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LEAD (II) BIOSORPTION BY A METAL TOLERANT STREPTOMYCES STRAIN

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Abstract

The aim of the present study is the optimization of biosorption conditions using a metal tolerant actinomycete (strain 723) for the removal of lead ions from waste water in batch and dynamic flow mode. The optimum conditions for lead biosorption by the strain were determined as initial pH 3.0, biomass amount 2.0 g L^{-1}, and initial metal concentration 400 mg L^{-1} in the batch condition. The maximum lead biosorption performance was obtained with a 19 mm diameter column, a 1 mL min^{-1} flow rate, and 400 mg L^{-1} initial metal concentration in the packed-bed column. The dried and wet mycelia biomasses of the strain were also compared in batch and packed-bed column systems to determine the best biosorbent types. In addition, dried cells immobilized with agar and Ca-alginate were also used in the packed-bed column studies. The Freundlich isotherm model fitted experimental data the best. In experiments for reusing capacity, Ca-alginate immobilized biosorbent had a significant residual adsorption capacity in Pb^{+2} sorption (q: 116.00 mg g^{-1}) even after five biosorption-desorption cycles. A biosorption yield of 91% was obtained at 400 mg L^{-1} initial Pb^{+2} concentration in large scale studies in reactor systems. The assignment of the studied biosorbent, actinomycete strain 723, to *Streptomyces* genus was supported by using cell wall and 16S rRNA analysis.

Key words: batch, biosorption, dynamic flow mode, lead, *Streptomyces*

1. Introduction

Heavy metal pollution is a serious environmental problem throughout the world. Intensive industrial usage has caused the accumulation of metals, such as cadmium, lead, mercury, copper, nickel, chrome, and iron, which have higher toxicity than natural geochemical cycles can tolerate (Balan et al., 2010; Dumbrava and Birghila, 2009; Hlihor and Gavrilescu, 2009; Nriagu and Pacyma, 1988). Lead has traditionally been used in many products such as gasoline, paint, solder, pottery, crystal, and plumbing hardware. The metal has negative effects on the central nervous system,

growth and development, the kidneys and human behaviour. The most vulnerable groups are pregnant women, infants and children up to 6 years of age. The World Health Organisation (WHO) recommends a 0.01 mg L^{-1} maximum acceptable concentration of lead in drinking water. Therefore, the removal of lead from water is of a significant interest. As an alternative process, biosorption is a simple and cost effective method for metallic pollutant elimination from waste water. The development and characterization of low-cost adsorbents for metallic and other pollutants to wastewater treatment has been reviewed by Gupta et al. (2009). Researchers have achieved a number of successful biosorption studies

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for metals such as silver (Ag) (Mattuschka and Straube, 1993), chromium (Cr) (Gupta and Rastogi, 2008a, 2009), cobalt (Co) (Mattuschka and Straube, 1993), zinc (Zn) (Bal et al., 2003; Paduraru and Tofan, 2008), nickel (Ni) (Aksakal et al., 2008; Bulgariu et al., 2010; Gupta et al., 2010; Mattuschka and Straube, 1993), copper (Cu) (Brinza et al., 2005; Kicsi et al. 2006; Rehman et al., 2008), cadmium (Cd) (Kefala et al., 1999; Vimala and Das 2009), lead (Pb) (Bulgariu et al., 2008; Golab et al., 1991; Gupta and Rastogi, 2008b; Mondal, 2009) and uranium (U) (Golab et al., 1991). Different types of microorganism were intensively used as biosorbents for the removal of metals which include algae (Brinza et al., 2005, 2007; Gupta and Rastogi, 2008b, 2009; Gupta et al., 2010; Liu et al., 2009), bacteria (Chang et al., 1998; Congeevaram et al., 2007; Gupta and Rastogi, 2008a; Kang et al., 2008), yeast (Goksungur et al., 2005; Han, 2006), mould (Congeevaram et al., 2007; Sağ 2000a, 2000b; Sun and Shao, 2007), and macrofungi (Gabriel et al., 2001; Muraleedharan et al., 1995; Sarı and Tuzen, 2009). In these studies, live and/or dead forms of these microorganisms were used for the removal of various metals from aqueous solutions. However, a limited number of studies can be found in the literature with metal resistant microbial strains (Kujan et al., 2005; Li et al., 2010; Lu et al., 2006). This situation motivated us to obtain a new microbial strain from metal mine sites to use in remediation studies. In the present study, we are proposing a new bioremediation agent for lead which is a metal tolerant *Streptomyces* strain 723.

2. Experimental

2.1. Isolation and selection of the strain

The microorganism tested in this study was isolated from an industrial copper mine, Küre, Kastamonu (Black Sea Copper Enterprises). The metal content of the soil sample was determined as (mg Kg^{-1}); 0.59 Pb^{2+}, 94.96 Cu^{2+}, 5.90 Ni^{2+}, 33.42 Co^{2+}, 267.37 Mn^{2+}, 2669.75 total Fe. The isolation was carried out using the dilution plate technique in Starch Casein Agar which contained (per litre) starch 10 g; casein 0.3 g; KNO$_3$ 2 g; NaCl 2 g; K$_2$HPO$_4$ 2 g; MgSO$_4$.7H$_2$O 0.05 g; FeSO$_4$.7H$_2$O 0.01 g; CaCO$_3$ 0.02 g and agar 18 g (Küster and Williams, 1964). The pH of the medium was adjusted to 7.2 prior to sterilization. The medium was supplemented with 50 μg mL^{-1} each of cycloheximide and rifampicine (Goodfellow and Williams, 1983; Mc Carthy and Williams, 1990; Williams and Cross, 1971). Colonies were selected during the incubation period at 25 ºC, over 14 days. The isolated actinomycete strain was maintained at 4 ºC as slant and/or at -20 ºC as spore suspension.

The strain was selected according to its higher degree of tolerance to elevated concentrations of different heavy metals, including lead, zinc, nickel, iron, cadmium, mercury, cobalt, and copper. One of the strains (strain 723) exhibited a higher resistance to a variety of metals than the others. This strain was selected for further biosorption studies.

2.2. Identification of the strain 723

To identify the strain 723, morphological, chemotaxonomic, and molecular techniques were used. To determine the diaminopimelic acid isomer type of the strain, whole-cell hydrolysates were used (Lechevalier and Lechevalier, 1970).

2.2.1. DNA extraction

One mL of culture suspension was centrifuged and resuspended in 1 mL of TE (tris-HCL, 50 mM; EDTA, 20 mM; pH 8.0). Lyses solution, 0.38 mL, was added, followed by 0.40 mL of sodium perchlorate solution. Phenol-chloroform was added to fill a 2 mL centrifuge tube, and the culture was extracted. The aqueous upper phase was transferred into another tube and extracted with chloroform-isoamyl alcohol. Following this, 2 mL of 95% ethanol was added to the aqueous phase, and the DNA was spooled out, washed in 80% ethanol, and air dried. The DNA was resuspended in 0.1x SSC (15 mM sodium chloride, 15 mM sodium citrate; pH 7.0). RNase was added to the final concentration of 1 mg mL^{-1}. The mixture was extracted once again with chloroform-isoamyl alcohol and centrifuged. Then, the aqueous phase was transferred to another tube, and the SSC was added (1x, final concentration). The DNA was then dissolved in 500 μl of TE.

