Cellular & Molecular Biology
*Cell. Mol. Biol.* 2014; 60 (5): 110-118
Published online December 24, 2014 (http://www.cellmolbiol.com)
Received on August 17, 2014, Accepted on September 21, 2014.
doi : 10.14715/cmb/2014.60.5.18

# Role of free living, immobilized and non-viable biomass of *Nostoc muscorum* in removal of heavy metals: An impact of physiological state of biosorbent

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**Abstract**

Biosorption of Pb and Cd by using free living, immobilized living and non-viable forms of *Nostocmuscorum* was studied as a function of pH (3-8), contact time (5-240 min) and metal concentration (10-100 µg $ml^{-1}$), to find out the most efficient physiological formfor metal removal. Results revealed that optimum conditions for biosorption of both the metals by different states of biosorbentwere almost same (contact time- 30 min, metal concentration- 100 µg $ml^{-1}$ and pH- 5.1 and 6, for Pb and Cd, respectively) however, the immobilized biomass of *N. muscorum* was found to be more suitable for the development of an efficient biosorbent as evident from the$q_{max}$(1000 mg $g^{-1}$protein) and $K_f$ (0.08 mg $g^{-1}$protein) values obtained from the Langmuir and Freundlich isotherms. A pseudo second order kinetics was found more suitable for describing the nature of biosorption of both the metals by all the three forms of *N. muscorum*. An analysis of correlation revealed that as the metal concentration increases, the removal of Pb and Cd by *N. muscorum* also increases significantly. The regression analysis showed that the rate of removal of Pb by free living and dead biomass was 1.89 and 1.58 times higher than the rate of removal of Cd by respective biomass. In contrast, the rate of removal of Cd by immobilized biomass was 1.46 times higher than that of Pb.

***Key words:*** Alignate beads, Immobilized, Isotherm, Kinetics, *Nostoc muscorum.*

## Introduction

The presence of certain heavy metals in the environment specifically in various water resources is of major concern because the threat to human health. The most dangerous metal include so called 'toxic trio' (Cd, Pb and Hg), which are not assigned any biological function(1).The International Agency for Research on Cancer (IARC) classified both cadmium and lead as a 'possible human carcinogen'. There is need of a treatment method for heavy metal removal from the waste water which is simple, effective and inexpensive(2).

Many researchers have addressed this problem, but there is still need for effective remedial technology(3,4). Scientists have discovered that various biomass can be used to clean up heavy metal pollution (5,6,7).Biological treatment has been receiving attention lately as an innovative, cost effective alternative to the more established treatment methods used at waste sites(8). The intrinsic capability of both living and dead microorganisms to sequester and possibly accumulate high levels of metal ions from dilute aqueous solution has attracted much attention over the years (9).Biosorption technology based on the utilization of dead biomass offers certain major advantages such as lack of toxicity constraints, non requirement of nutrient supply, no need to provide suitable growth conditions and recovery of bound metal species by an appropriate desorption method (7, 10). However, one major problem associated with microbial biosorbents is separation and harvesting of the biomass after metal removal. Immobilization of the organism in some suitable matrix like silica gel, polyurethane or alginate has proved useful in tackling this problem (4).The physical entrapment of the organism inside a polymeric gel in the form of beads is one of the most widely used techniques for immobilization which not only tackles the above problem but also provides mechanical strength, rigidity and porosity characteristics to the biosorbents(11). Further, the metal can be recovered from the loaded beads using appropriate desorption techniques, thereby, minimizing the possibilities of environmental contamination (12, 13).

There has been tremendous progress in the field of application of cyanobacteria as bioremedial agent to overcome the heavy metal related health hazard (4, 14, 15, 16). Cyanobacteria are suggested to have some added advantages over other microorganisms because of their large surface area, greater mucilage volume with high binding affinity and simple nutrient requirements (4, 15, 17)**.**

Though removal of Pb and Cd were studied by many microorganisms, no comparative study on biosorption mechanism of heavy metalsby using different physiological forms of an organism has been reported. In the present study, biosorption of Pb and Cd by a $N_2$ fixing cyanobacteria *Nostocmuscorum* was studied using its free living, immobilized and non-viable biomass. Some reports showed that dead cells could remove more metal than live cells (18),while other suggest that live biomass is more useful in remediation of heavy metals (19). In view of these conflicting results, it was assumed that application of isotherm models might be helpful in providing a suitable explanation for nature of metal biosorption.

