Bioresource Technology 100 (2009) 1533–1538

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Biosorption of chromium, copper, manganese and zinc by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum

Rosa María Pérez Silva[a], Arelis Ábalos Rodríguez[a], José Manuel Gómez Montes De Oca[b,*], Domingo Cantero Moreno[b]

[a] *Study Centre of Industrial Biotechnology, Faculty of Natural Sciences, University of East Santiago of Cuba, CP 90500, Cuba*
[b] *Department of Chemical Engineering, Food Technology and Environmental Technologies, University of Cádiz, CP11510 Peurto Real, Cádiz, Spain*

ARTICLE INFO

Article history:
Received 5 April 2007
Received in revised form 23 June 2008
Accepted 24 June 2008
Available online 23 October 2008

Keywords:
Pseudomonas aeruginosa
Heavy metals
Biosorption

ABSTRACT

The study describes the sorption of Cr, Cu, Mn and Zn by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum and heavy metals. The concentrations studied were 50, 49, 60 and 70 (mg L^{-1}) for Cr, Cu, Mn and Zn, respectively. The solution pH and ionic strength were very important factors in the metal biosorption performance and the biosorption capacity of *P. aeruginosa* AT18 for Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+}. In aqueous solution, the biosorption increased with increasing pH in the range 5.46–7.72. The results obtained in the experimental assays show that *P. aeruginosa* AT18 has the capacity for biosorption of the metallic ions Cr^{3+}, Cu^{2+} and Zn^{2+} in solutions, although its capacity for the sorption of manganese is low (22.39 mg Mn^{2+}/g of biomass) in comparison to the Cr^{3+}, Cu^{2+} and Zn^{2+} ions, as shown by the individual analyses. However, 20% of the manganese was removed from an initial concentration of 49.0 mg L^{-1}, with a Q_m value similar to that obtained in solutions containing mixtures of Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+}. The chromium level sorbed by *P. aeruginosa* AT18 biomass was higher than that for Cu, Mn and Zn, with 100% removal in the pH range 7.00–7.72 and a Q_m of 121.90–200.00 mg of Cr^{3+}/g of biomass. The removal of Cr, Cu and Zn is also a result of precipitation processes.

1. Introduction

The presence of heavy metals in aquatic environments is known to cause severe damage to aquatic life, and the fact that these metals kill microorganisms during the biological treatment of wastewater causes a delay in the water purification process. Most heavy metal salts are soluble in water and form aqueous solutions and, as a consequence, cannot be separated by ordinary physical separation methods (Hussein et al., 2004a,b).

Physico-chemical methods such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange and membrane technologies have been widely used to remove heavy metal ions from industrial wastewater. These processes may be ineffective or expensive, especially when the heavy metal ions are in solutions dissolved heavy metal ions/L containing in the order of 1–100 mg (Volesky, 1990; Volesky and Holan, 1995). Biological methods such as biosorption/bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physico-chemical methods Kapoor and Viraraghvan, 1995).

Bioadsorption of heavy metals is one of the most promising technologies involved in the removal of toxic metals from industrial waste streams and natural waters. It is a potential alternative to conventional processes for the removal of metals, such as ion exchange processes. If the biomass employed is a waste material, then it represents a cheap alternative to conventional processes due to the use of a low cost sorbent material (Shumate and Strandberd, 1985).

Microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Zhou and Kiff, 1991; Andres et al. 1993; Fourest and Roux, 1992; Hussein et al., 2001, 2004a). Feasibility studies for large-scale applications demonstrated that biosorptive process is more applicable than the bioaccumulative processes, because living systems (active uptake) often require the addition of nutrients and hence increase biological oxygen demand (BOD) or chemical oxygen demand (COD) in the effluent. In addition, maintenance of healthy microbial population is difficult due to metal toxicity and other unsuitable environmental factors. In addition, the potential for desorptive metal recovery is restricted since metal may be intracellular bound, metabolic products may form complexes with metals to retain them in solution, and mathematical modeling of a non-defined system is difficult (Brown and Lester, 1982; Ajmal et al. 1996; Dilek et al. 1998).

The use of adsorbents of biological origin has emerged in the last decade as one of the most promising alternatives to conventional

---

* Corresponding author. Tel.: +34 956016381; fax: +34 956016411.
E-mail addresses: rmaria@cebi.uo.edu.cu (R.M. Pérez Silva), josemanuel.montes-deoca@uca.es (J.M. Gómez Montes De Oca).

0960-8524/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2008.06.057

heavy metal management strategies (Shumate and Strandberd, 1985; Eccles, 1990; Macaskie, 1990; Tsezos, 2001). The absence a of rational method for a priori prediction of the biosorption potential of a microorganism means that the only method for identifying and developing newer and more efficient biosorbents is the sustained screening of microbes (Muraleedharan and Venkobachar 1990).