2.2.2. 16S ribosomal RNA (rRNA) sequencing

Total DNA from the isolated actinomycete strains was prepared according to the method described by Hoffman and Winston (1987). Oligonucleotide primers with a specificity for eubacterial 16S rRNA genes [forward primer 8-27: 50-AGA GTT TGA TCC TGG CTC AG-30 (Weisburg et al., 1991) and reverse primer 1492: 50-GGTTAC CTT GTT ACG ACT T-30 (Heuer et al.,1997)] were used to amplify the 16S rDNA. The amplicons obtained were purified and sequenced by Macrogen (Korea). The 16S rDNA gene sequences of actinomycete strains have been deposited in GenBank. Sequence data were analysed by comparison with 16S rDNA genes in the GenBank databases using the MEGA4 programme package (Tamura et al., 2007). An evolutionary tree was constructed using the neighbour joining algorithm (Saitou and Nei, 1987). Evolutionary distance matrices for the Fitche Margoliash method were generated as described by Jukes and Cantor (1969).

2.2.3. PCR amplification

Amplification reactions were carried out in an automated thermal cycler (Perkin Elmer, model 9700, Applied Biosystems). PCR products were run on a 1.0% agarose gel, stained with ethidium bromide and then visualized using an Image Analyzer Gel Doc, BIORAD. The PCR amplification reaction mixtures consisted of a template DNA 100 ng, 50 mM KCl, 10

mM Tris-HCl (pH9.0), 0.1 % (w v^{-1}), 1.5 mM MgCl$_2$ 100 nM primer, and 1.5 U of *Taq* polymerase (Promega). Amplification was performed with an initial denaturation step of 4 min at 94 ºC and then 30 cycles of 1 min denaturation at 94 ºC, 30 s at 57 ºC for the primer annealing, and 1.5 min at 70 ºC for the primer extension. A 7-min extension and cooling to 4 ºC completed the reaction sequence.

2.3. Preparation of biosorbents

Different biosorbent types were compared for lead removal, namely dried, wet, and immobilized cells in Ca-alginate and agar. To prepare wet and dried biosorbent types, the strain 723 was grown on an ISP2 medium at 125 rpm, and at 27 ºC. After a week, the biomass was harvested by filtration. After rinsing with deionised water, the biomass was separated for a wet biosorbent type. The cells were dried at 60 ºC for the dried biosorbent type. For the purpose of immobilization with Ca-alginate, the cell suspension was mixed with 2% Na-alginate (1:1). The prepared solution was dropped into 0.7 M CaCl$_2$ (1:1). The beads were washed with distilled water and dried at 60 ºC. All the biosorbent types were grounded and sieved, using an ASTM standard sieve, to select particle sizes of 100 meshes and used. To prepare an agar immobilized biosorbent, 5 mL of cell suspension (10-30 mg dried cell mL saline^{-1}) was added to 45 mL liquid agar (prepared with saline). This solution was divided into Petri dishes and left to solidify. After the solidification, the agar was sieved and washed with 0.9% NaCl solution to separate the free cells. The immobilized cells were dried at 60 ºC.

2.4. Batch studies

In this study, optimization of lead biosorption was performed as a function of parameters (pH, biomass amount, initial metal concentration and contact time). Therefore, the parameters giving the best results were kept constant at next stages. The effects of pH (1.0 – 5.0), biomass amount (0.4 - 6.0 g L^{-1}) initial metal concentration (100 – 500 mg L^{-1}) and contact time (5 - 120 min) on the lead biosorption by the strain 723 were studied in batch conditions. The mixtures were mixed on a magnetic stirrer at a rate of 200 rpm. The suspended solids were separated from the biosorption medium by centrifugation at 4500 rpm for 3 min. After separating the biomass from the solutions, the residual metal ion concentration in liquid phase was determined using flame Atomic Absorption Spectroscopy (AAS; Hitachi 180-70). Na$^+$ and K$^+$ ion concentrations in the solution were also determined with AAS. The pH values of the solutions were measured with a pH meter (Eutech Ion 510).

2.5. Packed-bed column studies

The optimum biosorption condition for strain 723 was determined for immobilized and dried cell types in packed-bed columns. Lead stock solutions were prepared with Pb(NO$_3$)$_2$. All lead solutions were diluted from 1000 mg/L stock solution in this study. The pH of lead solutions was adjusted with 0.1 M HNO$_3$ and 0.1 M NaOH. In the packed-bed column studies, first the best biosorbent type of the strain 723 (dried or immobilized) was selected. Further parameters in the packed-bed columns, such as column diameter (9-19 mm), flowing rate (1-15 mL min^{-1}), and initial metal concentration (100-500 mg L^{-1}) were investigated with the biomass type that gave the maximum biosorption capacity. To determine biosorption capacity on a large scale (1000 mL), optimum biosorption conditions (400 mg L^{-1} Pb^{2+} solution) and biosorbent type (2 g L^{-1} biomass immobilized with Ca-alginate) was used.

Throughout the study, biosorption capacities were calculated using the formula (Eq. 1):

$$q = V(C_i - C_e)/M \qquad (1)$$

where: q: biosorption capacity (mg g^{-1}); V: solution volume (L); Ci : initial metal ion concentration (mg L^{-1}); Ce : equilibrium metal ion concentration (mg L^{-1}); M: biosorbent amount (g).

2.6. Determination of adsorption isotherms

Freundlich and Langmuir isotherm models were used to determine the adsorption equilibrium of the lead ions in the batch and packed-bed column systems. The Langmuir parameters can be derived from a linearized form of Eq. (2) represented by:

$$1/q_e = 1/q_{max} + (1/q_{max}K_L)1/Ce \qquad (2)$$

where q_e and q_{max} are the equilibrium and monolayer biosorption capacities of the sorbent (mol g^{-1}), respectively. C_e is the equilibrium biosorbat concentration in the solution (mol L^{-1}) and K_L is the biosorption capacity when all binding sites are occupied by metal ions, and allows comparison of biosorption performances.

Biosorption-partition constants were determined by Freundlich equation (Eq. 3).

$$\ln q_e = \ln K_F + 1/n \ln C_e \qquad (3)$$

where K_F (L g^{-1}) is the Freundlich constant, which indicates biosorbent capacity and n (dimensionless) is the Freundlich exponent, which is related to biosorbent intensity.

2.7. Desorption studies

After the biosorption studies, the metal loaded biosorbents were rinsed with metal free deionized water. To determine the desorption performance of different eluent solutions, proper amounts of 0.1 M HCl, HNO$_3$, and EDTA were used in packed-bed systems containing metal loaded biomass. After the

desorption process, the biomass was harvested by centrifuging and gentle agitation. Metal ions released into the supernatant were determined immediately. The desorption efficiency (%) was calculated using the formula (Eq.4):

$$Desorption\ efficiency\ (\%) = (D_r / D_a) \times 100 \qquad (4)$$

where: D_r - Released metal ion amount in the supernatant solution (mg); D_a - Initially adsorbed metal ion amount on the biosorbent (mg).

After the determination of the best eluent for the adsorption trial, the regenerated biomass was again exposed to solutions containing 400 mg L^{-1} lead. This adsorption/desorption cycle was repeated five times. In this way, the efficiency of the adsorption and the desorption system was determined. In the presented study, the chemicals used were obtained from Sigma-Aldrich and Merck and were of analytical grade.

