ISSN: 2229- 6905

Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © 2009 PHYCOSPECTRUM - ISSN: 2229 – 6905

Biosorption of lead(II) and chromium(VI) by immobilized cells of microalga *Isochrysis galbana*

***Sujin Jeba Kumar, T., Balavigneswaran, C.K., Arun Vijay.M. and Srinivasa Kumar.K.P.**

Inbiotics, Parvathipuram, Nagercoil – 629 003, Kanyakumari District, Tamil Nadu, India.

**Corresponding Author* : Sujin Jeba Kumar.T., Ph. No. : +91 9486629713, email : sujinphenom@gmail.com

Abstract

Immobilized cells of microalgae *Isochrysis galbana* was investigated for biosorption of lead (II) and chromium (VI). The maximum biosorption capacity of lead and chromium was found to be 30.54 ± 0.73 mg g^{-1} and 29.21 ± 0.54 mg g^{-1} respectively. The optimum pH for both removal was found to be at pH 4. The maximum removal of lead was achieved at 25°C where is maximum uptake of chromium was observed at 35°C. The Langmuir model showed better representation of data with correlation coefficient exceeding 0.98. Biosorption kinetics exhibited pseudo second order kinetics model and thermodynamics parameters were evaluated.

Key words : *Isochrysis galbana*, Biosorption, immobilize cells, Langmuir model

Introduction

Lead is widely used as industrial raw material in the manufacture of storage batteries, pigments, leaded glass, fuels, photographic materials, solder and steel products. (Nadeem, M. et.al. 2006). Lead accumulates mainly in bones, brain, kidney ailments and muscles and may cause many serious disorders like anemia, kidney diseases, nervous disorder sand sickness even death (L.W.H. Chua, et.al. 1999). It is therefore, highly essential to remove Pb(II) from wastewater before disposing it. The potential sources of chromium (VI) wastes are effluents from metallurgy, electroplating, leather tanning, textile dyeing, paint, ink, and aluminum manufacturing industries (Bhattacharyya, et.al., 2006). High exposure of chromium (VI) causes cancer in the digestive tract and lungs and may cause gastric pain, nausea, vomiting, severe diarrhea, and hemorrhage (Mohanty, K. et.al. 2005). It is therefore, essential to remove Pb(II) and chromium(VI) from the environment.

Traditional methods for removing heavy metals, including chemical precipitation and filtration, redox reactions, electrochemical treatments, reverse osmosis, ion exchange, adsorption and evaporation, are generally expensive or inadequate for treating highly diluted solutions. Biosorption is an alternative process for the treatment of this kind of effluents. It is defined as the passive binding of metals or other compounds on a biosorbent (biomass) containing high affinity active sites or functional groups (B. Volesky. 2003).

Alginate is a principal component of the outer cell wall of brown algae and, according to several studies (S.K. Mehta, et.al., 2005; E. Romera, et.al. 2006) is responsible for the high metal uptakes of brown algae when compared to other algae, bacteria and fungi. However, the immobilization of cells can represent an alternative for solving the problem as well as providing advantages such as an increase in the cell retention time on bioreactors and higher high metabolic rate. (Tam, N.F.Y., et.al. 1994).

ISSN: 2229- 6905

Experimental Section

Algae Culture

The algae *Isochrysis galbana*, was procured from CMFRI, Tuticorin. The algae were cultured in modified Walney's medium. The algae were grown under low illumination upto 50 μ mol $m^{-1}s^{-1}d$ and unlimited aerated condition for one week. Temperature was adjusted 25 ± 2 °C.

Immobililzation of algal cells

Once the microalgae were acclimatized, algal cells were har-vested by centrifugation at 5500 rpm for 5 min. The cells were resuspended in 50 mL of distilled water to form a concentrated al-gal suspension with a uniform cell density of 10107cells mL1. The algal suspension was then mixed with a 4% sodium alginate solution in 1:1 volume ratio to obtain a mixture of 2% algae–alginate suspension. The mixture was transferred to a 50 mL burette and drops were formed when ‘‘titrated” into a calcium chloride solution (2%). The beads were kept for hardening in the $CaCl_2$ solution for 4 h at 25 ± 2 C, then rinsed with sterile saline solution (0.85% NaCl) and subsequently with distilled water.

