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RESEARCH ARTICLE

Biosorption of heavy metals from single and multimetal solutions by free and immobilized cells of *Bacillus megaterium*

Meenakshi Sati*[1], Megha Verma[2], J.P.N. Rai[3]

[1,2,3]Ecotechnology laboratory, Department of Environmental Science, G. B. Pant University of Agriculture and Technology, Pantnagar, 263145 (India)

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Corresponding Author

Meenakshi Sati

Abstract

Biosorption capacity of immobilized and free cells of bacterium *Bacillus megaterium* has been assessed for the removal of Pb^{2+}, Cr^{2+} and Cd^{2+} ions from single, binary and ternary metal solutions at varying initial metal ion concentrations, incubation time, pH and temperature regimes. From single metal solution biosorption capacity followed the order Pb > Cd > Cr and it was maximum 96.83%, 77.16% and 55.28% for Pb^{2+}, Cd^{2+} and Cr^{2+} ions respectively by immobilized cells and 76.29%, 54.59% and 32.18% by free cells at optimum conditions i.e. pH 7, temperature 40^0C for 50 ppm metal ion concentration. Mutual interference effects were probed using equilibrium adsorption capacity ratios, q_e'/q_e where the prime indicates the presence of one or two other metal ions. The combined action of the metals was found to be antagonistic. Multimetal adsorption behavior was successfully described by extended Langmuir Model. In binary metal solution, 10, 30 and 50 ppm of Cr^{2+} ions inhibited both Pb^{2+} and Cd^{2+} ions, Pb^{2+} ions being more influenced. In contrast, Cr^{2+} ions sorption remained unaffected at varying concentrations of competing metals. Thus in binary system, sorption order was Pb>Cr, Cd>Cr and Pb~Cd while interference dominance followed the order Pb<Cr, Cd<Cr and Pb~Cd. In ternary metal system biosorption affinity was Pb>Cd>Cr, and inhibition dominance was found to be Cr>Pb>Cd. Study revealed that the findings could assist devising toxic metal cleansing by bacterial sorption especially in immobilized state.

INTRODUCTION

Heavy metal pollution, a prevalent environmental problem worldwide, occurs directly by effluent out falls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water and from the atmosphere via rain water (Vijayaraghavan and Yun 2008; Sarin and Sarin 2010). Heavy metals are particularly hazardous because of their toxicity, tendency to bioaccumulate via food chain, and their abundance and persistence in the environment. Wastewaters usually contain more than one metal, but most of the metal sorption studies deal with single component system (Kusvuran et al. 2011; Sulaymon et al. 2013). The presence of several metals in solution may have a synergistic or conversely antagonistic effect on their biosorption, which depends on many factors, such as the quantity of metal ions competing for the same binding sites, the concentration of metal ions, the nature and concentration of biosorbent (Wang and Yong 2005; Kadukova and Horvathova 2012; Maresova et al. 2011). Conventional methods for removing metals include chemical precipitation, chemical oxidation or reduction, ion exchange, electrochemical treatment, and membrane technologies, which may be ineffective or extremely expensive, especially when the metals are in diluted state (Olmezoglu et al. 2012; Wang and Chen 2009). In industrial effluent, three widely available metals viz. Pb, Cd and Cr are often encountered in mixed form during specific metal treatment operations, metal plating and finishing, iron and steel manufacturing, production of automobile parts, paint manufacturing, paint and ink formulation (Apiratikul et al. 2004). Alternative metal removal

and/or recovery methods are being considered which are based on metal-sequestering properties of certain natural materials of biological origin. Some microorganisms can retain relatively high quantities of metals by passive biosorption such as precipitation, physical adsorption, ion exchange, and complexation (Nanda et al. 2011). This is commonly known as biosorption. The active mode of metal accumulation by living cells is bioaccumulation which depends on the metabolic activity of the cell and be significantly affected by the presence of the metal ions. Bacterial surfaces have great affinity to adsorb and precipitate metals resulting in metal concentration on bacterial surface. Biosorption is caused by a number of different physicochemical mechanisms, depending on a number of external environmental factors as well as on the type of a metal, its ionic form in the solution, and on the type of a particular active binding site responsible for sequestering the metal (Sag et al. 2001). Besides, the immobilization of microbial cells also determines state of binding sites (Hong et al. 2012; Kumar and Rao 2011).

In the present study the biosorption ability of immobilized and free cells of *Bacillus megaterium* was compared as a function of pH, temperature and initial metal ion concentration and incubation time in single and multimetal systems. The mono component and multi component biosorption data have been analyzed using the Langmuir and extended Langmuir isotherm models.