The key factors that should be considered in the application of a technology for the removal of toxic metals from industrial waste solutions are the use of low cost waste biomass, the cost of biomass immobilization and the possibility of continuous re-use of the biomass (Volesky, 2003). Many microorganisms are able to accumulate heavy metals from solutions. Accumulation mechanisms vary, although a passive physico-chemical phenomenon appears to be the most common. This is based on adsorption, ion exchange, complexation and/or micro-precipitation (Volesky, 1986). Biomass cell walls, which mainly consist of polysaccharides, proteins and lipids, offer many functional groups that can bind metal ions and these include carboxylate, hydroxyl, phosphate and amino groups. In some cases, adsorption on the external cell surface is a biomass defence system against toxic heavy metals, with the microorganisms producing an external polymeric layer to avoid metal penetration through the cell wall (Scott and Karanjkar, 1992).

Metal sorption performance depends on some external factors such as pH, the presence of others ions in solution (which may be in competition), organic material in solution (such as complexing agents), cell metabolic products in solution (which may cause metal precipitation) and temperature (Kuyucak and Volesky, 1988).

Adsorption is a phenomenon that has been studied in the bioremediation of heavy metals diverse as, for example, cadmium, chromium, lead, nickel, zinc and copper (Macaskie et al. 1987; Jianlong et al., 2001; Liu et al., 2003; Srinath et al., 2002; Tangaromsuk et al., 2002). Microorganisms (bacteria, fungi and algae) utilised as biosorbents were isolated from polluted ecosystems, and retained the heavy metals in relatively short time frames in contact with these metals solutions. In this process, the biomasses capable of participating in the sorption process are extracted easily from the aqueous systems.

Biosorption of Cr, Cu, Mn and Zn by *Pseudomonas aeruginosa* AT18 (isolated from the sites contaminated with petroleum) in artificial samples has been studied. It was found that the heavy metals contained in these samples decreased with time (Pérez et al. 2006a; Pérez et al. 2006b). The aim of this paper is to study the biosorption of these heavy metal ions by *P. aeruginosa* AT18 strains in single and complex solutions at intervals of pH 3–7.72 units.

2. Methods

2.1. Microorganisms and culture harvest and use

2.1.1. Biomass production

P. aeruginosa AT18 strain was isolated from the petroleum-contaminated soil at the Petroleum Refinery "Hermanos Díaz" in Cuba (Pérez et al., 2008). This strain, deposited in the Culture Collection of the Industrial Study Biotechnology Centre in the West University (Santiago of Cuba), was maintained on nutrient agar slants. The stock culture was transferred weekly and stored at ambient temperature (30 °C). Biomass of *P. aeruginosa* AT18 was developed by growing in nutrient broth (pH 7.0) at room temperature (28 ± 3 °C) for 24 h. Cells were harvested by centrifugation at 10 509*g* for 15 min (Sorvall RC-5B plus centrifuge, 12166 rotor). Cell suspensions of *P.* aeruginosa AT18 were prepared by resuspending a cell pellet in distilled water for use in biosorption experiments. All the experiments were carried out with resting cells. Biomass concentration in cell suspensions was determined by dry weight at 105 °C.

2.2. Cultivation conditions for biodegradation experiments

The mineral medium used for *P. aeruginosa* AT18 contained (g L^{-1}) NH_4Cl, 0.1; K_2HPO_4, 0.1; KH_2PO_4, 0.05; $CaCl_2$, 0.001; KCl, 0.01; $FeSO_4 \cdot 7H_2O$, 0.001; and $MgSO_4 \cdot 7H_2O$, 0.05, and a mixture of oligoelements (0.05 ml L^{-1}), whose composition was B (0.02% v/v), Cu (0.05% v/v), Mn (0.05% v/v), Mo (0.006% v/v) and Zn (0.07% v/v). The carbon sources (0.2% w/v in all cases) used were M30PP, lubricant oil, kerosene, toluene and naphthalene. Controls without carbon sources were performed. The inoculum used was 2% (v/v) of a cellular suspension of a concentration of 0.5 McFarland (Jorgensen et al., 1999). The strain was incubated in 500 ml Erlenmeyer flasks containing 100 ml of the medium at 30 °C for 20 days with shaking at 150 rpm in a reciprocal shaker. All experiments were carried out in triplicate (Pérez et al. 2006b).

2.3. Analytical determination

2.3.1. Metal solutions

The heavy metal adsorbates used in this study were chromium (Cr_2O_3), copper ($Cu(NO_3)_2$), manganese ($MnSO_4$) and zinc ($ZnSO_4$). Single stock solutions of chromium (60 mg L^{-1}), copper (50 mg L^{-1}), manganese (50 mg L^{-1}) and zinc (80 mg L^{-1}) were prepared by dissolving appropriate quantities of pure metal powders in 1% nitric acid.