## Materials and methods

### *Chemicals and Equipments*

To examine the heavy metal biosorption by the test organism stock solutions of Pb (II) and Cd (II) were prepared by dissolving nitrate salts of these metals in milli-Q water in calculated amount. The resulting stock solution was further diluted to get desired concentrations for biosorption experiments. For pH adjustment 0.1N HCl and 0.1N NaOH solutions were used. All the chemicals used in the present course of study are of Analytical Reagent (AR) Grade and were products of SD-Fine chemicals (India), Sigma chemicals (USA) or Merck (Germany). The solutions of metals were analyzed by using an atomic absorption spectrophotometer (Varian AA 240 FS, Australia) at a wavelength of 217 nm for lead and 228.8 nm for cadmium.

### *Preparation of Biomass*

The culture of cyanobacteria (*Nostocmuscorum*) was obtained from the National Facility for Blue-Green Algae, Indian Agricultural Research Institute, New Delhi and was grown in modified Chu-10 medium (20) under controlled laboratory conditions (25±1˚C) with 16 hour light/dark cycle.For preparing dead biomass, fixed amount of cells of *N. muscorum*were kept in an oven at 60˚C for 24 hours. The cells of *N. muscorum* were immobilized using Ca-alginate via entrapment method as described by Singh *et al*. (21). The immobilized beads were placed inChu-10 medium for overnight and were kept under illuminated condition so that organism comes out of stress. The amount of protein per bead was calculated by the method described by Lowry *et al.* (22) modified by Herbert *et al.* (23).

### *Biosorption Studies*

The free living, immobilized living and dead cells of *Nostocmuscorum*were suspended in the flasks containing different concentrations (10-100 µg ml$^{-1}$) of Cd and Pb metals. They were light incubated for 120 minutes for biosorption of metals. To check the absorption by alginate matrix, the beads of alginate without algal entrapment were also prepared. A set of alginate beads and a control set (without metal) was also run simultaneously. After 30 min incubation period, samples were centrifuged and supernatant was collected in test tubes. Supernatant of all the three forms were digested by the method described by Martin (24). The residual concentration of Pb and Cd in the medium was determined by AAS and that was subtracted from the initial metal ion concentration to get the amount of total absorbed metal.

To observe the effect of pH on biosorption of Pb (II) and Cd (II) by cyanobacterium*Nostocmuscorum*, initial pH values were maintained between pH 3-8. Experiments were not performed above pH 8 to avoid the hindrance in biosorption due to precipitation of the hydroxides of metal.

### *Adsorption isotherms*

To obtain adsorption isotherms (Langmuir and Freundlich) the biosorbent under study (*N. muscorum*) was suspended in metal solutions of different concentrations (10-100 µg ml$^{-1}$) at room temperature. The biosorption procedure was same as described above.

*Freundlich Isotherm*

This isotherm is derived from empirical consideration and expressed as

$$\mathrm{q_e} = \mathrm{K_f} C_e^{1/n}$$

where, qe is the amount adsorbed (mg g$^{-1}$), Ce is equilibrium concentration (mg L$^{-1}$), $K_f$ is the adsorption coefficient [Freundlich constant (mg g$^{-1}$)], which is a measure of adsorption capacity or fundamental effectiveness of the adsorbent. It is directly related to the standard free energy change, empirical constant 'n' is a measure of the adsorption intensity.

The model can be linearised logarithmically as

$$\mathrm{Log\,q_e} = (1/n)\,\mathrm{logC_e} + \log \mathrm{K_f}$$

Thus a plot between log $q_e$ and log $C_e$ is a straight line. Values of $K_f$ and1/n were calculated from plotting of graph between log $q_e$and log $C_e$, which is residual heavy metal concentration.

A high $K_f$ and high 'n' value are indicative of high adsorption throughout the concentration range. A low $K_f$ and high 'n' value indicates low adsorption throughout the studied concentration range. A low 'n' value indicates high adsorption at strong solute concentration.

*Langmuirisotherm*

Langmuir isotherm is obtained from a kinetic derivation of equilibrium between adsorbed and desorbed molecules. This give

$$\mathrm{q}_e = \mathrm{q_m}.K_A.C_e + \mathrm{K}_A.C_e$$

where, $q_e$ is the amount of adsorbate adsorbed per unit amount of adsorbent at equilibrium; $K_A$ the adsorption coefficient (a measure of adsorption energy) and $q_m$ the amount of adsorbate adsorbed per unit amount of adsorbent required for mono layer adsorption (limiting adsorbing capacity).