Materials and methods

Biomass used

Pure culture of *Bacillus megaterium* was obtained from the Department of Microbiology at G.B. Pant University of Agriculture and Technology Pantnagar. The slant cultures were prepared with Nutrient agar (NA) medium containing peptone 0.5% and yeast extract 0.3%. The slant cultures were incubated at 35^0 C for 24h.

Bacterial biomass immobilization

For cell immobilization, the modified method of Shide et al. (2005) was used. The exponentially growing cells of the culture were harvested aseptically into a 1 L capacity blender, using a spatula. The harvested cells were homogenized and 17.5 ml of the cell homogenate was added to 87.5 ml of distilled water into a 250 ml conical flask and mixed thoroughly. The mixture was allowed to settle and after 10 min, exactly 3.063 g of sodium alginate was added into the supernatant (concentrated cells). The mixture was subsequently pumped through a 5 ml syringe drop wise, into a flask containing sterilized 100 ml of 0.12 M calcium chloride solution. This mixture was allowed to settle for 1 h to complete precipitation that formed spherical beads. The immobilized cells were removed and stored until use at 4°C in 5 mM $CaCl_2$ solution.

Stock solutions of heavy metal

Stock lead(II), cadmium(II) and chromium(IV) solutions of a concentration of 1000 mg/L were prepared by dissolving $Pb(NO_3)$, $Cd(NO_3)\cdot 4H_2O$ and $K_2Cr_2O_7$ in distilled water respectively. The binary and ternary metal solutions were prepared using stock solutions containing combinations of 30 ppm primary cation (Pb^{2+}, Cd^{2+} or Cr^{2+} ions) and varying concentrations (10-50 ppm) of secondary cation (Pb^{2+}, Cd^{2+} or Cr^{2+} ions). The pH of working solutions was adjusted to 7 by addition of the necessary amount of either 1.0 M HCl or 0.1 M NaOH solution.

Biosorption studies

The biosorption of Pb(II), Cd(II) and Cr(II) ions was studied in batch system. All experiments were carried out with 50 mg biosorbent and 50 mL solution in a 250 mL flask at 40 ^{o}C and with a stirring speed of 120 rpm for 48 h. Each experiment was conducted in triplicate, and average values were used for the data analysis. In the first set of experiments, the biosorption capacity of immobilized and free cells of *Bacillus megaterium* for Pb^{2+}, Cd^{2+} and Cr^{2+} ions was determined separetly. Initial heavy metals concentrations were varied from 10 to 90 ppm for different incubation periods (24, 36, 48 and 60 h). The saturation value for the sorption was found to be about 50 ppm initial concentration of the metal ions and further experiments were carried out at this initial concentration. To determine the optimum temperature for biosorption, the temperature was varied from 30 to 45 ^{0}C. The effect of pH on the biosorption capacity of the bacterium was investigated in the pH range from 3 to 9 at 40^0C for 48 h with 50 ppm of initial metal ion loading. After incubation the cultures were centrifuged at 1500 rpm for 10 min. After centrifugation, the supernatants were digested using nitric acid-perchloric acid digestion according to APHA (2005) and were used for determination of residual metal ion concentration by using Atomic Absorption

Spectrophotometer (AAS). The amount of metal ions adsorbed per gram of *B. megaterium* was calculated as follows:

$$q_e = (C_o - C_e)\ V/m \qquad 1.$$

where q_e is the amount of metal ions adsorbed onto *B. megaterium* (mg g^{-1}), C_o is the initial metal ion concentration in solution (ppm), C_e is the concentration of non-adsorbed metal ions in solution (ppm), V is the volume of the medium (L) and m is the amount of *B. megaterium* used in the reaction mixture (g).

Binary system

In each experiment, immobilized cells of *B. megaterium* were brought into contact with a 50 ml solution containing initial concentration of primary metal at constant value of 30 ppm, while secondary metal concentration was varied between 10 to 50 ppm for each of the combinations: Pb-Cd, Cd–Cr and Pb–Cd. Each of the metal ions was analyzed as described above.