3. Results and Discussions

3.1. Phylogenetic analysis of strain 723

The studied strain showed L-diaminopimelic acid as diagnostic diamino acid in its peptidoglycan. This amino acid distinguishes *Streptomyces* species from other actinomycetes. Chemotaxonomic and morphological analysis confirms that the strain 723 belongs to the *Streptomyces* genus. The assignment of the strain to the genus *Streptomyces* is supported by 16S rRNA (Fig. 1).
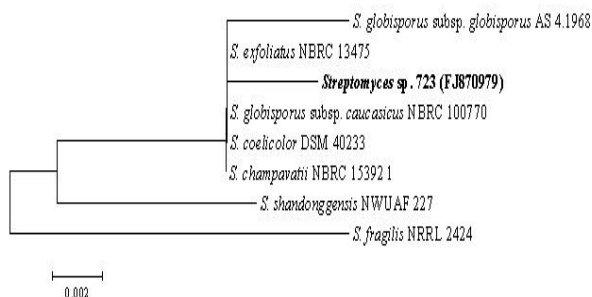

Fig. 1. The phylogenetic tree based on 16S rRNA gene sequences of strain 723 and representatives of the *Streptomyces* available in the database

A comparison of the almost complete 16S rRNA gene sequence of the tested strain with corresponding *Streptomyces* sequences from the GenBank database shows that the strain 723 lies in the evolutionary clade of *Streptomyces* allied taxa (Fig. 1).

A high similarity value (higher than 99%) is observed in *Streptomyces* strain 723 (accession number: FJ870979) in 16S rRNA gene sequences with *S. champavatii* NRRL B-5682, *S. globisporus* subsp. *caucasicus* (NBRC 100770), *S. globisporus* subsp. *globisporus* AS 4.1968, *S. exfoliatus* NBRC

13475 as well as *S. coelicolor* DSM 40233, whereas with *S. shandonggensis* NWUAF 227 and *S. fragilis* NRRL 2424 has an identity of 97 and 96% respectively.

3.2. Batch studies

Initial pH, amount of biomass, contact time, and initial metal concentration were investigated in batch conditions. The effect of initial pH on Pb^{+2} biosorption and Na$^+$ - K$^+$ ion concentrations in biosorption medium are presented in Figs 2, 3, respectively.
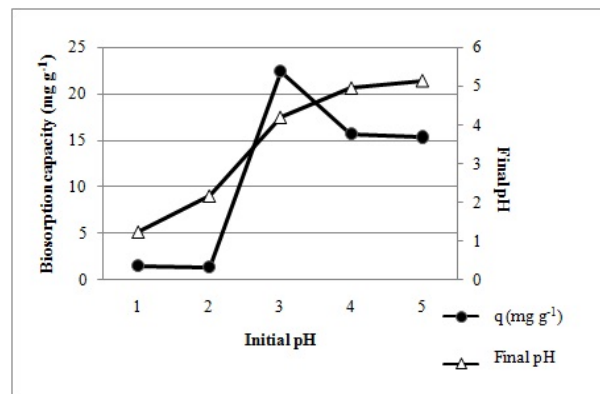

Fig 2. The effect of initial pH on Pb^{+2} biosorption (q) and pH change during biosorption with *Streptomyces* sp. 723 (Dry biomass amount: 2 g L^{-1}; Contact time: 60 min.; Initial Pb^{+2} concentration: 100 mg L^{-1})
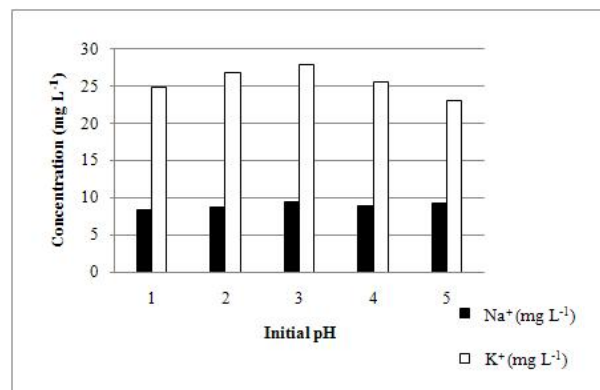

Fig. 3. Released Na$^+$ and K$^+$ ion concentrations at different pH

The biosorption capacity of the *Streptomyces* sp. 723 reached a maximum value (22.39 mg g^{-1} biomass) at pH 3.0. Lower pH values presented a very low capacity (Fig. 2). At the higher pH values, although slightly reduced to 15.36 mg g^{-1} biomass, the biosorption capacity remains constant. At the higher pH values (about 6.0), the lead ions tend to precipitate in the form of hydroxide. Therefore, biosorption studies could not be conducted on higher pH values. To determine the release of Na$^+$ and K$^+$ ions into the media, ion concentrations were measured during biosorption. In all treatments for pH values, except 1 and 2, the final pH values vary

between pH 4.2 - 5.0. Furthermore, the release of Na^+ and K^+ ions reaches a maximum value at pH 3.0 (Fig. 3). There was statistical difference between pH 3 and other pH groups for K^+, but there was not between 3, 4 and 5 for Na^+. So we can argue that Pb^{++} and K^+ exchange mechanism should be mainly effective in the biosorption process in batch conditions. Protons compete with positively charged Pb^{+2} ions to attach to biosorbent surfaces by reason of H^+ ions concentration which are very high at low pH value. As a result, biosorption is restricted by reason of the electrostatic thrust between protons on the biosorbent surface and Pb^{+2} ions in the media. The results are in accordance with the relevant literature (Holan and Volesky, 1994).

It is known that the amount of biosorbent is an important parameter for biosorption capacity and removal efficiency. The effect of the amount of biomass on Pb^{+2} biosorption was examined in the range of 0.4-6.0 g L^{-1} and the results are presented in Fig. 4. The increase of used biomass amount from 0.4 to 2.0 g L^{-1} brought about arises in biosorption capacity from 4.85 mg g^{-1} to 22.18 mg g^{-1}. Because of an increase of available binding sites, the removal efficiency went up. But, in the higher biomass amount, more than 2 g L^{-1}, biosorption capacity drastically decreased.

After a certain amount of metal ions, the biomass reaches saturation. At the higher biomass value, there were no free lead ions in the medium for the additional biomass. Therefore, an increase of biomass amount causes a reduction of biosorption capacity (q value). A biomass amount of 2 g L^{-1} was found to be most suitable in this study for lead biosorption and subsequent studies were carried out using this amount (Fig. 4).

The effect of the contact time on metal biosorption is an important parameter. Lead biosorption reached equilibrium after approximately 50 minutes by the *Streptomyces* sp. 723, and after this period a remarkable change in capacity was not observed (Fig. 5). This situation can be explained as the result of saturation of the entire biosorbent surface. The majority of Pb^{+2} ions were removed within the first 30 minutes after contact with the biosorbent. The establishment of biosorption equilibrium in a short time can be accepted as an advantage for the applicability of this strain.

The effects of initial metal ion concentrations on Pb^{2+} biosorption with bacterial biomass are presented in Fig. 6. Initial metal ion concentrations had a positive effect on the biosorption of lead ions at 100-500 mg L^{-1} ranges with dried biomass of *Streptomyces* sp. 723.

