process. Cayllahua and Torem (2010) indicated a displacement of the vibration bands of hydroxyl, amino, carbonyl, carboxyl and phosphate groups of *Rhodococcusopacus* during the biosorption of aluminum. Similar data were obtained by Mohamed et al. (2014) during biosorption of cadmium on *Bacillus cereus* DAA54 and *Pseudomonas aeruginosa* DAA86.

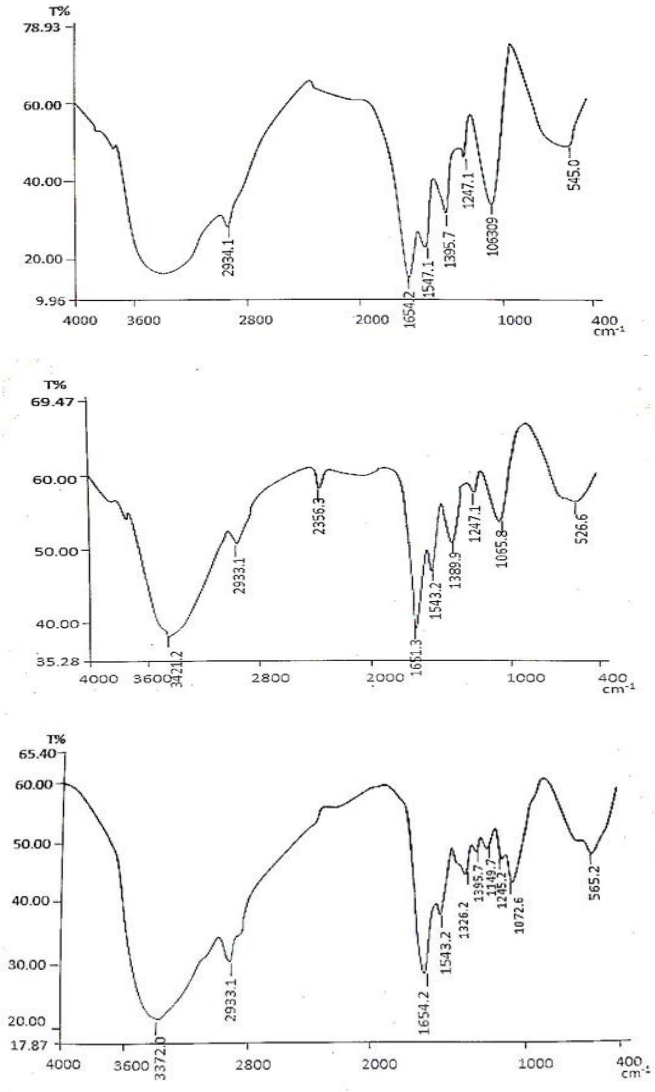

Figure 2. FTIR analysis for the bacterial metal free biomass (a) and biomass after 40 ppm of Cd^{2+} (b) and Zn^{2+} (c) – loaded *Klebsiella pneumonia* KM609983.

Effect of pH on biosorption

The influences of pH on the sorption of metals were depicted in figure 3 (a,b). In all cases, metal uptake by the biomass increases with increasing pH till it reaches a maximum after which the metal uptake decreases. The optimal adsorption of Cd^{2+} and Zn^{2+} by living and lyophilized biomass was achieved at pH 7.The pH of the metal solution is an important parameter in biosorption capacity. Marandi et al,(2010)and Saglam (1999),stated that the pH of medium affects the solubility of metal ions and the ionization state of the functional (carboxylate, phosphate, and amino) groups on the microbial cell wall. At low pH values, large quantities of protons compete with the metal cations for the adsorption sites (Kapoor et al, 1999&Saglam et al, 2002). Dissociation of protons from functional groups of on the cell wall occurs by increasing the pH providing more negative groups for complexing the metal cations. High pH values may cause hydrolysis of metal ions and complex formation (Fourest and Roux, 1992).