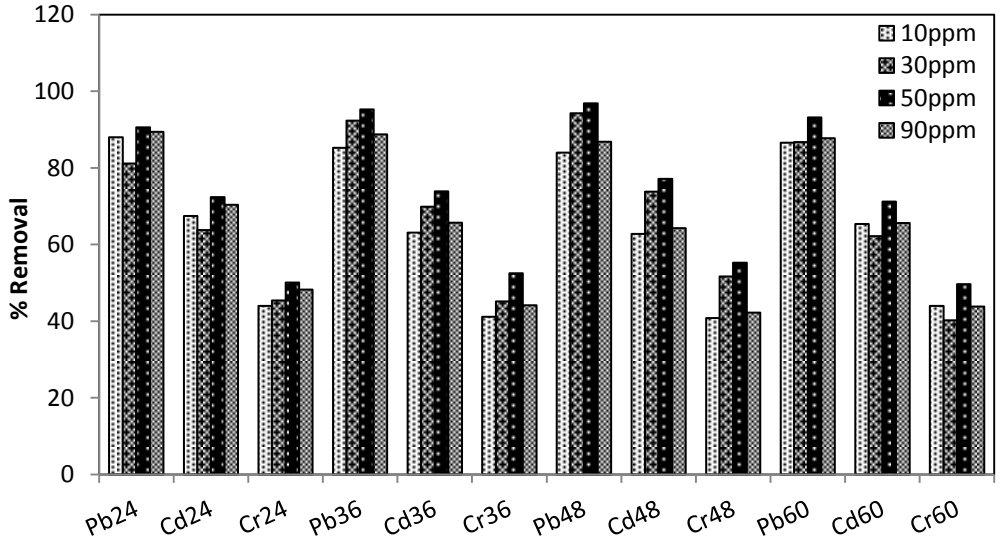
Ternary system

Biosorption in the ternary system was assessed by using composite solutions containing 30 ppm of primary metal ion with varying concentration of secondary metal ion ranging from 10 to 50 ppm. The experiments were carried out in similar conditions as those in binary sorption systems. Each of the three cations was analyzed by flame atomic absorption spectrometry as above.

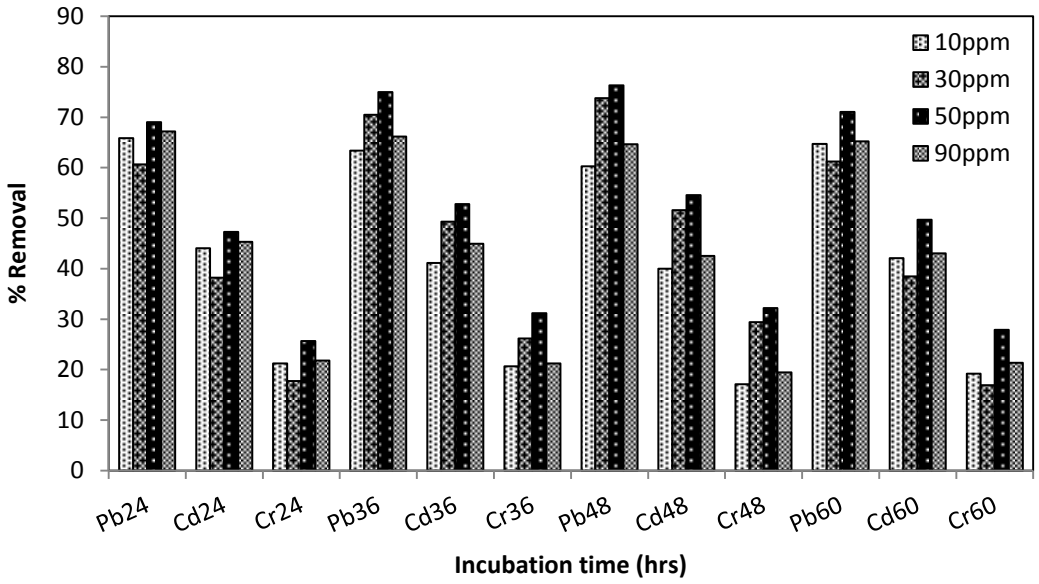
Results and Discussion

Biosorption in single metal solutions

Among the three metals Pb^{2+} ions were maximally biosorbed by both immobilized and free cells of *B. megaterium* but the extent of biosorption by former was significantly greater than the later. Maximum biosorption of Pb^{2+} ions 96.83% followed by Cd^{2+} ions 77.16% and minimum biosorption of Cr^{2+} 55.28% was recorded after 48 hrs of incubation with 50 ppm metal ion concentration [Fig. 1(a)]. This may be due to increasing electrostatic interactions, resulted into progressive decreased affinity for metal ions by sites (Muter et al. 2001; Donmez and Aksu 2001). At 90 ppm Pb^{2+} ions concentration there was no increase in metal uptake, indicating that the binding sites were completely saturated by Pb^{2+} ions.