2.3.2. Stock solutions

Solutions were adjusted to the desired pH values with 0.1 M sodium hydroxide and 0.1 M nitric acid. Chromium, copper, manganese and zinc concentrations were determined using an Iris Intrepid Thermo Elemental Inductive Coupling Plasma Atomic Emission Spectrophotometer (ICP-AES).

2.3.3. Biomass

The qualitative analysis of the biomass obtained after 1 h in the experiments of bioadsorption by *P. aeruginosa* AT18 strain was realised by Energy Dispersive Spectrometry (EDS) analysis using instruments such as scanning electron microscope FIE Quanta-200 (Philips) and an X-ray micro-analyser EDAX 9100/60 (Philips, Holland). Samples of biomass were dried and coated with gold prior to the EDS analysis.

2.4. Procedure of sorption experiments

2.4.1. Metal sorption studies

A batch equilibrium method was used to determine the sorption of chromium, copper, manganese and zinc by P. aeruginosa AT18. A set of 250 mL Erlenmeyer flasks containing 100 mL of metal solution was used in the experiments. Cell suspensions (10 mL) were exposed to metal solutions for 72 h on a rotary shaker at 150 rpm. Biomass was separated by centrifugation at 10 000 rpm for 15 min, and the supernatants were analysed for residual metal concentration. Metal adsorbed by P. aeruginosa AT18 biomass (Q, mg metal/g dry biomass) was calculated as

$$Q = V(C_0 - C_e)/X, \tag{1}$$

where Q is the specific metal uptake (mg metal/g biosorbent), V is the volume of the metal solution (mL), C_0 is the initial concentration of metal in the solution (mg metal/L), C_e is the final concentration of metal in the solution (mgL^{-1}), and X is the dry weight of the biomass (g).

The metal sorption ability of the biomass was determined by the above-mentioned procedure, in all the following experiments, unless stated otherwise.

2.4.2. Effect of pH

To check the effect of pH on biosorption, the biomass of *P. aeruginosa* AT18 strain was conditioned to different pH environments (ranging between 3 and 8). Suspensions of pH-conditioned biomass (10 mL) were then contacted with metal solutions of the corresponding pH.

2.4.3. Effect of contact time

To examine metal biosorption kinetic, 10 mL of cell suspension (10 mg of dry cell biomass) was contacted with 100 mL aliquots of metal solutions in 250 mL Erlenmeyer flask. Experiments were performed in duplicate along with appropriate controls run simultaneously. Flasks were incubated on a rotary shaker at room temperature (26–30 °C). Samples of metal solutions were withdrawn from each flask at different time intervals (0–72 h) and were analysed for residual metal content. The study is realised in individual samples for each metal, and the solution mixture of metals is studied.

3. Results and discussion

3.1. Growth P. aeruginosa AT18 on heavy metals

To examine the tolerance of heavy metals by *P. aeruginosa* AT18, the cells were cultivated in nutrient broth with concentrations of Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+}. It appears that the cells were able to survive metal concentrations as high as 300 mg L^{-1} for Cu^{2+}, 150 mg L^{-1} for Mn^{2+}, 100 mg L^{-1} for Cr^{3+} and 320 mg L^{-1} for Zn^{2+}.

Fig. 1 shows the growth curves of *P. aeruginosa* AT18 in the presence of heavy metals. The observed growth is similar for all cases, and the obtained specific growth rates are: $1.23 \times 10^{-1}\ h^{-1}$ for Cr^{3+}, $1.33 \times 10^{-1}\ h^{-1}$ for Cu^{2+}, $1.06 \times 10^{-1}\ h^{-1}$ for Mn^{2+} and $1.5 \times 10^{-1}\ h^{-1}$ for Zn^{2+}. However, with the presence of the mixture of four heavy metals, the growth rates of *P. aeruginosa* AT18 were inferior to the realised individual heavy metals. The growth was major in Zn^{2+} followed by Cu^{2+}, Cr^{2+} and Mn^{2+}. The resistance of *P. aeruginosa* AT18 to high concentration of the four heavy metals suggests its suitability for heavy metal treatment.

3.2. Adsorption of Cr³⁺, Cu²⁺, Mn²⁺ and Zn²⁺ by P. aeruginosa AT18 (CCCEBI 1044)

During the study on the influence of Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} in biodegradation of petroleum by *P. aeruginosa* AT18, it was observed that the concentration of these metals in the medium studied was considerably low, and that chromium was more attractive. What is more *Pseudomonadaceas*, for example *P. aeruginosa*, has demonstrated to be efficient in the accumulation of different heavy metals present in the contaminated effluents (Hussein, 1999; Hussein et al., 2001, 2005). For this, the study of bioadsorption of the heavy metals such as Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} by *P. aeruginosa* AT18 strain was realised.