Effect of time on biosorption

Figure 4 (a, b) shows the amount of adsorbed metal plotted against time at the optimum pH. It can be seen that biosorption consists of two phases: a primary rapid phase that accounts for the major part in the total metal biosorption, and a second slow phase that contributes to a relatively small part which suggests that most of the metal

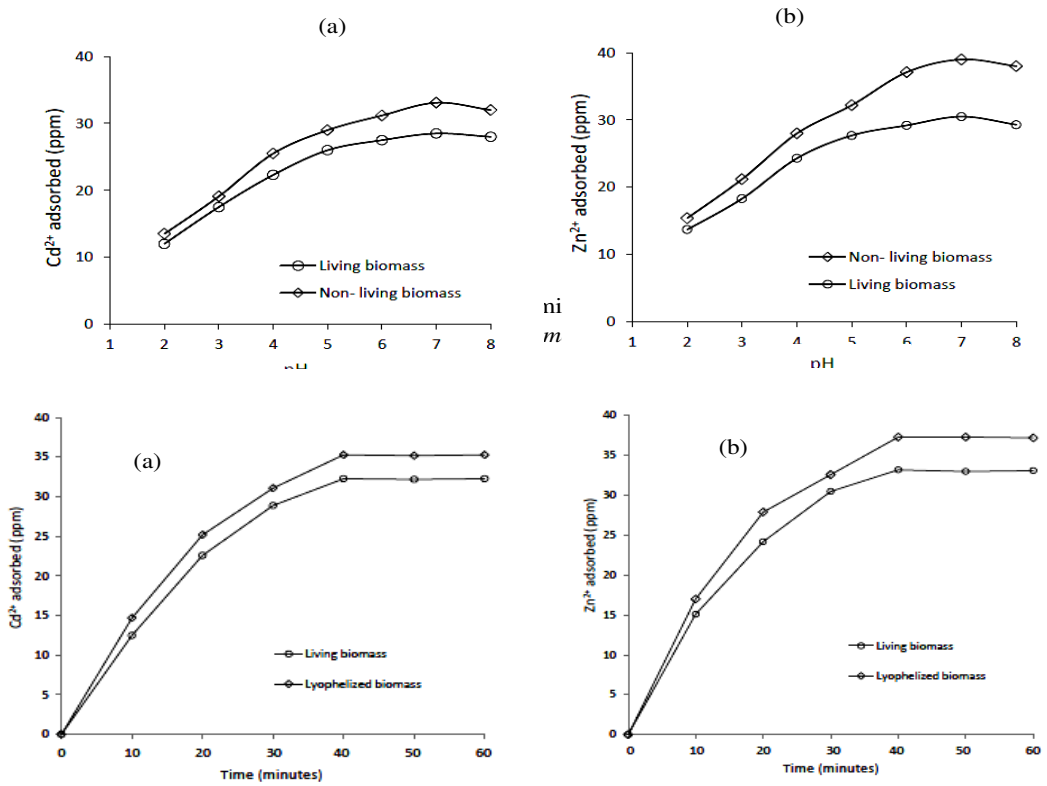

Figure 4. Effect of different time on cadmium (a) and zinc (b) biosorption by living and lyophilized cells of *Klebsiella pneumonia* KM609983.

ions are adsorbed within the first 10min.This may be due to the interaction with functional groups located on the surface of the cells. After that the rate decreases till we reach a constant value of metal concentration after 40min.This represents the equilibrium time at which an equilibrium metal ion concentration is presumed to be attained. This short time required for biosorption is in accordance with the result given by other authors (Hassan et al., 2009; Gabr et al.2008; Zouboulis et al., 2004; Pardo et al., 2003 and Sar et al., 1999).

Adsorption isotherms

The equilibrium capacity of Zn^{2+} appeared to be significantly higher than Cd^2.The isotherms indicate that the biosorption rate increases with an increase in equilibrium concentration of the sorbate. Thus, there was an increase in metal uptake as long as binding sites were free. Adsorption follows both Langmuir and Freundlich isotherms (Figures .5, 6).