(a)



(b)

Fig. 1 Percent biosorption of heavy metals by (a) immobilized cells and (b) and free cells of *B. megaterium* at varying metal concentration and sorption duration

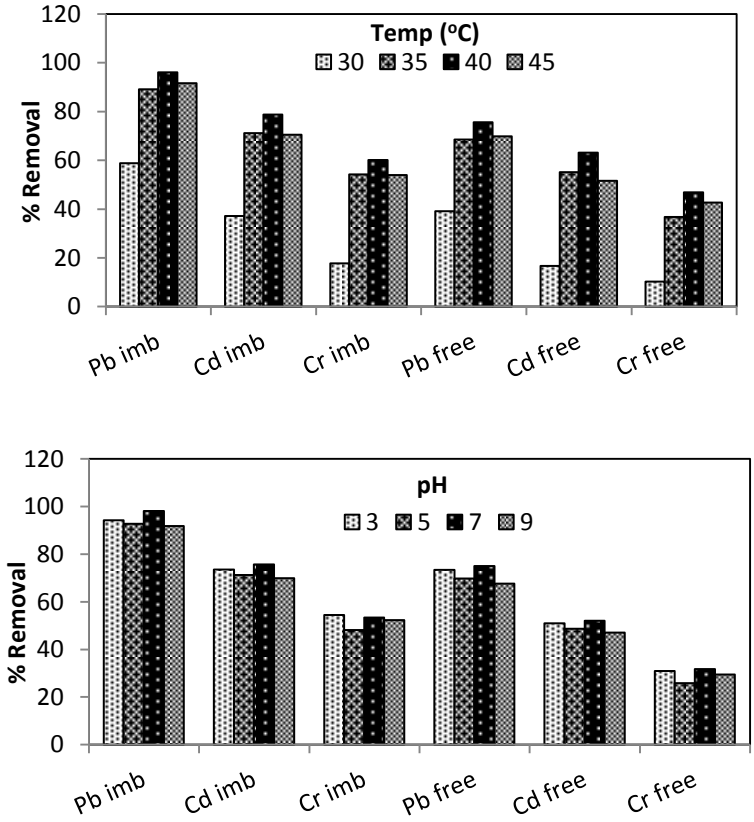


Fig. 2 Percent biosorption of heavy metals by immobilized and free cells of *B. megaterium* at varying (a) temperature and (b) pH regimes

The effect of various temperature regimes on the biosorption showed that the maximum biosorption is of Pb^{2+} ions 96.15% followed by Cd^{2+} ions 78.82% and least by Cr^{2+} ions 60.12% at 40^0C temperature by immobilized cells from 50 ppm of initial ion concentration [Fig 2(a)]. Temperature above 40^0C resulted in lower biosorption of metals, which may be due to the fact that higher temperatures usually reduce sorption due to the decrease surface activity and kinetic energy of the solute (Sag and Kutsal 2001). Moreover, physical damage to the biosorbent can also be expected at higher temperatures. Besides, an exothermic nature of some adsorption processes, may also reduce the biosorption capacity of the biomass at higher temperature (Rai et al. 2002).

pH is one of the major parameters which affect biosorption capacity of the biosorbent (Tuzen et al. 2007). The effect of pH on Pb^{2+}, Cd^{2+} and Cr^{2+} ions biosorption with *B. megaterium* [Fig. 2(b)] exhibited maximum (98.10%) Pb^{2+} ions removal at pH 7.0 by immobilized cells from 50 ppm of initial metal ion concentration. Initial increase in pH from acidic to neutral enhanced metal biosorption, which decreased above pH 7.0. The reason for this is that, at lower pH, the cell surface became more positively charged, reducing attraction between biomass and metal ions (Beveridge and Murray 1976; Mann 1990). Further at alkaline pH, biosorption decreased due to formation of insoluble oxides, hydroxides and carbonates, which reduced the free metal ions available for biosorption (Schiewer and Volesky 1995).

Interference effects of one metal ion on the biosorption of other metal ion

The q_e'/q_e ratios were used to investigate the sorption dynamics of the binary and ternary metals solution, where the prime denotes the presence of other metal ions. Generally, three possible behavior types are exhibited: $q_e'/q_e > 1$, synergism (the effect of the mixture is greater than that of the individual adsorbates in the mixture); $q_e'/q_e < 1$,antagonism (the effect of the mixture is less than that of each of the individual adsorbates in the mixture) and $q_e'/q_e = 1$, non-interaction (the mixture has no effect on the adsorption of each of the adsorbates in the mixture) (Mahamadi and Nharingo 2010).