As a result of an increase of initial metal ion concentrations from 100 to 500 mg L^{-1}, biosorption capacity increased from 23.10 to 86.65 mg g^{-1}. The biosorption capacity of the dried biomass remained almost stable at higher than 400 mg L^{-1} of initial metal ion concentrations (Fig. 6). This situation can be explained by saturation of the biomass with metal ions.
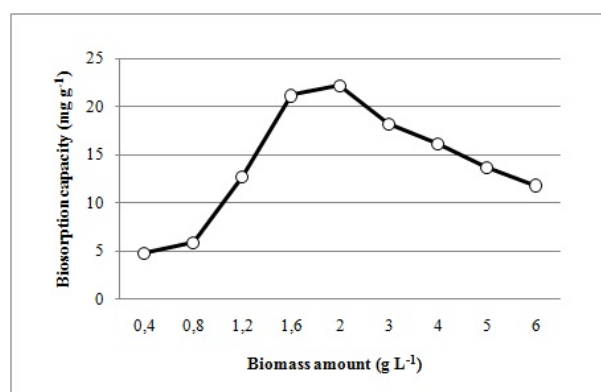

Fig. 4. The effect of dry biomass amount on Pb^{2+} biosorption (q) with *Streptomyces* sp. 723 (pH: 3; Contact time: 60 min.; Initial Pb^{+2} concentration: 100 mg L^{-1})
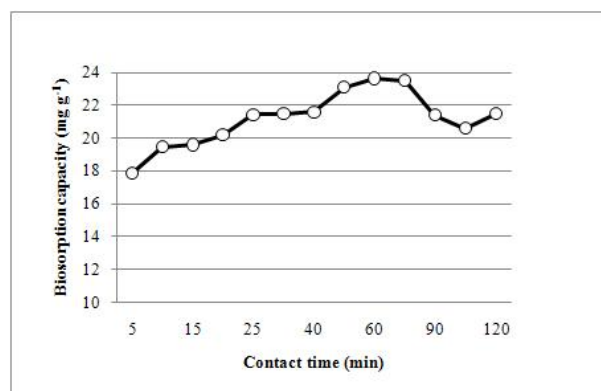

Fig. 5. Pb^{2+} biosorption (*q*) with *Streptomyces* sp. 723 as a function of contact time (pH: 3; Dry biomass amount: 2 g L^{-1}; Initial Pb^{+2} concentration: 100 mg L^{-1})
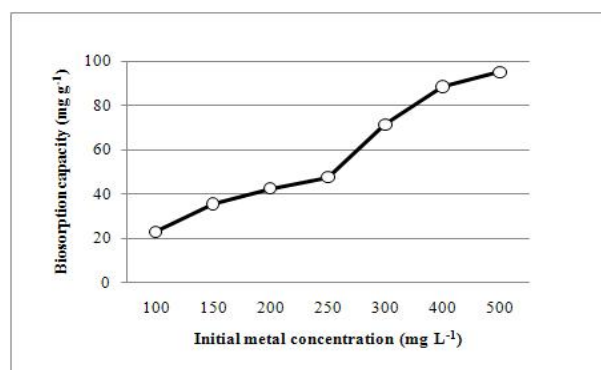

Fig. 6. Initial metal concentration effect on Pb^{2+} biosorption (*q*) with *Streptomyces* sp. 723 (pH: 3; Dry biomass amount: 2 g L^{-1}; Contact time: 50 min.)

3.3. Packed-bed column studies

The dried biomass of *Streptomyces* sp. 723 used in the batch system was also examined in the packed-bed column which allows for more efficient sorbent utilization.

The optimized pH and amount of biomass values obtained in the batch processes were used in packed-bed column conditions. Both processes were compared in terms of biosorption performance. In an effort to determine the lead biosorption performance

of *Streptomyces* sp. 723 in the packed-bed column; the diameter of column, flow rate, and initial metal concentration were investigated. Related data in turn with diameter of column, flow rate and initial metal concentration are presented in Figs 7-9, respectively.

An experiment concerning the effect of column diameter on the biosorption was carried out with 9, 11, 13 and 19 mm columns using the constant flow rate (3 mL min^{-1}). A positive correlation was observed between the column diameter and the biosorption capacity. With an increase of column diameter from 9 to 19 mm, an increase in biosorption capacity was observed from 6.80 to 16.45 mg g^{-1} (Fig. 7). When increase in the surface area in column, number of the functional group of surface increase for lead biosorption. Because the used biomass amount is constant, an increase in the surface area of the biosorbent may result in an increased interaction between the biosorbent surface and metal ions. Therefore, a 19 mm column was used for further studies. To investigate the effect of the flow rate on Pb^{+2} ions, the biosorption capacity of the bacterial biomass was compared in five different flow rates. The flow rate of the solution changed from 1 to 15 mL min^{-1} through a peristaltic pump. As clearly depicted in Fig. 8, the biosorption capacity decreased sharply from 21.50 to 3.30 mg g^{-1} as the flow rate increased from 1 to 15 mL min^{-1}. There was a negative correlation between the flow rate and the biosorption capacity. The longer contact time seems to have resulted in Pb^{+2} ions which can be more successful in the adsorption on the biosorbent. On the other hand, with the increase in flow rate, the resistance of the hydrodynamic boundary layer decreases. For this reason, 1 mL min^{-1} was decided upon as an optimum flow rate.

The effect of the initial metal concentration on the biosorption capacity was investigated using similar conditions of batch optimization. As shown in Fig. 9, with the increase of the initial metal ions, the biosorption capacity of the biosorbent increased as with the batch conditions. The equilibrium data obtained from the batch and packed-bed column studies at different initial Pb^{2+} concentrations were evaluated in Freundlich and Langmuir isotherm models, which are widely applied to understand the sorption mechanisms. Freundlich and Langmuir isotherms belonging to the lead biosorption graphics are presented in Figs 10 and 11, respectively. Calculated isotherm constants from these graphics are presented in Table 1. From Table 1 r^2 values were compared; bacterial biomass and biosorption of Pb^{2+} ions fitted both the Freundlich and Langmuir isotherms. However, maximum biosorption capacity (q_{max}) values calculated from the Langmuir model were not compatible with maximum biosorption capacity values obtained empirically. Therefore, the bacterial biomass and biosorption of Pb^{2+} defined with the Freundlich model is appropriate and we can conclude that the used biosorbent might follow a heterogeneous biosorption system with different active sites in this study.
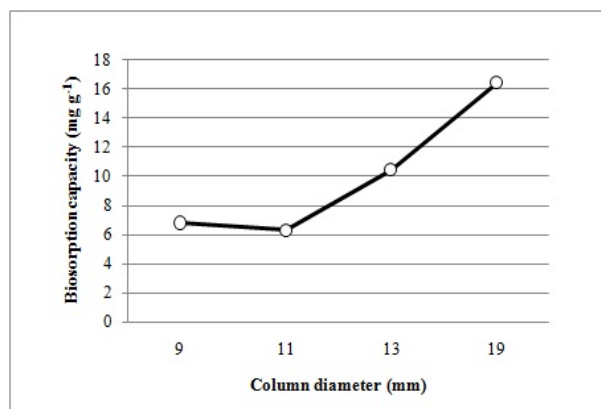