The above equation can also be written in linear form as

$$\frac{\mathrm{C_e}}{\mathrm{q_e}} = \frac{1}{\mathrm{q_{max}b}} + \frac{\mathrm{C_e}}{\mathrm{q_{max}}}$$

Where, $C_e$ is equilibrium concentration (mg L$^{-1}$), $q_e$ is amount adsorbed per gram of adsorbent at equilibrium (mgg$^{-1}$), $q_{max}$is Langmuir constant related to the maximum adsorption capacity and b is energy of adsorption. The value of $q_{max}$and b were calculated from the slope and intercept of the graph.

*Determination of Equilibrium parameter ($R_L$)*

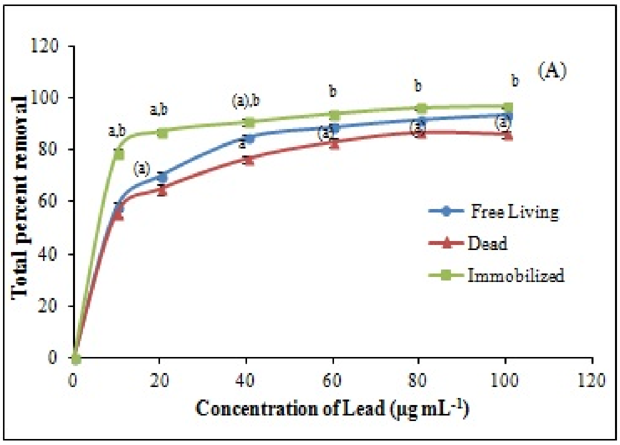
Equilibrium parameter $R_L$ which describes the type of Langmuir isotherm is represented as given by Hall *et al*. (25).

$$\mathrm{R}_L = \frac{1}{(1 + \mathrm{b\,C_0})}$$

Where b is the Langmuir constant (L mg $^{-1}$) and $C_0$ is the initial concentration of Pb and Cd (mgL$^{-1}$). A value of $R_L$ between 0 and 1 indicates a favourable sorption isotherm, if the $R_L$ =0, the adsorption is considered irreversible.

### *Kinetic Study*

Kinetic study of adsorption by cyanobacterium un-



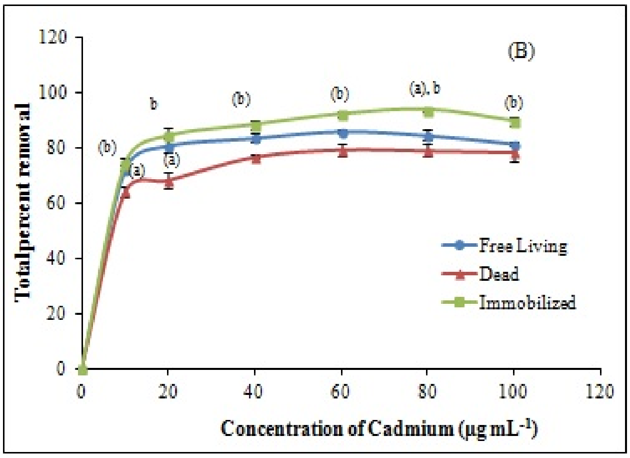




**Figure 1.** Total percent removal of Lead (A) and Cadmium (B) (10-100 µg $mL^{-1}$) by free living, dead and immobilized living biomass of *Nostocmuscorum.*All the values are mean ± SE of three replicates. (a) - $p<0.05$ or a – $p< 0.001$ as compared to live (b) - $p<0.05$ or b – $p< 0.01$ as compare to dead.

der study was carried out at 25, 50 and 100 µg $ml^{-1}$ initial concentrations of both Pb and Cd metals at room temperature wherein the extent of metal adsorption was analyzed at regular time interval.

### *Statistical analysis of the data*

Experimental data obtained in triplicate were summarized as mean ± SE and were shown graphically. Groups were compared by two factor analysis of variance (ANOVA) and the significance of mean difference within and between the groups was done by Newman-Keuls post hoc test. Based upon the nature of the data, association between dose and response was also evaluated by using simple linear/non-linear correlation and regression analysis. A two-sided (α=2) $p<0.05$ was considered statistically significant. All analyses were performed on STATISTICA windows version 6.0 (StatSoft, Inc., USA).