Biosorption in binary solutions

The q_e'/q_e ratios for sorption of one metal in the presence of another metal were all <1 (Table 1) indicating antagonistic behavior, hence the biosorption of the metals was depressed by the presence of other metal ions in the multimetal solutions. The q_e'/q_e ratios for the sorption of Pb^{2+} ions in the presence of Cr^{2+} and Cd^{2+} ions were 0.36 and 0.65, respectively indicating a greater suppression effect of Cr^{2+} ions on Pb^{2+} ions biosorption. A similar trend was observed for the effect of Cr and Pb on the sorption of Cd (qe'/qe = 0.58 and 0.7, respectively). However, the suppression effect of Pb^{2+} and Cd^{2+} ions on Cr^{2+} ion adsorption was not significant (0.8 and 0.85). Thus, the results showed that in all cases, there was an inhibitory effect of one metal on binding of the other, with Cr^{2+} ions showing the greatest effect. Both Pb^{2+} and Cd^{2+} ions sorption were inhibited by Cr^{2+} ions, the former being more affected. In presence of 50 ppm Cr^{2+} ions at pH 7.0 and temperature 40^0C, biosorption capacity of *B. megaterium* for Pb^{2+} ions was decreased from 60 mg/g to 47.62 mg/g, while Cd^{2+} ions at 50 ppm reduced the adsorption capacity of bacterium for Pb^{2+} ions from 60 mg/g to 55.55 mg/g which was not much visible. Interestingly, the adsorption capacity of bacterium for Cr^{2+} ion sorption remained almost unaffected (14.91 mg/g and 16.66 mg/g from 18.92 mg/g) in the presence of different concentrations of Pb^{2+} and Cd^{2+} ions (10, 30 and 50ppm). Overall, sorption order in binary systems was found to be Pb>Cr, Cd>Cr and Pb~Cd while the interference dominance follow the order Pb<Cr, Cd<Cr, Pb~Cd.

Biosorption in ternary solutions

The ternary metal biosorption of Pb^{2+}, Cd^{2+} and Cr^{2+} ions by *B. megaterium* was investigated to establish the competitive effect of two metal ions on the biosorption of one metal ion. The biomass had the highest affinity for Pb^{2+} ions (q_m = 43.48 mg/g) and the least affinity for Cr^{2+} ions (q_m = 13.56 mg/g). The biosorption affinity order was found to be: Pb>Cd>Cr, while the interference dominance followed Cr>Pb>Cd. As expected, it can be observed that there was greater suppression of metal uptake in the ternary system than the binary system. For instance, binary qe'/qe ratios for the uptake of Pb^{2+} ions in the presence of Cr^{2+} and Cd^{2+} ions were 0.36 and 0.65, respectively, whilst the value decreased to 0.32 in the simultaneous presence of both metal ions. In the ternary systems, the greatest suppression effect was observed for the combined action of Cr^{2+} and Cd^{2+} on Pb^{2+} uptake (qe'/qe = 0.32). When compared to the value in binary sorption (qm = 47.62 and 55.55 mg/g in the presence of Cr^{2+} and Cd^{2+} ions, respectively), the decrease in q_m for Pb^{2+} ion biosorption in the ternary system could be attributed due to the increase in the electrostatic repulsion (screening effect) among the cations that would limit the sorption of the metal ion of interest. In competitive systems, binding of different metal ions on biosorbents having different functional groups

Table 1. Adsorption parameters for single, binary and ternary system

System	Metal ion (+interferent)	q_{max} (mg/g)	b (L/mg)	R^2	q_e'/q_e	Interective effect
a) Pb as primary metal ion						
Single	Pb	60	1.33	0.998	1	Non-interective
Binary	Pb(+Cr)	47.62	0.772	0.998	0.36	Antagonistic
	Pb(+Cd)	55.55	0.594	0.999	0.65	Antagonistic
Ternary	Pb(+Cr+Cd)	43.48	0.463	0.996	0.32	Antagonistic
b) Cd as primary metal ion						
Single	Cd	40.32	0.211	0.999	1	Non-interective
Binary	Cd(+Cr)	32.55	0.093	0.998	0.58	Antagonistic
	Cd(+Pb)	36.52	0.071	0.994	0.73	Antagonistic
Ternary	Cd(+Cr+Pb)	25.67	0.009	0.959	0.49	Antagonistic
c) Cr as primary metal ion						
Single	Cr	18.92	0.081	0.998	1	Non-interective
Binary	Cr(+Pb)	14.91	0.094	0.893	0.83	Antagonistic
	Cr(+Cd)	16.66	0.026	0.995	0.85	Antagonistic
Ternary	Cr(+Pb+Cd)	13.56	0.018	0.993	0.68	Antagonistic

depends on ionic properties such as electronegativity, ionic radius, potential, and redox potential of these metals (Naja et al. 2010). Sulaymon et al. (2011) showed that large ionic radius (molecular cross-sectional area) resulted in greater adsorption efficiency. Allen and Brown (1995) proposed that more electronegative metal ions will be more strongly attracted to the surface. The findings made in the binary and ternary sorption can be explained by the physicochemical properties of the ions. The preference of the biosorbent for the Pb^{2+} ions may be due to the fact that the metal has the largest atomic weight, two numbers of coordination (2, 4), being paramagnetic results the most electronegative ion which has the highest standard reduction potential compared to Cr^{2+} and Cd^{2+} ions. These ionic properties make Pb^{2+} ion most likely to be adsorbed (Fagundes-Klen et al. 2007; Perez-Marin et al. 2008). In contrast, the antiferromagnetic property of Cr^{2+} makes it the most interfering metal in multimetal systems.