Biosorption experiments

The stock lead(II) and chromium(VI) solutions of 1000 mg l^{-1} concentrations were prepared by dissolving, 1.6 g lead nitrate (Merck Germany) and 2.827 g potassium dichromate (Fischer Germany), respectively in one litre of distilled water. The stock solutions were further diluted to obtain, solutions of various known concentrations of lead(II) and chromium(VI). A variable speed shaker 20-500 rpm equipped thermostatically controlled heating water bath was used for batch experimentation. Initial concentrations of lead(II) and chromium(VI) solutions, used in the experiments were 100 mg l-1. The rotational speed of stirrer, in all the experiments was kept constant at 200 rpm. Except for the experiments to study the effect of dose, 0.5 g biosorbent was used in 100 ml of solution. After completion of each batch, the solution was filtered. The filtrate was analyzed, using Shimadzu 6800 Atomic Absorption Spectrophotometer, to determine the quantity of residual metals. The quantity of sorbed metal was found by material balance. The metal uptake, qt was determined using the following equation:

$$q_t = V (C_o - C_f) / m \quad [1]$$

Where, C_o and C_f are the initial and final concentration of metal in solution (mg $l-^1$), V is the volume of solution (l) and m is the mass of biosorbent (g). All chemicals used were of analytical reagent grade.

Effect of pH

In heavy metal biosorption, pH is the most important parameter. The solution pH was varied in the range from 1 to 6, by using 0.1 M nitric acid and 0.1 M ammonia as buffers and the corresponding metal uptake of the immobilized algal cells were studied.

Effect of biosorbent dose

The removal efficiency and specific uptake of metals depend on type and quantity of the bisorbent . In order to find the optimal dose experimentally, the quantity of biosorbent was varied from 1-50 $gl-^1$ and the percentage removal with increase in biosorbent dose was tabulated.

Models of Biosorption equilibrium

The Langmuir model is expressed as:

$$q_e = q_{max} b C_e / 1 + b C_e \qquad [2]$$

Where, q_{max} represents the maximum biosorption capacity and b is an affinity parameter, related to the energy of biosorption (Lagergren, S., 1898).

The Freundlich model (Freundlich, 1928) is expressed mathematically as:

$$q_e = K_F(C_e)^{1/n} \quad (3)$$

Where; K_F and n are the Freundlich coefficients. The constant K_F provides an indication of the biosorption capacity of biosorbent and n is related to the intensity of biosorption (Freundlich, H., 1928).

Initial concentration of both metals was varied from 10 to 1000 $mgl^{-1}$and quantity of biosorbent was kept constant at 0.5 g. Equilibrium concentration, C_e and equilibrium capacity, q_e were determined. C_e was plotted against C_e / q_e and straight lines were fitted by regression and the obtained Langmuir were given in the table . In order to see the applicability of Freundlich model, ln C_e was plotted against ln q_e and straight lines were fitted to the plots and the obtained Freundlich parameters were shown in the table and the applicability of the model to the system were studied.

Biosorption kinetics

In order to investigate the biosorption kinetics, the Lagergren first order (Langmuir, I., 1918) and pseudo second order kinetics models (HO, Y.S. and MCKAY, G., 1999) were applied.

The expression for the Lagergren first order model is:

$$dq_t / dt = k_1(q_e - q_t) \quad [4]$$

Where, k1is the Lagergren rate constant for adsorption (min^{-1}), q_e is the amount of metal biosorbed at equilibrium (mg g^{-1}) and q_t is the amount of metal biosorbed (mg g^{-1}) at any time t.