However, the equilibrium data fitted well with the Langmuir and Freundlich adsorption isotherms for Cd^{2+} and Zn^{2+} at various initial metal concentrations. Data in tables (2,3) show the values of Langmuir and Freundlich parameters. These data in table (2) showed that the q_{max} values obtained for Cd^{2+} and Zn^{2+}uptake by the lyophilized bacterial biomass were 227.3 and 243.9 mgg^{-1}, respectively, which were higher than those obtained for living biomass:212.8 and 217.4 mgg^{-1}, respectively. It is worth mentioning that the correlation coefficients (R) for all the cadmium and zinc *Klebsiella* systems were found to be close to unity (≥ 0.95).The Freundlich analysis (table 3) determined that the biosorptive capacity (kf) values for $Cd^{2+}$and Zn^{2+} were 33.04 and 43.65, respectively, of lyophilized biomass as well as 12.3 and 15.8, respectively, for living biomass.

In general, these data indicate that the sorption capacity increased with increasing the initial metal-ion concentration for metal ions on the biomass surface. The comparison of metal sorption capacities showed that the selectivity order for metal ion towards the studied biomass matrices is Zn^{2+}> Cd^{2+} for a given initial metal ion concentration .

The selectivity order for metal ion towards the studied biomass (Zn^{2+}> Cd^{2+}) for a given initial metal ion concentration might be due to the difference in their ionic radii .The ionic radius of Zn^{2+} is 74 A°, while that of Cd^{2+} is 97A° .Tobin et al .,1984; Vinod and Anirudhan, 2001; Horsfall and Spiff, 2005, demonstrated that ions having a smaller ionic radius could be more quickly adsorbed onto a fixed adsorption area. Moreover, according to the Pearson

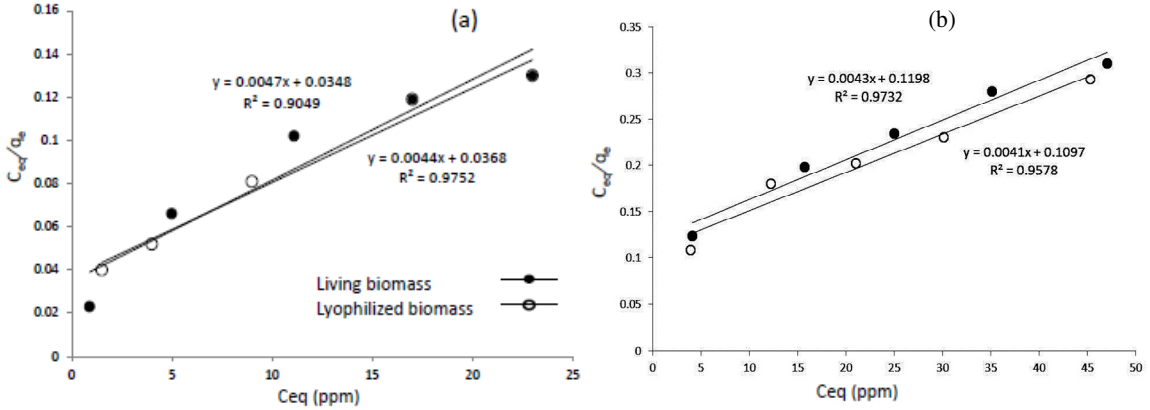

Figure 5. Linear form of Langmuir adsorption isotherm of Cd^{2+}**(a)** and Zn^{2+}**(b)** by living and lyophilized biomass of *Klebsiella pneumonia* KM609983.