Equillibrium models

Langmuir model was used to explain the sorption equilibrium data for Pb^{2+}, Cd^{2+} and Cr^{2+} ions respectively, in single component system (Fig. 3). Langmuir isotherm, which assumes that finite numbers of binding sites are distributed homogeneously over the surface of the biosorbent, adsorption energy of all the sites is identical and independent of the presence of adsorbed species on neighboring sites, can be represented as:

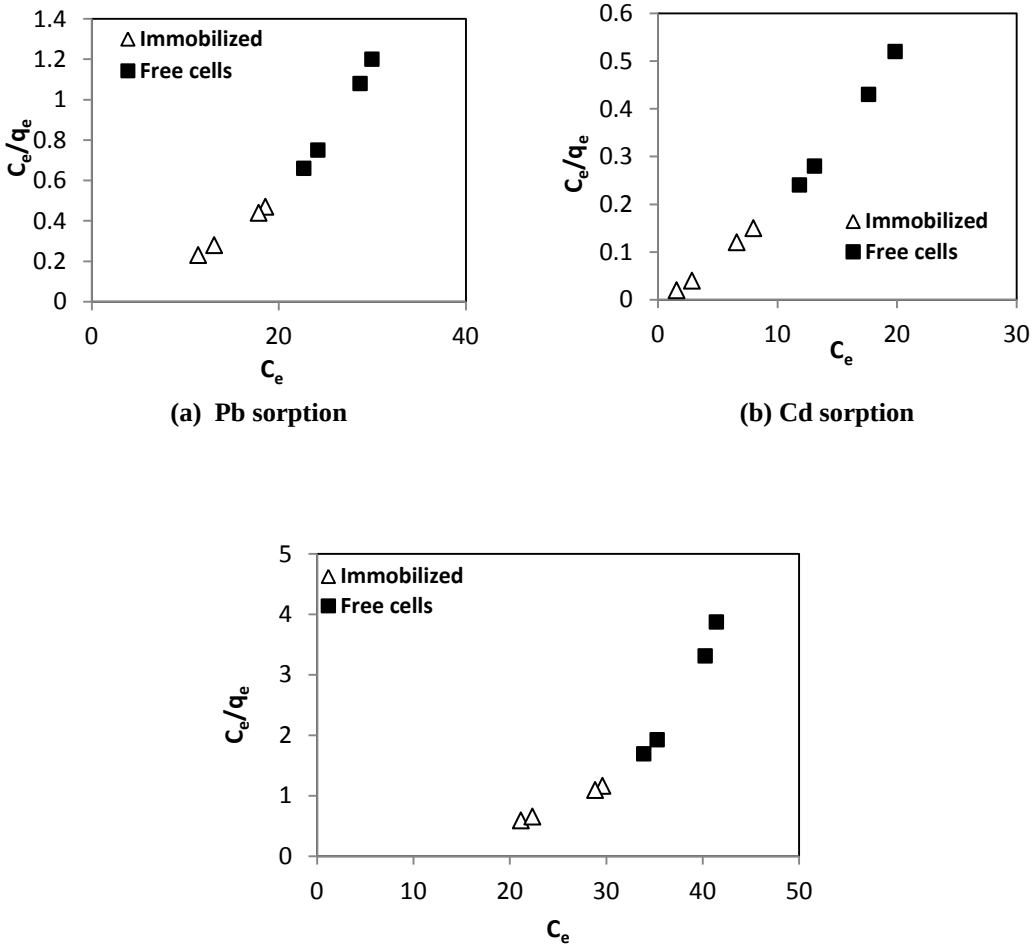
$$q_e = q_m b C_e /(1+ bC_e) \qquad 2.$$

where C_e is the residual metal concentration (mM/L); q_e is amount of metal adsorbed (mM/g); q_m (mg/g) and b (L/mg) are Langmuir constants showing the adsorption capacity and energy of adsorption, respectively (Langmuir

1918). The figure shows a well fitting of the model by immobilized cells due to high R^2 values. To analyse the nature of competition among Pb^{2+}, Zn^{2+} and Cd^{2+} ions, the multi component Langmuir model was applied to the binary and ternary sorption equilibrium data as follows (Bellot and Condoret 1993; Chong and Volesky 1996).

$$q_{e,i} = q_{m,i}b_iC_{e,i} / 1+b_jC_{e,i}+b_jC_{e,j}+b_kC_{e,k}+b_lC_{e,l} \quad 3.$$