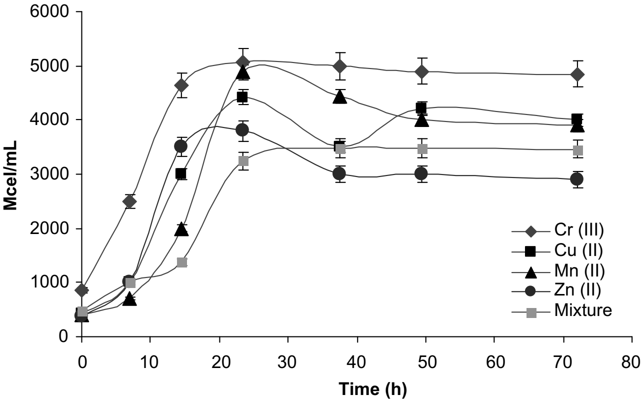

Fig. 1. Growth of *Pseudomonas aeruginosa* AT18 in nutrient both with 300 mg L^{-1} for Cu^{2+}, 150 mg L^{-1} for Mn^{2+}, 100 mg L^{-1} for Cr^{3+} and 320 mg L^{-1} for Zn^{2+}.

3.3. Capacity of P. aeruginosa AT18 for adsorption of Cr³⁺, Cu²⁺, Mn²⁺ and Zn²⁺

The microorganisms and these products can be very efficient accumulators of metals. For this reason, the technologies based in the microorganisms are often the alternative treatments that are viable or and that helping conventional techniques in the elimination and/or recuperation of metals.

The experience on the study of capacity of biomass *P. aeruginosa* AT18 in the adsorption of the metallic ions Cr^{3+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} in aqueous solution was realised in triplicate.

3.4. Bioadsorption of Cr³⁺, Cu²⁺, Mn²⁺ and Zn²⁺ of mixture dissolution by P. aeruginosa AT18

The biosorption of a mixture of metal ions Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} using *P. aeruginosa* AT18 was investigated. The concentration of each metal was 50, 60 and 70 mg L^{-1} for copper, manganese, chromium and zinc, respectively.

The Langmuir and Freundlich adsorption models were used to evaluate the adsorption behaviour on biosorbent P. aeruginosa AT18. The Langmuir model gave the maximum sorption data of Cr^{3+} Cu^{2+}, Mn^{2+} and Zn^{2+} by *P. aeruginosa*. The *b* constant affinity is represented between the sorbent and the sorbet, and the maximum capacity of adsorption is estimated.

$$\frac{C_e}{Q} = \frac{K_d}{Q_m} + \frac{1}{Q_m} C_e \qquad (2)$$

$$\ln Q = \ln K + \frac{l}{n} \ln C_e \qquad (3)$$

Table 1
Kinetic parameters obtained in Langmuir and Freundlich models applied the study bioadsorption in metallic ions Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} by *Pseudomonas aeruginosa* AT18 in mixture solution

Metal	pH	Langmuir			Freundlich		
		K_d	Q_m	r^2	K	n	r^2
Cr^{3+}	3.09	0.770	5.85	0.9093	0.290	0.6890	0.9190
	5.46	1.290	59.50	0.9064	0.100	0.7280	0.9933
	6.25	0.121	99.68	0.9973	0.010	0.7370	1.0000
	7.0	0.152	121.90	0.9975	0.020	0.7670	1.0000
	7.72	0.203	200.00	0.9957	0.022	0.8580	1.0000
Cu^{2+}	3.09	0.751	2.20	0.8890	0.345	0.7151	0.9011
	5.46	0.004	55.25	1.0000	5×10^{-7}	0.8418	1.0000
	6.25	0.104	86.95	0.9994	6×10^{-5}	0.9762	1.0000
	7.0	0.051	5.25	1.0000	5×10^{-6}	0.8423	1.0000
Mn^{2+}	3.09	0.441	6.60	0.8990	0.369	0.3599	0.9071
	5.46	6.510	13.70	0.9010	0.537	0.5374	0.9781
	6.25	4.440	17.70	0.9531	0.304	0.3045	0.9972
	7.0	0.443	20.32	0.9744	0.225	0.2267	1.0000
	7.72	0.275	12.38	0.9722	0.354	0.3541	0.9821
Zn^{2+}	3.09	1.791	1.90	0.8956	0.745	0.2032	0.9626
	5.46	6.192	10.00	0.9967	0.844	1.1355	0.9922
	6.25	7.334	21.9	1.0000	0.716	0.0949	0.9924
	7.0	4.871	74.5	0.9872	0.657	0.7312	0.9999
	7.72	6.332	56.4	0.9679	0.790	0.0834	0.9932

r^2, regression coefficient of kinetic curves obtained of Langmuir and Freundlich models.