Time, t was plotted against log (q_e - q_t) and straight lines, were fitted by regression. The values of k_1 and q_e were determined from the slope and intercept of lines.

The equation of pseudo second order model is:

$$dq_t / d_t = k_2(q_e - q_t)^2 [5]$$

Where, k_2 is equilibrium rate constant of second order kinetics model (g $mg^{-1}min^{-1}$), q_e is the equilibrium capacity and qt is the biosorption capacity at any time t. The time, t was plotted against t/q_t and straight lines were fitted to these plots. And the equilibrium rate constant, k_2 and equilibrium capacity, q_e were determined from the slope and intercept of the lines and pseudo second order model parameters were found out.

Effect of temperature

The effect of temperature on biosorption of lead(II) and chromium(VI), was studied by varying the temperature in the range 20-45ºC . The biosorbent contains more than one type of sites for metal binding. The biosorption equilibrium constant, kdis described thermodynamically by Van't Hoff equation as (Verma,A. et.al. J.K., 2006)

$$\ln k_d = -\Delta H / RT + \Delta S / R \quad [6]$$

The values of Gibbs free energy can be calculated by using the following equation.

$$\Delta G = - RT \ln k_d \; [7]$$

Where, ΔH, ΔS and ΔG are the change in enthalpy, entropy and Gibbs free energy of the system, respectively. T is the absolute temperature (K), R is the gas constant (8.314 J $mole^{-1}$ K^{-1}) and k_d is the equilibrium constant given by the following equation (Bektas, Nihal; et.al. 2004)

$k_d = q_e / C_e$ [8]

Values of $k_d(l^{g-1})$ were calculated at different temperatures using the Equation 8 The plot of reciprocal temperature (1000/T) versus ln kd yielded straight lines, and correlation coefficient were found out. The values of ΔH and ΔS were determined from the slope and intercept of lines in. The values of ΔG at different temperatures were calculated using Equation 7. The values of ΔH, ΔS and ΔG were found out

Statistical Analysis

The results are expressed as mean values ± standard deviation (SD).

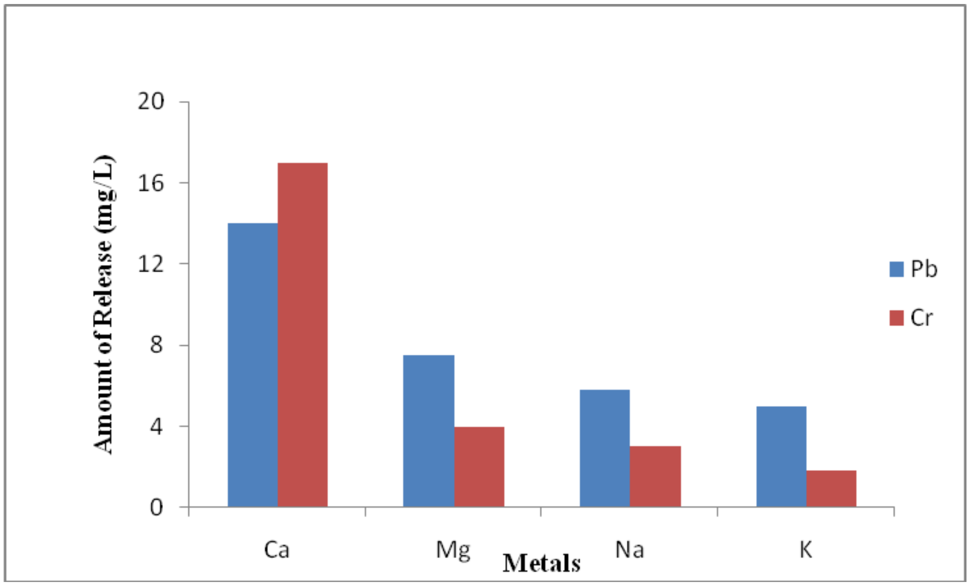
Results and Discussions

Characteristics of immobilized algal cells

The larger number of cells at the surface of the beads caused a greater self-shading effect to the cells inside the beads, limiting its growth but not pigment production (Lau, P.S., et.al. 1997). The method used by us produced approximately 6600 uniform algal beads of approximately 2.4 mm diameter with a initial cells number of 3.5 x 10^5 cells bead1for every 100 mL of the algae–alginate mixture. The approximate volume of each bead was 0.01528 mL.