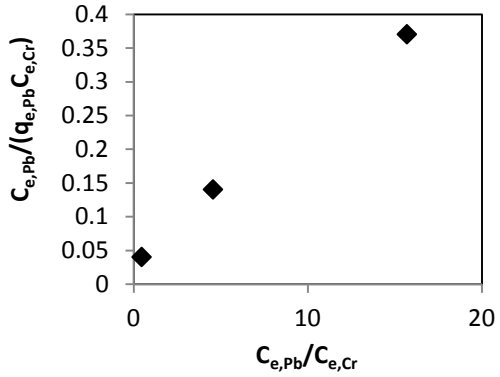
where $q_{m,i}$ and b_i are physical parameters, and $C_{e,i,}$ are equilibrium concentrations in the mixture of the solutes. The graphs plotted using Eqs. (2) and the adsorption parameters obtained are shown in Figs. 4 and 5 and Table 1, respectively. It is evident from the table that in binary system, Cr(II) competitively interfere with Pb(II) and Cd(II) adsorption on the biosorbent, while interference by Pb^{2+} and Cd^{2+} ions on Cr^{2+} ion adsorption was not significant. Further, most R^2 values for binary system are 0.99 indicating the better suitability of the model. On the basis of q_m values the interference dominance followed the order: Pb<Cr, Cd<Cr and Pb~Cd. Fitting ternary equilibrium sorption data to the multi component Langmuir model yielded the coefficients of determination: 0.99, 0.99, and 0.94 for the adsorption of Pb^{2+}, Cd^{2+} and Cr^{2+} ions, respectively, which indicate better fitting of the model in ternary system. Based on the q_m values, the biosorption affinity followed the order Pb > Cd > Cr.



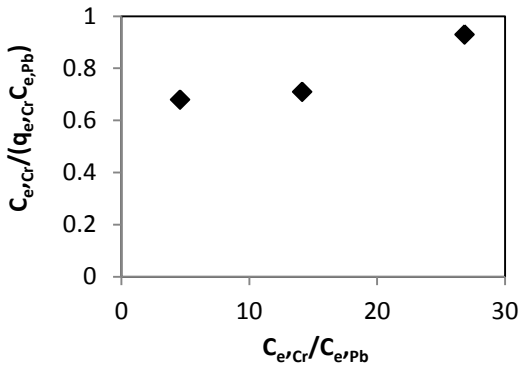
(a) Pb sorption **(b) Cd sorption**

(c) Cr sorption

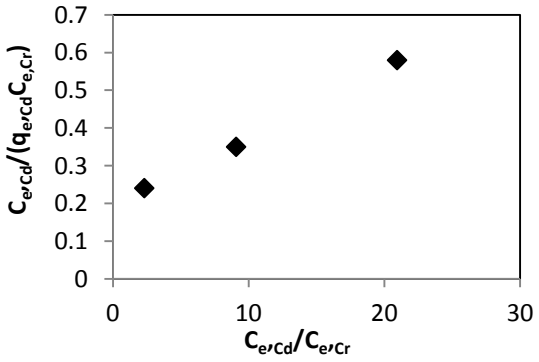
Fig. 3 Mono component Langmuir models for single sorption