## Results

### *Effect of initial metal concentration*

The impact of various states of culture on the accumulation of different concentration of Pb and Cd by *N. muscorum*was studied to find out the efficient state of biosorbent for biosorption. The metal accumulation potential of immobilized living cells of *N. muscorum* was always higher than the free living and non-living cells for both the metals(Fig. 1). The total percent removal of metals (Pb and Cd) increased in all the forms of biosorbentin a concentration dependent manner up to 80, 60 and 100 µg $ml^{-1}$ for free living, non-living and immobilized biomass, respectively. Immobilized biomass removes a maximum of 96.5 % Pb and 93.6 % Cd, whereas non-living and free living biomass removed 86, 93.7 % Pb and 80.3, 88.5 % Cd, respectively. On comparing percent removal of Pb and Cd it was observed that biosorption of Pb was always higher than that of Cd by all the forms of tested biosorbent.The biosorption by alginate beads alone (without biomass) was also recorded for each condition. The biosorption of both the metals by alginate beads was found almost negligible and was substracted from the total biosorption by immobilized cells of *N. muscorum*.

### *Study of correlation between Total percent removal of Pb and Cd by free living, dead and immobilized biomass of Nostocmuscorum*

The removal of Pb and Cd by free living, dead and immobilized biomass of *Nostocmuscorum*was analyzed by simple linear correlation and regression analysis(Fig. 2). The correlation analysis revealed that as concentration increases the removal of Pb by free living ($r=0.97$, $p<0.001$), dead ($r=0.96$, $p<0.001$) and immobilized ($r=0.90$, $p<0.001$) biomass of *N.muscorum*also increasessignificantly (Fig.2a). Further, regression analysis revealed that the rate of removal of Pb by free living was highest (b=1.0362%/µg/ml) followed by dead (b=0.913%/µg$ml^{-1}$) and immobilized the least (b=0.4602%/µg$ml^{-1}$) **(**Fig. 2a).

Similarly, as concentration increases the removal of Cd by free living ($r=0.85$, $p<0.001$), dead ($r=0.87$, $p<0.001$) and immobilized ($r=0.97$, $p<0.001$) biomass of *N.muscorum*also increasessignificantly **(**Fig.2b). Further, regression analysis revealed that the rate of removal of Cd by immobilized was highest (b=0.6736%/µg$ml^{-1}$) followed by dead (b=0.0.5769%/µg$ml^{-1}$) and by free living the least (b=0.5487%/µg$ml^{-1}$) (Fig. 2b**).** Regression analysis also showed that the rate of removal of Pb by free living cells was 1.89 times higher than the Cd. Similarly, the rate of removal of Pb by dead biomass was 1.58 times higher than the Cd. In contrast, the rate of removal of Cd by immobilized was 1.46 times higher than the Pb.

### *Adsorption isotherm*

Adsorption of Pb and Cd was studied as a function of different physiological state (free living, dead and immobilized) of *N. muscorum* to understand the nature of adsorption by fitting the experimental data to Langmuir and Freundlich models, which are widely used to analyze data for water and waste water treatment applications. During modeling of the adsorption of both Pb and Cd on free living, dead and immobilized cell biomass as biosorbent the related Langmuir constants ($q_{max}$ and $b$) and Freundlich constants ($K_F$ and $n$) along with the regression coefficient ($R^2$) were calculated as shown in table 1. Based on the $R^2$ values, it was observed that the nature of adsorption of Pb and Cd could be described by both Langmuir and Freundlich model.