Fig. 7. The effect of column diameter on lead biosorption (*q*) with *Streptomyces* sp. 723 in packed-bed column (pH:3; Dry biomass amount: 2 g L^{-1}; Initial Pb^{+2} concentration: 100 mg L^{-1}; Flow rate: 3 mL min^{-1})
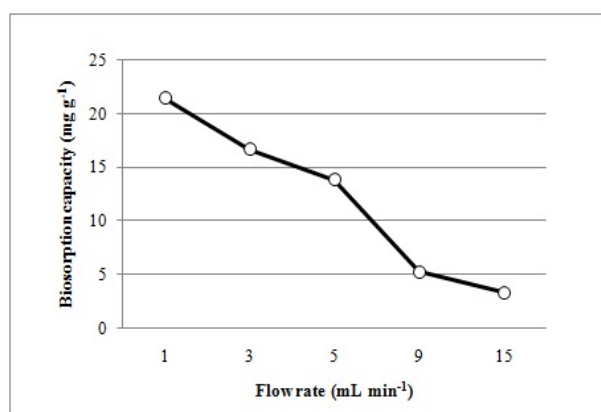

Fig. 8. The effect of flow rate on lead biosorption (*q*) with *Streptomyces* sp. 723 in packed-bed column (pH:3; Dry biomass amount: 2 g L^{-1}; Initial Pb^{+2} concentration: 100 mg L^{-1}; Column diameter: 19 mm)
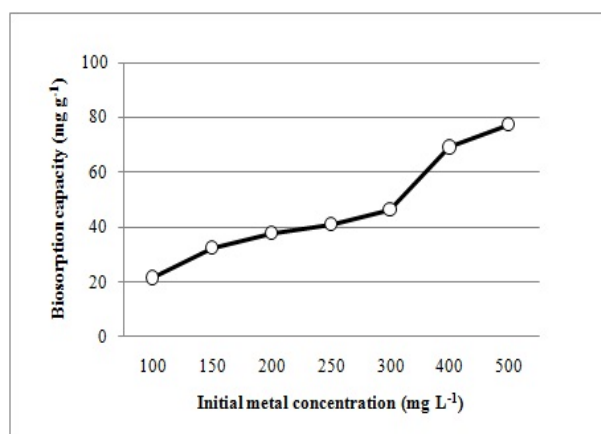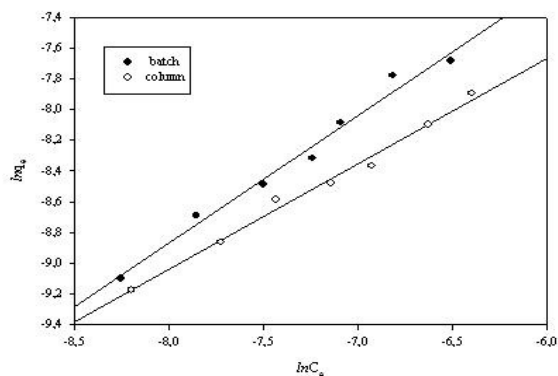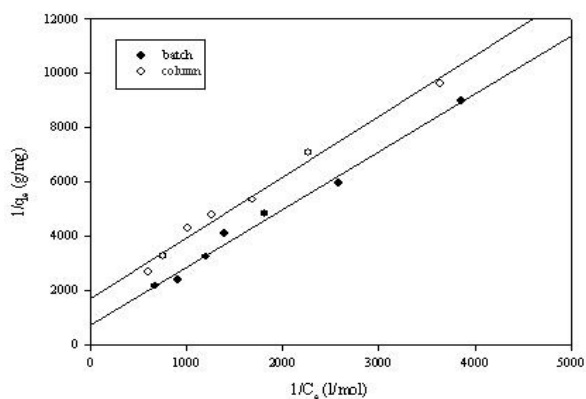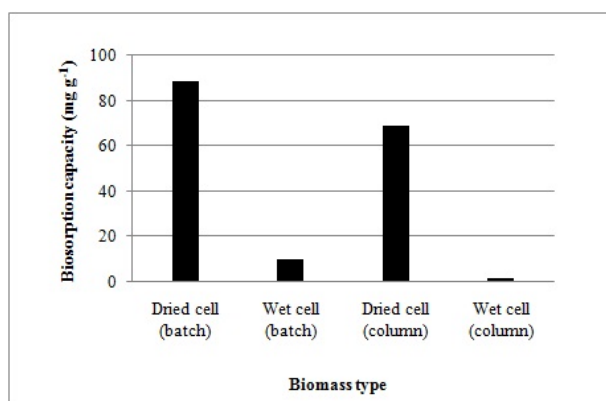

Fig. 9. The effect of initial metal concentration on Pb^{2+} biosorption with *Streptomyces* sp. 723 in packed-bed column (pH:3; Dry biomass amount: 2 g L^{-1}; Column diameter: 19 mm; Flow rate: 1 mL min^{-1})

The biosorption capacity was designated with dried (dead) and wet (live) cells in the batch and packed-bed column and the data obtained is presented in Fig. 12. The biosorption capacity of dried cells was higher when compared with wet cells.

Table 1. Freundlich and Langmuir isotherm constant for the Pb^{2+} biosorption with *Streptomyces* sp. 723 batch and packed-bed column

Biosorption model	$q_{exp.}(mg\ g^{-1})$	Langmuir				Freundlich		
		q_{max} $(mg\ g^{-1})$	K_L $(L\ mol^{-1})$	r_L^2	R_L	n	K_F $(L\ g^{-1})$	r_F^2
Batch	95.25	275.58	3.54×10^2	0.98	0.539	1.20	1.08×10^{-1}	0.98
Column	77.20	119.97	7.73×10^2	0.98	0.348	1.45	2.92×10^{-2}	0.99

Fig. 10. Freundlich isotherm model for lead biosorption with *Streptomyces* sp. 723 in batch and packed-bed column

Fig. 11. Langmuir isotherm model for Pb^{2+} biosorption with *Streptomyces* sp. 723 in batch and packed-bed column

Fig. 12. Lead biosorption with dried (dead) and wet (live) cells of *Streptomyces* sp. 723 in batch and packed-bed column (pH:3; Biomass amount: 2 g L^{-1}; Contact time: 50 min; Initial Pb^{+2} concentration: 400 mg L^{-1}; Column diameter: 19 mm; Flow rate: 1 mL min^{-1})

The literature suggests that, because of their higher biosorption capacity, dead bacterial cells can be more promising and efficient biosorbents than live ones. This is explained since wet cells put up resistance to heavy metals. Furthermore, biosorption studies carried out with dried biomass are advantageous because this biomass does not need a nutrition source and is not affected from toxicity (Yan and Viraraghavan, 2001). The utilization of immobilized biomass prominences at practical applications because of high mechanic stability, high desorption efficiency, repeat utilization and high biosorption capacity (Stoll and Duncan, 1997; Valdman et al., 2001; Yan and Viraraghavan, 2001). For this reason, the dried biomass used was immobilized with Ca-alginate and agar. The biosorption capacities of free, Ca-alginate immobilized, and agar immobilized bacterial biomass are shown in Fig. 13. The *Streptomyces* sp. 723 biomass immobilized with Ca-alginate had the highest biosorption capacity (Fig. 13). It surpasses the biosorption performance of alginate alone and the free *Streptomyces* sp. 723 biomass because of its metal affinity. Therefore, desorption and repeat utility studies were performed with Ca-alginate immobilized biomass.