Metal release during Biosorption of Lead and Chromium

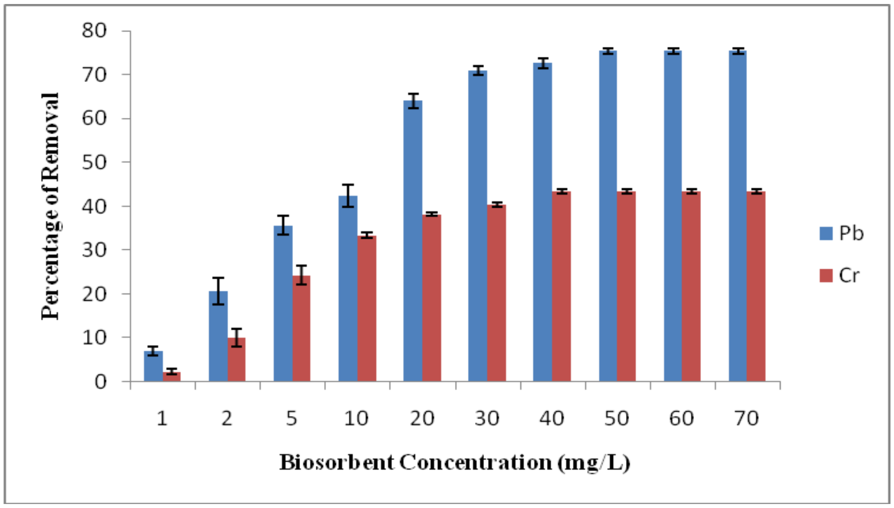
Fig.1 Metal release during Biosorption of Lead and Chromium



During the biosorption of lead and chromium by immobilized cells of *Isochrysis galbana* results in the release of light metals like Ca, Mg, Na and K ions as shown in fig 1, but predominantly Ca and Mg ions but during the biosorption of chromium small amount OH^- ions are also released indicating the involvement of ion exchange mechanism.