*Freundlich Isotherm*

In case of Freundlich isotherm, $K_F$ represents the

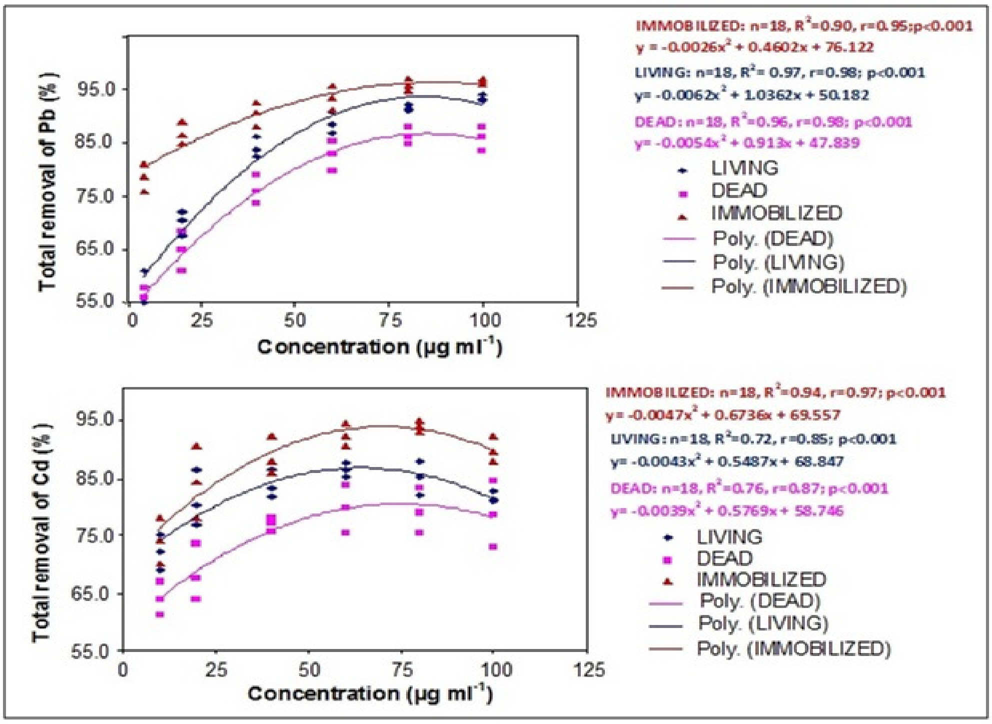



**Figure 2.** Study of Correlation between total percent removal of Pb and Cd by free living, dead and immobilized biomass of *Nostocmuscorum.*

adsorption coefficient and $n$ is related to the effect of concentration of metal ions. The nature of metal adsorption could be defined by both $K_F$ and $n$ values. To some extent, the $n$ values contribute more significantly. Plots of Log qe and Log Ce(Fig.3) for the adsorption of both the metal ions in all the physiological forms of biomass were found to be linear throughout the concentration range studied.

The $K_F$ values for adsorption of Pb ion was about 4 times higher for the free living cells and 8 times for the immobilized cell biosorbent than that observed for Cd ions whereas the $K_F$ value for dead cell biomass was twofold greater for Cd than that for Pb. The results indicated higher adsorption coefficients for Pb than Cd ions. Among all the physiological states Pb have higher adsorption coefficient on the immobilized biosorbent. The values of $n$ for Pb uptake by living, dead and immobilized biomass were 1.01, 1.10 and 1.00 respectively, while the corresponding values for Cd were 1.07, 1.40 and 1.00, indicating a favourable adsorption of both the metal ions by *N. muscorum*.

*Langmuir Isotherm*

The Langmuir model assumes monolayer biosorption onto a surface with a finite number of identical sites. Langmuir isotherm showed linear plots 1/qe versus 1/Ce(Fig. 4). Langmuir constants $q_{max}$ and $b$ defined the total adsorption capacity and metal binding affinity of cell biomass, respectively, for both the Pb and Cd metals.

The $q_{max}$ values for both the metals were found to be in sequence of immobilized> free cells > dead cell biosorbent(Table 1). Based on $q_{max}$, it was revealed that *N. muscorum* cells in all the physiological states (living, dead and immobilized) showed higher biosorption capacity for Pb than the Cd. On comparing of living and dead biomass of *N. muscorum,* it was observed that living biomass could adsorb two times more Pb and three times more Cd than the dead biomass. Between both the