To determine the regeneration capacity of the exhausted biosorbent, desorption performance of the system was investigated with different eluent solutions, such as 0.1 M HCl, HNO₃ and EDTA. Having the highest recycle performance, 0.1 M HNO₃ was selected as an effective eluent in this study (Fig. 14).
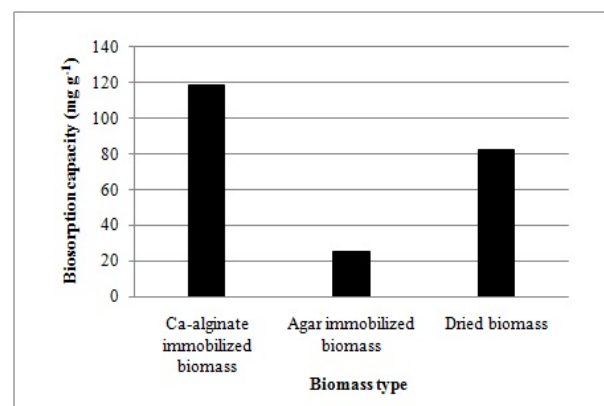
Fig. 13. Biosorption capacity of Ca-alginate immobilized, agar immobilized and dried bacterial biomass (pH:3; Biomass amount: 2 g L^{-1}; Initial Pb^{+2} concentration: 400 mg L^{-1}; Column diameter: 19 mm; Flow rate: 1 mL min^{-1})
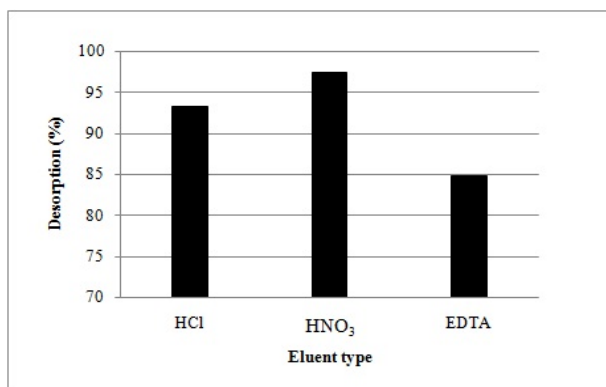
Fig. 14. Desorption performance of the system with different eluent solutions (pH:3; Biomass amount: 2 g L^{-1}; Contact time: 50 min; Initial Pb^{+2} concentration: 400 mg L^{-1}; Column diameter: 19 mm; Flow rate: 1 mL min^{-1}; Biosorbent type: Ca-alginate immobilized dry cell)

The biosorption-desorption cycle was investigated five times repeatedly with the same column. As clearly revealed in Fig. 15, the immobilized system can be used five times when 0.1 M HNO$_3$ solution was used as a desorption agent. Repeated utilization of the same biosorbent is an important property of biosorbents for biosorption in terms of economy. Utilization of the biosorbent in higher volumes was also confirmed with the Bioflo 100 model reactor (New Brunswick) in 1 litre scale (Fig. 16).

Metal tolerant strains of actinomycetes have been isolated from soil (Amoroso et al., 2000; Mengoni et al., 2001), fresh water (Amoroso et al., 1998) and sea (Ravel et al., 1998) in various studies. The presence of responsible genes and plasmids associated with metal tolerance (Amoroso et al., 2000; Amoroso et al, 2001; Ravel et al., 1998) were determined in the actinomycete cells. Endo et al (1996) identified the capability of these strains for bioremediation studies. However, there is lack of biosorption studies concerning metal tolerant actinomycete isolates not only in our country but also throughout the world. The biosorption performance of dried and Ca-alginate immobilized biomass obtained from *Streptomyces* sp. 723 was very high in the batch and packed-bed column systems.

A comparison of Pb^{2+} biosorption by *Streptomyces* sp. 723 and other biosorbent types reported earlier is presented in Table 2. When these results are considered, it can be clearly seen that *Streptomyces* sp. 723 could be a good alternative for the bioremediation of industrial waste waters, because of its high biosorption and desorption capacity in comparison with other adsorbents.
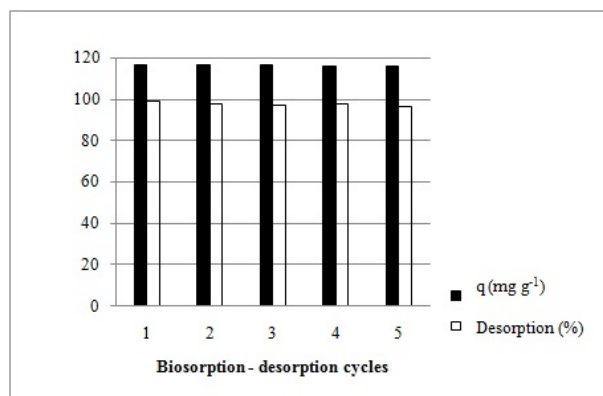

Fig. 15. Biosorption-desorption cycles of immobilized biosorbent systems (pH:3; Biomass amount: 2 g L^{-1}; Contact time: 50 min; Initial Pb^{+2} concentration: 400 mg L^{-1}; Column diameter: 19 mm; Flow rate: 1 mL min^{-1}; Biosorbent type: Ca-alginate immobilized dry cell; Eluent type: HNO$_3$)
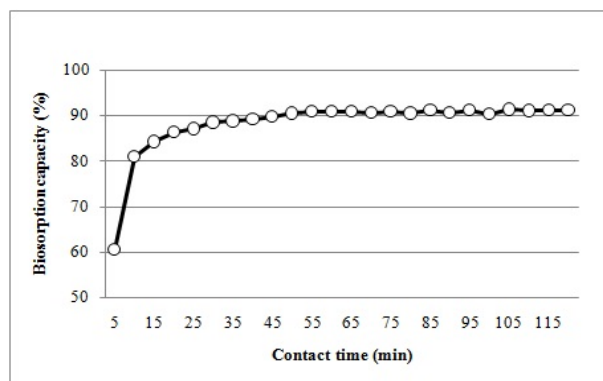

Fig. 16. Biosorption yield in dynamic flow mode in 1 liter scale (pH: 3; Biomass amount: 2 g L^{-1}; Initial Pb^{+2} concentration: 400 mg L^{-1}; Biosorbent type: Ca-alginate immobilized dry cell; Volume: 1 liter)

Table 2. Uptake of Pb^{2+} by some biological materials and microbial biomass

Biosorbent	Experimental conditions		Lead adsorption capacity (mg g-1)	References
	pH	Lead concentration (mg L-1)		
Streptomyces sp. 723	3.0	400	116.00	This study
Agaricus bisporus	5.0	10-100	33.78	Vimala and Das, 2009
P. aeruginosa ASU 6a	6.0	0-160	123.00	Gabr et al., 2008
Calophyllum inophyllum	4.0	25-400	34.51	Lawal et al., 2010
Parmelina tiliaceae	5.0	25-400	75.80	Uluozlu et al., 2008
Aspergillus niger	-	250	54.046	Iskandar et al., 2011
Maize stalk sponge	6.0	5-100	80.00	García-Rosales and Colín-Cruz, 2010
KOH treated pine cone powder	5.0	60-120	32.26	Ofomaja et al., 2010
Amanita rubescens	5.0	10-400	38.4	Sari and Tuzen, 2009

4. Conclusions

As a conclusion, it can be argued that:

1. *Streptomyces* sp. 723 has a high Pb^{2+} biosorption performance in the condition of 3.0 initial pH, 2.0 g L^{-1} biomass amount, 50 min contact time, 400 mg L^{-1} initial metal concentration, 19 mm diameter column, and 1 mL min^{-1} flow rate.