The Langmuir and Freundlich constant models, and thus the correlation coefficients for the bioadsorption of copper by the biomass of *P. aeruginosa* AT18 are listed in Table 1. The parameters of Langmuir model were dependents of initial valour of pH of solution. The maximum capacity (Q_m) of the metal and the constant of equilibrium (K_d) increase with increase of initial pH and decrease when the pH valour is superior to 7.0, except for chromium.

The initial pH in the mixed metal ion solution is the most important factor for the capture of metal ions by the *P.* aeruginosa AT18 biomass. The low adsorption at pH 3.09 increases with the increase in the pH. The highest level of adsorption was observed for the Cr^{3+} ions, and the maximum adsorption capacity of 200.00 mg Cr^{3+}/g of biomass was obtained at pH 7.72, which represents the removal of 99.6% of Cr^{3+} present in the solution.

The sorption of copper at low pH is indicative of competition with the excess protons in the cell wall (Puranik and Paknikar, 1999). An increase in the sorption of copper was observed on increasing the pH, with the highest removal obtained at a pH of 6.25. However, higher pH values led to a decrease in removal efficiency. A study at pH 7.0 was not carried out because copper hydroxide would precipitate out of solution at this pH. *P. aeruginosa* AT18 removed 95.0% of the Cu^{2+} ions at pH 6.25, with a sorption capacity of 86.95 mg of Cu^{2+}/g of biomass. Lower values were obtained both at lower and at higher pH, demonstrating that 6.25 is the optimum pH for the adsorption of copper by *P. aeruginosa* AT18.

The sorption of Zn^{2+} ions by the *P. aeruginosa* AT18 biomass in the mixture was lower than 15 mg of Zn^{2+}/g of biomass at pH values below 6.0. However, at higher pH, Zn^{2+} is sorbed by the biomass, and pH 7.0 proved to be the optimum pH with 87.7% of the metal removed and with a maximum biosorption capacity of 77.5 mg of Zn^{2+}/g of biomass. At pH values above 7, a decrease was observed in the removal of this metal by the strain under investigation.

In the experiments, it was found that manganese was adsorbed to the lowest extent by the *P.aeruginosa* AT18 biomass, with a maximum biosorption capacity of 38.2 mg of Mn^{2+}/g of biomass and a removal of only 21.69% at pH 7.0. It is believed that this difference in behaviour is due to the fact that Mn2+ is more soluble than Cr^{3+}, Cu^{2+} and Zn^{2+}, meaning that a proportion of the Mn^{2+} was taken up by the functional groups in the cell wall – this metal can subsequently be desorbed into solution. However, of the metals studied, manganese has the highest atomic mass and this parameter could also influence the adsorption process (Chen et al. 2000).

The maximum sorption capacity of *P. aeruginosa* AT18 at pH values below 6.0 units was low. This dependence of biosorption on pH is caused by the presence of functional groups in the cell wall and the chemical configuration of the metal. The cell wall contains amines, amides and carboxylic acid functional groups that are either protonated or deprotonated, depending on the pH of the aqueous medium. Increasing the pH increases the negative charge at the surface of the cells until all relevant functional groups are deprotonated, a situation that favours electrochemical attraction and sorption of cations. Furthermore, the increase in metal uptake on increasing the pH may be the result of more efficient competition between cations and H^+ for the binding sites on bacteria. Anions would be expected to interact more strongly with the cells as the concentration of positive charges increases, as a result of protonation of the functional groups at low pH values (Puranik and Paknikar, 1999).

Microscopy electronic analysis and EDS of this sample of biomass taken after 1 h incubation showed the presence of metallic ions Cr^{3+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} in the biomass, besides other constituent groups of the cell wall. Among the ionic metals, chromium was found in major proportion in the cell wall and manganese was found in low proportion, demonstrating the result obtained in the experiment.

3.5. Bioadsorption of Cr3+, Cu2+, Mn2+ and Zn2+ by P. aeruginosa AT18 in independent solutions

The optimum obtained time at the pH for the adsorption of Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} in the solution mixture of these metals was prudently realized in the study of adsorption for each metal individually, with the purpose of knowing a lot about the influence that could cause the mixture of these metals in the adsorption process by *P. aeruginosa* AT18. The study was realized in a period of 72 h at the initial pH corresponding to each metal.

During the adsorption of Cr^{3+} ions by *P. aeruginosa* AT18, the cellular concentration changes depending on time (Fig. 2). It was also observed that at the one hours of contact of dissolution with biomass, the concentration decrease from 60 mgL^{-1} to 3.8 mgL^{-1}, and the bioadsorption in equilibrium was determined at 28 h. The constant kinetic parameters of adsorption (Kd) and the maximum capacity (Qm) for the adsorption of Cr^{3+} by *P. aeruginosa* AT18 were determined by experimental data (Table 2). *P. aeruginosa* AT18 in the assay conditions has the maximum capacity of adsorption of 200.45 mg Cr^{3+} /g of biomass at a pH of 7.72, similar to that obtained in the solution mixture (200.00 mg Cr^{3+}/g of biomass), showing that the mixture of the four metals in this study does not have significant influence on the bioadsorption of Cr^{3+}. *P. fluorescens* bioadsorbs Cr^{3+} obtaining 13.26 mg Cr^{3+}/g of biomass to a maximum capacity of bioadsorption (Hussein et al., 2005).