Effect of biosorbent dose

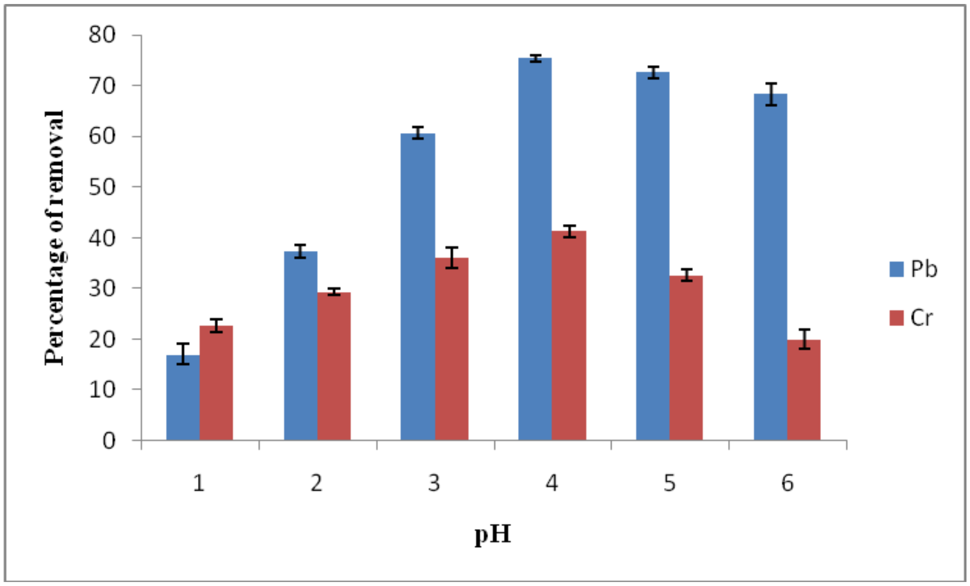
Fig.2 Optimal dose for Biosorption



To determine the effect of adsorbent dose, different amounts (0.05–10.0 g/L) of adsorbent were suspended in 10mLlead solution in which the concentration of lead was 100 and 200 mg/L. The effect of adsorbent dose on the extent of removal of lead and chromium at optimum pH (4.0) is shown in Fig. 2. The amount of adsorbent significantly influenced the extent of lead adsorption. The amount of lead adsorbed decreased with biosorbent concentration and increase in biosorbent concentration was ineffective after 40 mg/l for chromium and after 50 mg/l for lead adsorption. Various reasons have been suggested to explain the decreased adsorption capacity at increasing biomass including availability of solute, electrostatic interactions, interference between binding sites, and reduced mixing at higher biomass densities (Meikle, A.J., et.al. 1990; Fourest, E., et.al. 1992).

Effect of pH

Fig.3 Effect of pH on Biosorption of Lead and Chromium



It is well known that pH could affect the protonation of the functional groups on the biomass as well as the metal chemistry. The effect of pH on lead and chromium adsorption capacity of immobilized *Isocrysis galbana* sps. is shown in Fig. 3. As the pH of the lead solution and chromium solution increased, the adsorption capacity increased and it was maximum at pH 4 and decreased above pH 4. At pH higher than 5, the precipitation of insoluble metal hydroxides takes place restricting the true biosorption studies. The results showed strong pH dependence of biosorption. The cell wall matrix of green algae contains complex heteropolysac-charides that can provide amino, carboxyl and sulphate groups. At low pH, cell wall ligands are protonated and restrict the approach of metal cations as a result of the repulsive force. As pH increases, more ligands such as amino, phosphate and carboxyl groups would be exposed and carry negative charges with subsequent attraction of metal ions (G.C.D¨onmez, et.al. 1999; Z. Aksu, 2001).

Biosorption isotherm

The studies of isotherm on lead adsorption by were carried out. The adsorption isotherm was obtained at constant biomass dose (1 gl^{-1}) and at solution pH value (pH 4).Table 1 shows Langmuir and Freundlich constants and correlation regression coefficients of lead bio-sorption.

Table. 1Langmuir model and Freundlich model, parameters for lead (II) and chromium (VI) biosorption

Metal	The Langmuir model parameters			Freundlich model parameters		
	q$_{max}$ (mg g^{-1})	b (l mg^{-1})	R^2	n	K$_F$	R^2
Pb	30.54 ± 0.73	0.012 ± 0.004	0.99	1.7 ± 0.18	0.89 ± 0.15	0.89
Cr	29.21 ± 0.54	0.0065 ± 0.0006	0.98	1.5 ± 0.15	0.53 ± 0.07	0.95