2. Ca-alginate immobilized cell was the most preferable biomass type and Freundlich isotherm is more appropriate to explain the equilibrium sorption phenomena.

3. Because of its high capacity (*q*: 116.00 mg g^{-1} after five biosorption-desorption cycles), the biosorbent can be used on a larger scale to treat wastewater charged with P^{b+2}.

References

Aksakal O., Ucun H., Kaya Y., (2008), Removal of nickel from aqueous solution by Nordmann Fir (*Abies nordmanniana* (STEV.) SPACH subsp. *nordmanniana*) cones, *Environmental Engineering and Management Journal*, **7**, 359-363.

Amoroso M.J., Castro G.R., Carlino F.J., Romero N.C., Hill R.T., Oliver G., (1998), Screening of heavy metal-tolerant actinomycetes isolated from the Sali River, *Journal of General and Applied Microbiology*, **44**, 129-132.

Amoroso M.J., Schubert D., Mitscherlich Schumann P., Kothe E., (2000), Evidence for high affinity nickel transporter genes in heavy metal resistant *Streptomyces spec.*, *Journal of Basic Microbiology*, **40**, 295-301.

Amoroso M.J., Castro G.R., Durán A., Peraud O., Oliver G., Hill R.T., (2001), Chromium accumulation by two *Streptomyces spp.* isolated from riverine sediments, *Journal of Industrial Microbiology and Biotechnology,* **26**, 210-215.

Bal Y., Bal K.E., Lalam A., (2003), Removal of Bi (III) and Zn (II) by nonliving *Streptomyces rimosus* biomass from nitric solutions, *The European Journal of Mineral Processing and Environmental Protection*, **3**, 42-48.

Balan C., Bulai P., Bilba D., Macoveanu M., (2010), Sphagnum moss peat: a green and economical sorbent for removal of heavy metals (Cd and Cr) from wastewaters, *Environmental Engineering and Management Journal*, **9**, 469-478.

Brinza L., Dring M., Gavrilescu M., (2005), Biosorption of Cu^{2+} ions from aqueous solution by *Enteromorpha* sp.**,** *Environmental Engineering and Management Journal***, 4**, 41-50.

Brinza L., Dring M.J., Gavrilescu M., (2007), Marine micro and macro algal species as biosorbents for heavy metals**,** *Environmental Engineering and Management Journal*, **6**, 237-251.

Bulgariu L., Răţoi M., Bulgariu D., Macoveanu M., (2008), Equilibrium study of Pb(II) and Hg(II) sorption from aqueous solutions by moss peat, *Environmental Engineering and Management Journal*, **7,** 511-516**.**

Bulgariu L., Bulgariu D., Macoveanu M., (2010), Kinetics and equilibrium study of nickel (II) removal using peat moss, *Environmental Engineering and Management Journal,* **9**, 667-674.

Chang J.S., Huang J.C., Chang C.C., Tarn T.J., (1998), Removal and recovery of lead fixed-bed biosorption with immobilized bacterial biomass, *Water Science and Technology*, **38**, 171-178.

Congeevaram S., Dhanarani S., Park J., Dexilin M., Thamaraiselvi K., (2007), Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates, *Journal of Hazardous Materials*, **146**, 270–277.

Dumbrava A., Birghila S., (2009), Analysis of some heavy metal levels in Danube River water, *Environmental Engineering and Management Journal*, **8**, 219-224.

Endo G., Ji G., Siver S., (1996), *Heavy metal resistance plasmids and use in bioremediation*, In: *Environmental Biotechnology Principles and Applications,* Moo-Young M., Anderson W.A. Chakrabarty A.M. (Eds.), Kluwer Academic Publishers, Dordrecht / Boston / London, 47-63.

Gabr R.M., Hassan S.H.A., Shoreit A.A.M., (2008), Biosorption of lead and nickel by living and non-living cells of *Pseudomonas aeruginosa* ASU 6a, *International Biodeterioration and Biodegradation*, 62**, 195-203.**

Gabriel J., Baldrian P., Hladokova K., Hakova M., (2001), Copper sorption by native and modified pellets of wood-rotting basidiomycetes, *Letters in Applied Microbiology*, **32**, 194-198.

García-Rosales G., Colín-Cruz A., (2010), Biosorption of lead by maize (*Zea mays*) stalk sponge, *Journal of Environmental Management*, **91**, 2079-2086.

Goksungur Y., Uren S., Guvenc U., (2005), Biosorption of cadmium and lead ions by ethanol treated waste bakers' yeast biomass. *Bioresource Technology*, **96**, 103-109.

Golab Z., Orlowska B., Smith R.W., (1991) Biosorption of lead and uranium by *Streptomyces sp.*, *Water Air and Soil Pollution*, **60**, 99-106.

Goodfellow M., Williams S.T., (1983), Ecology of Actinomycetes, *Annual Review of Microbiology*, **37**, 189-216.

Gupta V.K., Rastogi, A., (2008a), Sorption and desorption studies of chromium (VI) from nonviable cyanobacterium *Nostoc muscorum* biomass, *Journal of Hazardous Materials*, **154**, 347-354.

Gupta V.K., Rastogi, A., (2008b), Biosorption of lead(II) from aqueous solutions by non-living algal biomass *Oedogonium* sp. and *Nostoc* sp.-A comparative study, *Colloids and Surfaces B: Biointerfaces,* **64**, 170-178.

Gupta V.K., Rastogi, A., (2009), Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogonium hatei* from aqueous solutions, *Journal of Hazardous Materials*, **163**, 396-402.

Gupta V.K., Carrott P.J.M., Ribeiro Carrott M.M.L., Suhas (2009), Low-Cost Adsorbents: Growing Approach to Wastewater Treatment-a Review, C*ritical Reviews in Environmental Science and Technology*, **39**, 783-842.

Gupta V.K., Rastogi A., Nayak, A., (2010), Biosorption of nickel onto treated alga (*Oedogonium hatei*):

Application of isotherm and kinetic models, *Journal of Colloid and Interface Science*, **342**, 533-539.

Han R., Li H., Li Y., Zhang J., Xiao H., Shi J., (2006), Biosorption of copper and lead ions by waste beer yeast, *Journal of Hazardous Materials,* **B137**, 1569-1576.

Heuer H., Krsek M., Baker P., Smalla K., Wellington E.M., (1997), Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel electrophoretic separation in denaturing gradients, *Applied and Environmental Microbiology*, **63**, 3233-3241.

Hlihor R.M., Gavrilescu M., (2009), Removal of some environmentally relevant heavy metals using low-cost natural sorbents, *Environmental Engineering and Management Journal*, **8**, 353-372.

Hoffman C.S., Winston F., (1987), A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli. Gene*, **57**, 267-272.

Holan Z.R., Volesky B., (1994), Biosorption of lead and nickel by biomass of marine algae, *Biotechnology and Bioengineering*, **43**, 1001-1009.

Iskandar N.L., Zainudin N.A.I.M., Tan S.G., (2011), Tolerance and biosorption of copper (Cu) and lead (Pb) by filamentous fungi isolated from a freshwater ecosystem, *Journal of Environmental Sciences*, **23,** 824-830.

Jukes T.H., Cantor C.R., (1969), *Evolution of protein molecules*, In: *Mammalian Protein Metabolism, Vol. 3*, Munro H.N. (Ed.), Academic Press**,** New York, **21-132**.