The kinetic curves of Langmuir and Freundlich have the lineal comportment with correlation coefficients proximate to 1, being superior in the Freundlich model.

Table 2 shows the kinetic parameters calculated by applying the kinetic Langmuir and Freundlich models on the basis of the experimental data obtained during the process bioadsorption of Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} by *P. aeruginosa* AT18.

The copper ions were removed (96%) from the solution by *P. aeruginosa* AT18 at optimum pH with the adsorption being superior (113.64 mg/g of biomass) to that obtained in the solution mixture (86.95 mg/g of biomass), the other metal ions in the medium

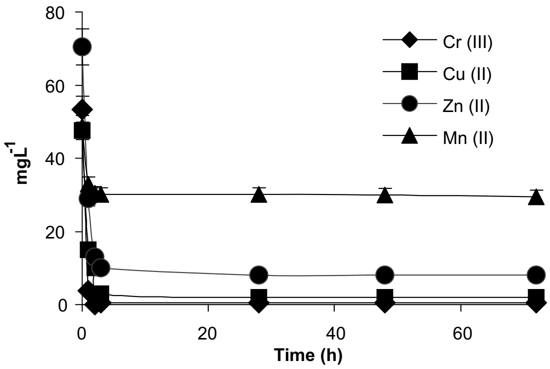

Fig. 2. Concentration of metallic ions: Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+}, evolution in the solution on the biomass of *Pseudomonas aeruginosa* AT18.

Table 2
Kinetic parameters obtained of Langmuir and Freundlich models applied the study of bioadsorption of Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} by *Pseudomonas aeruginosa* AT18

Metal	pH	Langmuir			Freundlich		
		K_d	Q_m	r^2	K	n	r^2
Cr^{3+}	7.72	0.195	200.45	0.9957	0.0002	0.7621	1.0000
Cu^{2+}	6.25	0.159	113.64	0.9994	4×10^{-5}	0.7151	1.0000
Mn^{2+}	7.00	0.443	22.39	0.9744	0.0002	0.9757	1.0000
Zn^{2+}	7.00	2.877	87.72	0.9872	0.0020	0.8883	0.9999

(Cr^{3+}, Mn^{2+} and Zn^{2+}) can compete with Cu^{2+} ions for capturing upon the functional group of the cell wall of *P. aeruginosa* AT18, thereby obtaining much less capacity. Saxema et al., 2001 studied removal of Cu^{2+} in wastewater by *P. putida* S4, obtaining that this strain is capable of removing more than 80% of Cu^{2+} present in the wastewater. The initial pH of the medium has an influence on the ability of the *Bacillus*, *Pseudomonas* and *Micrococcus* bacteria isolated from activated sludge. In this process, the adsorption of Cu^{2+} is superior the more high is the pH (>20 mg/g of biomass at pH > 3.5 units) (Low et al., 1998). Savvaidis et al. (2003) also studied the bioadsorption of Cu^{2+} by the *Pseudomonas* genus strain isolated from the environment contaminated with heavy metals. The study was realised in the intervals of 3–8 pH unit and obtained that *P. cepacia* showed high capacity bioadsorbance with Q_m of 20.32–25.4 and 40.0–45.72 mg/g for a biomass of 317.5 mg L^{-1} and 635 mg L^{-1}, respectively, for initial concentrations (Savvaidis et al. 2003).

The adsorption of Zn^{2+} ions by *P. aeruginosa* AT18 in the individual solution of this metal is higher than the solution mixture of Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+}. *P. aeruginosa* AT18 adsorbed 87.72 mg Zn^{2+}/g of biomass, higher than that obtained in the solution mixture (74.5 mg Zn^{2+}/g of biomass), the 94% of zinc present in the solution was removed. *Brevibacterium sp.* HZM-1, isolated from one of the soils of zinc mine showed to high adsorption this metal, with a Q_m of 41.85 mg of Zn^{2+}/g of biomass. This biomass was also capable of adsorbing other metallic ions such as Cu^{2+} (Taniguchi et al., 2000). *Thiobacillus ferroxidans* also presented a high capacity of adsorbing Zn^{2+} ions in aqueous solution (82.61 mg/g of biomass) for a period of 30 min (Celaya et al., 2000).