Biosorption kinetics

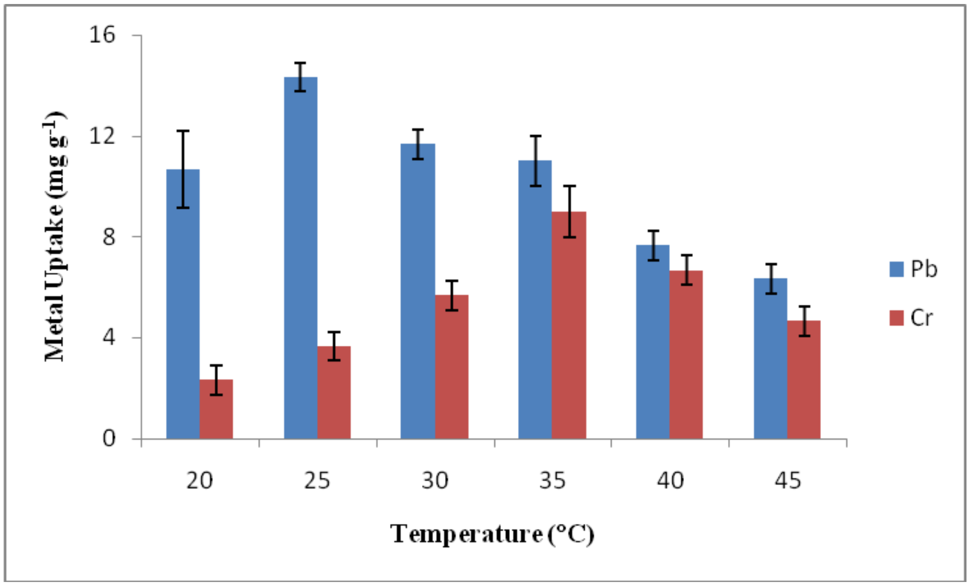
From Table 2 correlation coefficients of 0.99 indicated the applicability of pseudo second order model to the present system. The applicability of this model suggested that biosorption of lead and chromium, on immobilized algal cells was based on chemical reaction, between metals and active sites of the immobilizes algal cells. The biosorption of lead ions on the immobilized algal cells follows second-order biosorption kinetics

Table.2 Langergren first order and pseudo second order model parameters for lead (II) and chromium (VI) biosorption

Metal	The Langergren first order model parameters			The pseudo second order model parameters		
	k$_1$ (min^{-1})	**q$_e$** (mg g^{-1})	R^2	**k$_2$** (g mg^{-1} min^{-1})	**q$_e$** (mg g^{-1})	R^2
Pb	0.064 ± 0.005	12.14 ± 0.40	0.96	0.0082 ± 0.0006	15.55 ± 0.25	0.99
Cr	0.047 ± 0.003	7.23 ± 0.35	0.96	0.0150 ± 0.002	8.71 ± 0.24	0.99

Effect of temperature

Fig.4 Effect of Temperature on Biosorption of Lead and Chromium



The maximum removal of lead was achieved at 25°C where is maximum uptake of chromium was observed at 35°C .The effect of temperature on biosorption capacity was shown in fig 4.The values of ΔH, ΔS and ΔG are listed in Table 3. Positive values of ΔH, ΔG and higher removal capacities at elevated temperatures, indicated that biosorption of chromium was endothermic in nature. At higher temperatures the energy of system seemed to facilitate the chromium attachments onto biosorbent surfaces. On the other hand enthalpy change for lead was negative and there was a vivid decrease in removal capacity was observed with increase in temperature from 20ºC to 45ºC. This indicated that biosorption of lead was exothermic.

Table. 3 The thermodynamics parameters for lead (II) and chromium (IV) bisorption

Metal	T (°C)	D H (kJ mol $^{-1}$)	D S (kJ mol $^{-1}$ K^{-1})	D G (kJ mol $^{-1}$)	R^2
Pb	20	- 48.45 ± 01.27	- 0.15 ± 0.025	1.44 ± 0.05	0.96
	30			2.40 ± 0.08	
	40			4.75 ± 0.14	
Cr	20	14.8 ± 0.92	0.04 ± 0.004	5.89 ± 0.13	0.95
	30			5.76 ± 0.22	
	40			5.30 ± 0.16	

Conclusion

From our studies, immobilized cells of *Isochrysis galbana* were found to be well suited for the removal of lead and chromium from aqueous solution. This is due to its rapid rate of biosorption as well as its high capacity of biosorption. It worked well at wide range of temperature and pH. Moreover this method of biosoprtion proved to be eco-friendly and inexpensive but with better efficiency than other method. So, it will be a good alternative against harmful mettalic pollutants like lead and chromium.