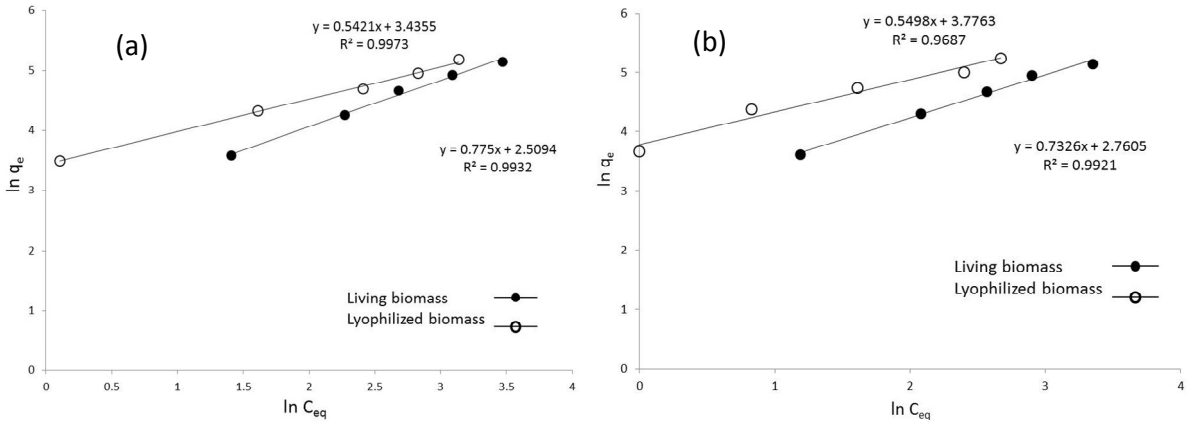

Figure 6. Linear form of Freundlich adsorption isotherm of Cd^{2+}**(a)** and Zn^{2+}**(b)** by living and lyophilized biomass of *Klebsiella pneumonia* KM609983.

Table 2. Langmuir adsorption constants obtained from the Langmuir adsorption isotherms of Cd^{2+} and Zn^{2+} by living and lyophilized biomass of *Klebsiella pneumonia* KM609983.

Biosorbents	Cadmium			Zinc		
	q_{max} (mg/g)	b (l/mg)	r	q_{max} (mg/g)	b (l/mg)	r
living cells	212.8	0.135	0.9049	232.4	0.036	0.9732
lyophilized cells	227.3	0.119	0.9752	243.9	0.037	0.9578

Table 3. Freundlich adsorption isotherms of Cd^{2+} and Zn^{2+} by living and lyophilized biomass of *Klebsiella pneumonia* KM609983.

Biosorbents	Cadmium			Zinc		
	K_f mg metal/g	n	r	K_f mg metal/g	n	r
living cells	12.3	1.29	0.9921	15.81	1.37	0.9932
lyophilized cells	33.04	1.84	0.9687	43.65	1.82	0.9973

classification, metals are categorized into three types, those that are polarizable or "soft", those that are non - polarizable or "hard" and those that are borderline (Williams et al .1998) .Zn^{2+} is classified in the borderline category according to this classification, while Cd^{2+} ions fall into the soft category (Sen Gupta, 2002).Soft cations form more stable complexes with soft donors, while hard cations prefer hard donors (Buffle, 1988).It was remarkable that the uptake of heavy metals using lyophilized cells is more efficient than that using living cells. Similar data were reported by Kureck et al. (1982), who compared the Cd^{2+} sorption by dead and active bacterial cells of *Serratiaarcescens* and *Paracoccus*sp., data revealed that dead cells sorbed Cd^{2+} much more readily than living cells. Our data are also in accordance with the results given by other authors (Sar and D'Souza 2001; Choi and Yun, 2004;Öztürk et al., 2004;Tunali et al., 2006).Moreover, lyophilized cells are not affected by the toxicity of the metal ions or by adverse operating conditions, they are also easier to handle (Chu and Hasim 2004; Karakagh et al 2012).

CONCLUSIONS

The adsorption equilibrium data fitted well the Langmuir and Freundlich models for metal ions in the studied concentration range. The biosorption mechanism includes mainly ionic interactions and formation of complexes between metal cations and acidic sites in the cell wall of bacterium. The results demonstrate that the bacterial isolate *Klebsiella pneumonia* KM609983 could be used as a promising biosorbent for the removal of Cd^{2+} and Zn^{2+} ions from aqueous solutions.

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