Solution pH and ionic strength play crucial roles in determining the metal bioadsorption performance. Bioadsorption capacity of *P. aeruginosa* AT18 for Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} in water increased with increasing pH in the pH range of 5.46–7.72. The results obtained from experimental assay showed that *P. aeruginosa* AT18 has the capacity of adsorbing the metallic ions Cr^{3+}, Cu^{2+} and Zn^{2+} in the solutions presents; however, it showed a low capacity for the adsorption of manganese, as in the analyses of individual solutions low level of adsorption was obtained (22.39 mg Mn^{2+}/g of biomass) compared with the Cr^{3+}, Cu^{2+} and Zn^{2+} ions. However, the removal of 20% of manganese from the initial concentration of 49.0 mg L^{-1} with Q_m similar to that obtained in the solution mixture of Cr^{3+}, Cu^{2+}, Mn^{2+} and Zn^{2+} was studied.

4. Conclusions

The removal of Cr, Cu and Zn is also a result of precipitation processes. The study demonstrated that competition besides sorption and precipitation processes can play a role in the removal of these heavy metals. This competition at rising pH is possibly due to enhanced alkali ion concentration and to the complexation of the functional groups at the cell wall of *P. aeruginosa*. Consequently, the metal removal above a pH of 5.5–5.8 is frequently originated from mechanisms other than biosorption, as well as the decreasing removal data above pH 7.0. Further research work will be necessary to elucidate the contribution of the particular processes.

References

Ajmal, M., Rafaqat, A.K., Bilquees, A.S., 1996. Studies on removal and recovery of Cr(VI) from electroplating wastes. Water Res. 30, 1478–1482.

Andres, Y., MacCordick, H.J., Hubert, J.C., 1993. Adsorption of several actinide (Th, U) and lanthanide (La, Eu, Yb) ions by *Mycobacterium smegmatis*. Appl. Microbiol. Biotechnol. 39, 413–417.

Brown, M.J., Lester, J.N., 1982. Role of bacterial extracellular polymers in metal uptake in pure bacterial culture and activated sludge. Water Res. 16, 1539–1548.

Celaya, R.J., Noriega, J.A., Yeomans, J.H., Ortega, L.J., Ruiz-Manriquez, A., 2000. Biosorption of Zn(II) by *Thiobacillus ferrooxidans*. Bioprocess Eng. 22 (6), 539–542.

Chen, B.Y., Utgikar, V.P., Harmon, S.M., Tabak, H.H., Bishop, D.F., Grovind, R., 2000. Studies on biosorption of zinc(II) and copper(II) on *Desulfovibrio desulfuricans*. Int. Biodeterior. Biodegrad. 46, 11–18.

Dilek, F.B., Gokcai, C.F., Yetis, U., 1998. Combined effects of Ni(II) and Cr(VI) on activated sludge. Water Res. 32 (2), 303–312.

Eccles, H., 1990. Biosorption of heavy metals: a scientist's dream or a commercial reality. J. Chem. Tech. Biotechnol. 49, 330–334.

Fourest, E., Roux, J.C., 1992. Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. Appl. Microbiol. Biotechnol. 37 (3), 399–403.

Hussein, H.M.A., 1999. Influence of Heavy Metal Ions on the Biodegradation of Hazardous Waste Water Components. Ph.D. Thesis, Faculty of Science, Alexandria University, Alexandria, Egypt.

Hussein, H., Krull, R., Abou El-Ela, S.I., Hempel, D.C., 2001. Interaction of the different heavy metal ions with immobilized bacterial culture degrading xenobiotic wastewater compounds. In: Proceedings of the Second International Water Association World Water Conference, Berlin, Germany, 15–19, October, 2001.

Hussein, H., Farag, S., Moawad, H., 2004a. Isolation and characterization of *Pseudomonas* resistant to heavy metals contaminants. Arab. J. Biotechnol. 7, 13–22.

Hussein, H., Farag, S., Kandeel, K., Moawad, H., 2004b. Biosorption of heavy metals from waste water using *Pseudomonas* sp. Electron. J. Biotechnol., 7 (1) ISSN: 0717–3458.

Hussein, H., Farag, S., Kandil, K., Moawad, H., 2005. Tolerance and uptake of heavy metals by *Pseudomonads*. Process Biochem. 40, 955–961.

Jianlong, W., Xinmin, Z., Decai, D., Ding, Z., 2001. Bioadsorption of lead (II) from aqueous solution by fungal biomass of *Aspergillus niger*. J. Biotechnol. 87, 273–277.

Jorgensen, Turnidge, J.D., Washington, J.A., 1999. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray, P.R., Pfaller, M.A., Tenover, F.C., Baron, E.J., Yolken, R.H. (Eds.), Manual of Clinical Microbiology, seventh ed. ASM Press, Washington, DC, pp. 1526–1543.

Kapoor, A., Viraraghvan, T., 1995. Fungal biosorption: an alternative treatment option for heavy metal bearing wastewater: a review. Bioresour. Technol. 53, 195–206.