Acknowledgement

We acknowledge Inbiotics, Parvathipuram, Nagercoil-629003, Kanyakumari District, Tamil Nadu, Inida for providing laboratory facilities and necessary guidance.

References

Aksu, Z., 2001, Equilibrium and kinetic modelling of cadmium(II) biosorption by *C. vulgaris* in a batch system: effect of temperature, Sep. Purif. Technol. 21 (3):285–294.

Bektas, Nihal; AGIM, Burcu Akman and KARA, Serdar. 2004. Kinetic and equilibrium studies in removing lead ions from aqueous solution by natural sepiolite. Journal of Hazardous Materials, vol. 112, no. 1-2 : 115-122.

Bhattacharyya, Krishna G. and Gupta, 2006. Susmita Sen. Adsorption of chromium(VI) from water by clays. Industrial Engineering and Chemistry Research, vol. 45, no. 21:7232-7240.

Chua, L.W.H., K.H. Lam, S.P. Bi, 1999, A comparative investigation on the biosorption of lead by filamentous fungal biomass, Chemosphere 39:2723–2736

D'onmez, G.C., Z. Aksu, A. Ozturk, T.Kutsal, 1999. Acomparative study on heavymetal biosorption characteristics of some algae, Process Biochem. 34 (9)(1999) :885–892.

Fourest, E., Roux, J., 1992. Heavy metals biosorption by fungalmycelial by-product: Mechanisms and influence of pH. Appl. Microbiol. Biotechnol. 37:399–403.

Freundlich, H., 1928. Colloid and capillary chemistry. E.P. Dutton and Co., New York, 1928.

HO, Y.S. and MCKAY, G., 1999. Pseudo-second order model for sorption processes. Process Biochemistry, vol. 34, no. 5: 451-465.

Lagergren, S., 1898, Zur theone der sogenannten adsorption gelostn. Stoffe. Stcok. Ak. Handl. Bihay, 24(Afd. I), : 39.

Langmuir, I., 1918. The sorption of gases on plane surfaces of glass, mica and platinum. Journal of the American Chemical Society, vol. 40, :1361-1403.

Lau, P.S., Tam, N.F.Y., Wong, Y.S., 1997. Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized Chlorella vulgaris. Environ. Technol. 18:945–951.

Mehta, S.K., J.P. Gaur, 2005. Use of algae for removing heavy metal ions from wastew-ater, Crit. Rev. Biotechnol. 25:113–152.

Meikle, A.J., Gadd, G.M., Reed, R.H., 1990. Manipulation of yeast for transport studies: critical assessment of cultural and experimental procedure. Enzyme Microbiol. Technol.12:865–872.

Mohanty, K.; Biswas, M.N. and Meikap.B.C., 2005. Removal of chromium(VI) from dilute aqueous solutions by activated carbon developed from Terminalia arjunanuts activated with zinc chloride. Chemical Engineering Science, vol. 60, no. 11: 3049-3059.

Nadeem, M.; Mahmood, A.; Shahid,S.A.; Shah, S.S., Khalid, A.M. and Mckay, 2006. Sorption of lead from aqueous solution by chemically modified carbon adsorbents. Journal of Hazardous Materials, vol. 138, no. 3 : 604-613.

Romera, E., F. Gonzalez, A. Ballester, M.L. Blazquez and J.A. Mu˜ noz, 2006. Biosorption with algae: a statistical review, Crit. Rev. Biotechnol. 26 :223–235

Tam, N.F.Y., Lau, P.S., Wong, Y.S., 1994. Wastewater inorganic N and P removal by immobilized Chlorella vulgaris. Water Sci. Technol. 30, :369–374

Verma,A.; Chakraborty, S. and Basu. J.K., 2006. Adsorption study of hexavalent chromium using tamarind hull-based adsorbents. Separation and Purification Technology, July 2006, vol. 50, no. 3: 336-341.

Volesky, B. 2003. Sorption and Biosorption, BV-Sorbex, Inc., St. Lambert, Quebec.