Kang S.Y., Lee J.U., Kim K.W., (2008), Biosorption of Pb(II) from synthetic wastewater onto *Pseudomonas aeruginosa*, *Journal of Environmental Pollution*, **34**, 195-202.

Kefala M.I., Zouboulis A.I., Matis K.A., (1999), Biosorption of cadmium ions by Actinomycetes and separation by flotation, *Environmental Pollution*, **104**, 283-293.

Kicsi A., Cojocaru C., Macoveanu M., Bilba D., (2006), Optimization of batch process variables using response surface methodology for Cu^{2+} removal from aqueous solution by peat adsorbent *Environmental Engineering and Management Journal*, **5,** 1291-1300**.**

Kujan P., Prell A., Safar H., Sobotka M., Rezanka T., Holler P., (2005), Removal of copper ions from dilute solutions by *Streptomyces noursei* mycelium comparioson with yeast biomass, *Folia Microbiologia*, **50**, 309-313.

Küster E., Williams S.T., (1964), Selection of media for isolation of *Streptomycetes*, *Nature (London)*, **202**, 928-929.

Lawal O.S., Sanni A.R., Ajayi I.A., Rabiu O.O., (2010), Equilibrium, thermodynamic and kinetic studies for the biosorption of aqueous lead(II) ions onto the seed husk of *Calophyllum inophyllum*, *Journal of Hazardous Materials*, **177**, 829-835**.**

Lechevalier M.P., Lechevalier H., (1970), Chemical composition as a criterion in the classification of aerobic actinomycetes, *International Journal of Systematic Bacteriology*, **20**, 435-443.

Li H., Lin Y., Guan W., Chang J., Xu L., Guo J., Wei G., (2010), Biosorption of Zn(II) by live and dead cells of the *Streptomyces ciscaucasicus* strain CCNWHX 72-14, *Journal of Hazardous Materials,* **179**, 151-159.

Liu Y., Cao Q., Luo F., Chen J., (2009), Biosorption of Cd^{+2}, Cu^{+2}, Ni^{+2} and Zn^{+2} ions from aqueous solutions by pretreated biomass of brown algae, *Journal of Hazardous Materials* , **163**, 931-938.

Lu W.B., Shi J.J., Wang C.H., Chang J.S., (2006), Biosorption of lead, copper and cadmium by an indigenous isolate *Enterobacter* sp. J1 possessing high heavy-metal resistance, *Journal of Hazardous Materials*, **B134**, 80-86.

Mattuschka B., Straube G., (1993), Biosorption of metals by a waste biomass, *Journal of Chemical Technology and Biotechnology*, **58**, 57-63.

Mc Carthy A.J., Williams S.T., (1990), Methods for studying the Actinomycetes, *Methods in Microbiology*, **22**, 533-563.

Mengoni A., Barzanti R., Gonelli C., Gabrielli R., Bazzicalupo M., (2001), Characterization of nikel-resistant bacteria isolated from serpentine soil, *Environmental Microbiology*, **3**, 691-698.

Mondal M.K., (2009), Removal of Pb(II) ions from aqueous solution using activated tea waste: Adsorption on a fixed-bed column, *Journal of Environmental Management*, **90**, 3266-3271.

Muraleedharan T.R., Iyengar L., Venkobachar C., (1995), Screening of tropical wood-rotting mushrooms for copper biosorption, *Applied and Environmental Microbiology*, **61**, 3507-3508.

Nriagu J.O., Pacyma J.M., (1988), Quantitative assessment of world-wide contamination of air, water and soils by trace elements, *Nature*, **333**, 134-139.

Ofomaja A.E., Naidoo E.B., Modise S.J., (2010). Biosorption of copper (II) and lead (II) onto potassium hydroxide treated pine cone powder, *Journal of Environmental Management*, **91**, 1674-1685.

Paduraru C., Tofan L.**,** (2008), Investigations on the possibility of natural hemp fibers use for Zn(II) ions removal from wastewaters**,** *Environmental Engineering and Management Journal***,** **7,** 687-693**.**

Ravel J., Amoroso M.J., Colwell R.R., Hill R.T., (1998), Mercury resistant actinomycetes from Chesapeake Bay, *FEMS Microbiology Letters*, **162**, 177-184.

Rehman A., Farooq H., Hasnain S., (2008), Biosorption of copper by yeast, *Loddermyces elongisporus*, isolated from industrial effluents: its potential use in wastewater treatment, *Journal of Basic Microbiology*, **48**, 195-201.

Sag Y., Ozer D., Kutsal T., (1995), A comparative study of the biosorption of lead (II) ions to *Z. ramigera* and *R. arrhizus*, *Process Biochemistry*, **30**, 169-174.

Sağ Y., Ataçoğlu I., Kutsal T., (2000a), Equilibrium parameters for the single-and multicomponent biosorption of Cr(VI) and Fe(III) ions on *R. arrhizus* in a packed column, *Hydrometallurgy*, **55**, 165-179.

Sağ Y.,Yalçuk A., Kutsal T., (2000b), Mono and multi-component biosorption of heavy metal ions on *Rhizopus arrhizus* in a CFST, *Process Biochemistry*, **35**, 787-799.

Saitou N., Nei M., (1987), The neighbor-joining method, a new method for reconstructing phylogenetic trees, *Molecular Biology and Evolution*, **4**, 406-425.

Sarı A., Tuzen M., (2009), Kinetic and equilibrium studies of biosorption of Pb(II) and Cd(II) from aqueous solution by macrofungus (*Amanita rubescens*) biomass, *Journal of Hazardous Materials,* **164**, 1004-1011.

Stoll A., Duncan J.R., (1997), Comparison of the heavy metal sorptive properties of three types of immobilized, non viable *Saccharomyces cerevisiae* biomass, *Process Biochemistry*, **32**, 467-472.

Sun F., Shao Z., (2007), Biosorption and bioaccumulation of lead by *Penicillium* sp. Psf-2 isolated from the deep sea sediment of the Pacific Ocean, *Extremophiles*, **11**, 853-858.

Tamura K., Dudley J., Nei M., Kumar S., (2007), MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0., *Molecular Biology and Evolution*, **24**, 1596-1599.

Uluozlu O.D., Sari A., Tuzen M., Soylak M., (2008), Biosorption of Pb(II) and Cr(III) from aqueous solution by lichen (*Parmelina tiliaceae*) biomass, *Bioresource Technology*, **99,** 2972-2980**.**

Valdman E., Erijman L., Pessoa F.L.P., Leite S.G.F., (2001), Continuous biosorption of Cu and Zn by immobilized biomass *Sargassum sp*., *Process Biochemistry*, **36**, 869-873.

Vimala R., Das N., (2009), Biosorption of cadmium (II) and lead (II) from aqueous solutions using mushrooms: A comparative study, *Journal of Hazardous Materials,* **168**, 376-382.

Weisburg W.G., Barns S.M., Pellelier D.A., Lane D.J., (1991), 16S ribosomal DNA amplification for phylogenetic study, *Journal of Bacteriology*, **173**, 697-703.

Williams S.T., Cross T., (1971), Actinomycetes, *Methods in Microbiology*, **4**, 295-334.

Yan G., Viraraghavan T., (2001), Heavy metal removal in a biosorption column by immobilized *M. rouxii* biomass, *Bioresource Technology*, **78**, 243-249.