Kuyucak, N., Volesky, B., 1988. Biosorbents for recovery of metals from industrial solutions. Biotechnol. Lett. 10 (2), 137–142.

Liu, Y., Xu, H., Yang, S., Tay, J.A., 2003. General model for biosorption of Cd, Cu, and Zn by aerobic granules. J. Biotechnol. 102, 233–239.

Low, W., Cheung, K.C., Chua, Hong, Wong, M.F., Yu Peter, H.F., 1998. Copper ion sorption and desorption by activated sludge bacteria. Preprints of Extended Abstracts presented at the ACS National Meeting, American Chemical Society and Division of Environmental Chemistry, 38 (2), 40–43.

Macaskie, L.E., 1990. An immobilized cell bioprocess for removal of heavy metals from aqueous flows. J. Chem. Technol. Biotechnol. 49, 330–334.

Macaskie, L.E., Wates, J.M., Dean, A.C.R., 1987. Cadmium accumulation by a *Citrobacter* sp. immobilized on gel and solid supports: applicability to the treatment of liquid wastes containing heavy metal cations. Biotechnol. Bioeng. 30, 66–73.

Muraleedharan, T.R., Venkobachar, C., 1990. Mechanism of biosorption of copper (II) by *Ganoderma lucidum*. Biotechnol. Bioeng. 35, 320–325.

Pérez, R., Abalos, A., Gómez, J.M., Cantero, D., 2006a. Biodegradation of crude oil by *Pseudomonas aeruginosa* AT18 strain. Rev. Tecnología Química XXXI (1), 70–77.

Pérez, R., Abalos, A., Gómez, J.M., Cabrera, G., Ramírez, M., Cantero, D., 2006. Integrated system for heavy metals precipitation and petroleum biodegradation. In: Höflinger, W. (Ed.), Chemical Industry and Environment V, vol I. Vienna University of Technology, pp. 387–396. ISBN: 3-900-554-57-9.

Pérez, R., Camacho, M., Abalos, A., Gómez, J.M., Cabrera, G., Ramírez, M., Cantero, D., 2008. Aislamiento y selección de una cepa bacteriana degradadora de hidrocarburos a partir de suelos contaminados con petróleo. Revista CENIC Ciencias Biológicas 39 (1), 44–51.

Puranik, P.R., Paknikar, K.M., 1999. Influence of co-cations on biosorption of lead and zinc – a comparative evaluation in binary and multimetal systems. Bioresour. Technol. 70, 269–276.

Savvaidis, I.H., Martin, N., Poole, R.K., 2003. Copper biosorption by *Pseudomonas cepacia* and other strains. World J. Microbiol. Biotechnol. 19 (2), 117–121.

Saxema, D., Gowri, P., Hangola, N., Hugo, R., Srivestova, S., 2001. Renoval of copper by *Pseudomonas putida* strain isolated from copper mine. J. Exp. Biol. 39 (6), 590–593.

Scott, J.A., Karanjkar, A.M., 1992. Repeated cadmium biosorption by regenerated *Enterobacter aerogenes* biofilm attached to activated carbon. Biotechnol. Lett. 14 (8), 737–740.

Shumate, E.S., Strandberd, W.G., 1985. Accumulation of metals by microbial cells. Compr. Biotechnol. 13, 235–247.

Srinath, T., Verma, T., Ramteke, P.W., Garg, S.K., 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Chemosphere 48, 427–435.

Tangaromsuk, J., Pokethitiyook, P., Kruatrachue, M., Upatham, E.S., 2002. Cadmium biosorpion by *Sphingomonas paucimobilis* biomass. Bioresour. Technol. 85, 103–105.

Taniguchi, J., Hemmi, H., Tanahashi, K., Amano, N., Nakayama, T., Nishino, T., 2000. Zinc biosorption by a zinc-resistant bacterium, *Brevibacterium* sp. strain HZM-1. Appl. Microbiol. Biotechnol. 54 (4), 581–588.

Tsezos, M., 2001. Biosorption of metals. The experience accumulated and the outlook for technology development. Hydrometallurgy 59, 241–243.

Volesky, B., 1986. Biosorbent materials. Biotechnol. Bioeng. Symp. 16, 121–126.

Volesky, B., 1990. Biosorption of Heavy Metals. CRC Press, Inc.

Volesky, B., 2003. Sorption and Biosorption. BV-Sorbex, Inc., St. Lambert, Montreal, Quebec, Canada. ISBN: 0-9732983-0-8

Volesky, B., Holan, Z.R., 1995. Biosorption of heavy metals. Biotechnol. Prog. 11, 235–250.

Zhou, J.L., Kiff, R.J., 1991. The uptake of copper from aqueous solution by immobilized fungal biomass. J. Chem. Technol. Biotechnol. 52, 317–330.