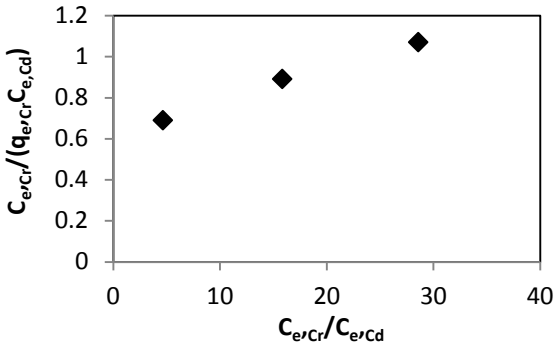
(a) Pb sorption in presence of Cr



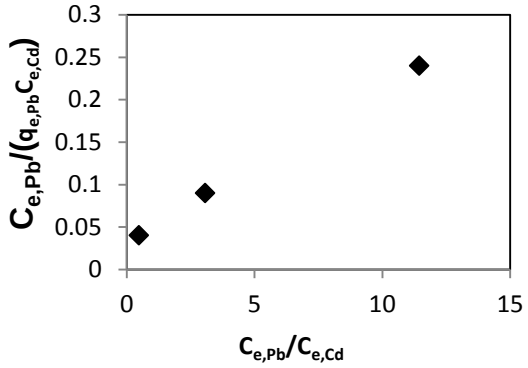
(b) Cr sorption in presence of Pb



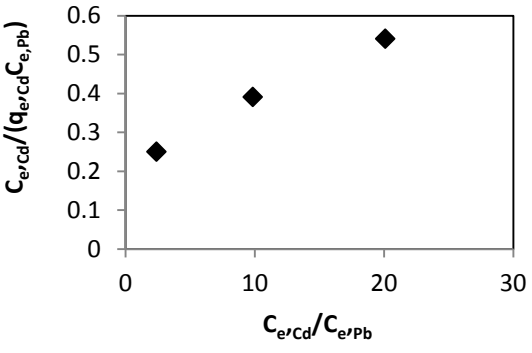
(c) Cd sorption in presence of Cr



(d) Cr sorption in presence of Cd

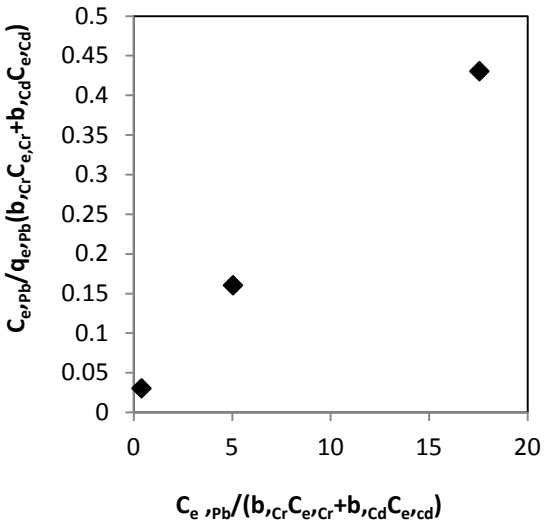


(e) Pb sorption in presence of Cd

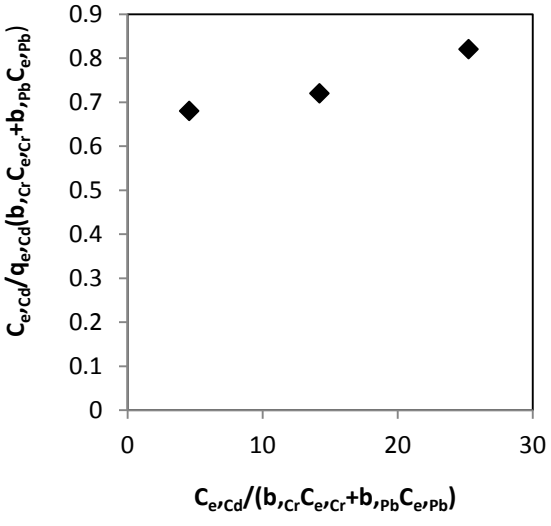


(f) Cd sorption in presence of Pb

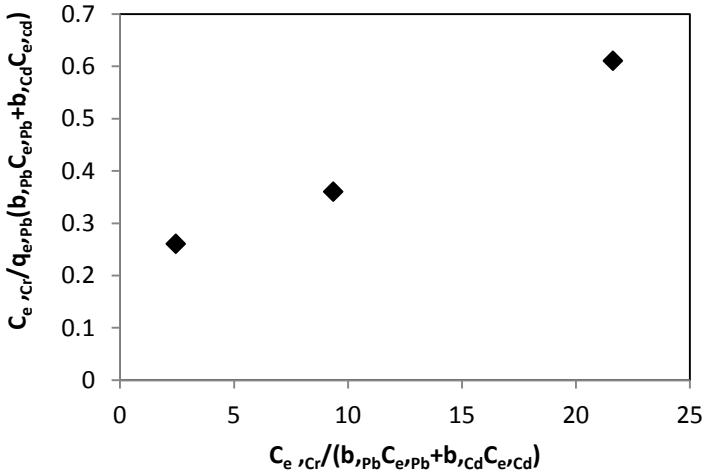
Fig. 4 Extended Langmuir models for binary sorption



(a) Pb sorption in presence of Cr & Cd



(b) Cd sorption in presence of Cr & Pb



(c) Cr sorption in presence of Pb & Cd

Fig. 5 Extended Langmuir models for ternary sorption

Conclusion

Experimental results demonstrated that immobilized cells of *B.megaterium* were more powerful biosorbents than its free cells for the biosorption of heavy metals. Further, Pb^{2+} ions were maximally biosorbed by the *B. megaterium* as compared to Cd^{2+} and Cr^{2+} ions in single and multimetal solutions. However Pb sorption was drastically inhibited in presence of Cr^{2+} ion, while the inhibition effect of Cd^{2+} was not so conspicuous. Moreover, in presence of Pb^{2+} and Cd^{2+} ions Cr^{2+} ion adsorption remain almost unaffected. All these depicted, *B. magaterium* an exellent biosorbent for the removal of toxic heavy metals from single and multimetal system and by immobilizing its biomass, the capacity of biosorption could be enhanced appreciably.

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