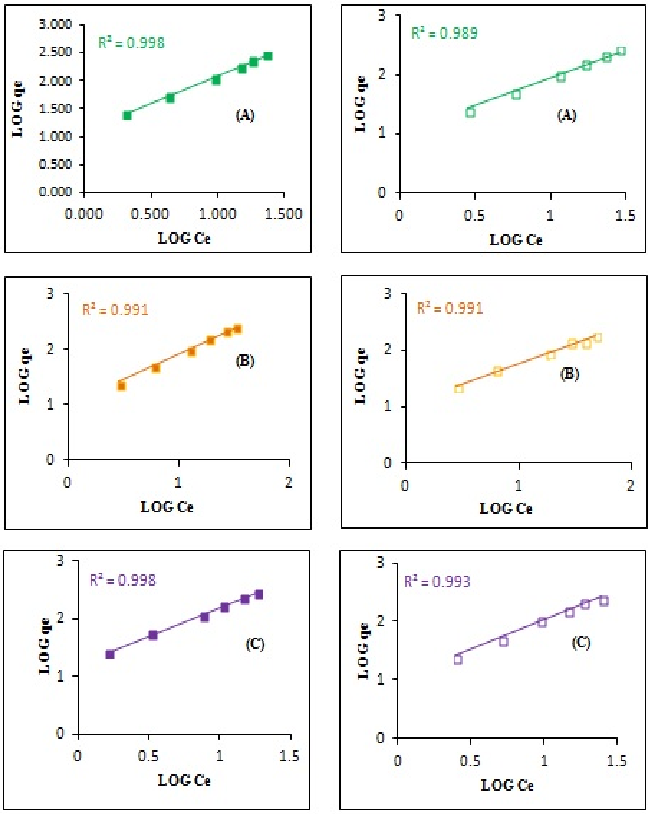



**Figure 3.** Freundlich Isotherm for the sorption of Pb(Closed symbols) and Cd (Open symbols) on Free living (A), Dead (B) and Immobilized (C) biomass of *Nostocmuscorum.*

free living and immobilized cell condition the immobilized cells adsorbed 1.2 times more Pb and 0.9 times more Cd than the free living cells.

The values of constant $b$, clearly indicated higher affinity of *N. muscorum* towards Pd ($b$= 0.0166) as compared to the Cd ($b$= 0.0134). Dead cells of *N. muscorum* showed least adsorption capacity ($q_{max}$) for both the metals in comparison to free living and immobilized biomass, but showed about three times greater binding affinity towards Cd when compared with that for free living and immobilized biomass.

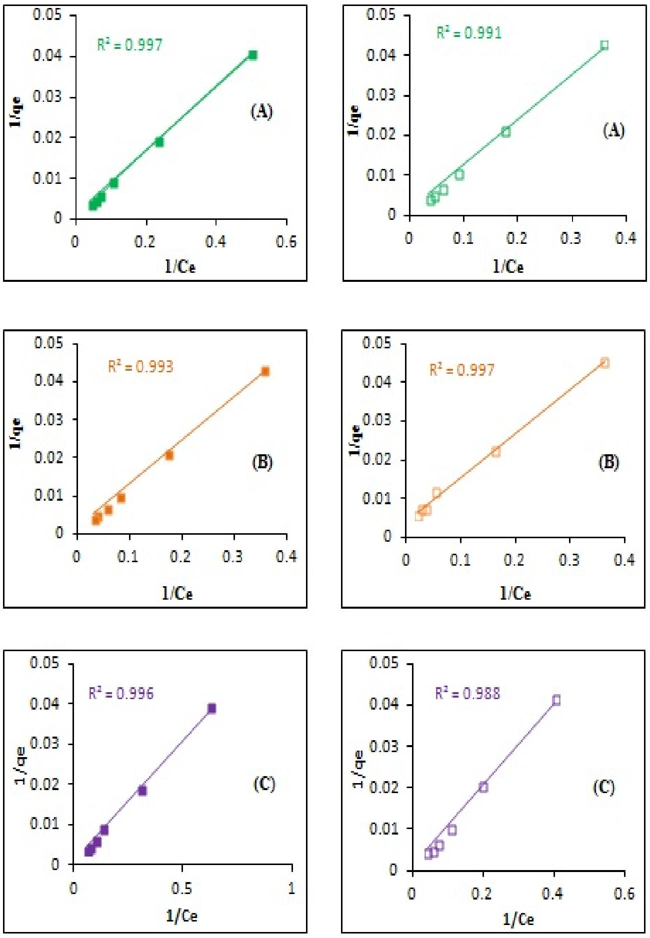


**Figure 4.** Langmuir Isotherm for the sorption of Pb(Closed symbols) and Cd (Open symbols) on Free living (A), Dead (B) and Immobilized (C) biomass of *Nostocmuscorum.*

### *Separation Factor ($R_L$)*

According to Hall and his coworkers, the shape of Langmuir isotherm can be expressed in terms of a separation factor ($R_L$). The values of $R_L$ obtained as shown in Table- 2 were between 0 and 1 for all types of adsorbents and initial concentrations of Pb and Cd, indicating a favorable process. These results suggested for applicability of Langmuir isotherm for the adsorption of both Pb and Cd onto *N. muscorum*cells. The $R_L$ vaues for adsorption of Cd by dead biomass of *N. muscorum* was relatively less throughout the concentration range as compared to the free living and immobilized biomass. This result supported the least adsorption capacity ($q_{max}$) for Cd in case of dead biomass.

### *Effect of pH*

Effect of external pH conditions (pH 3.0-8.0) on the biosorption of Pb and Cd by freeliving, immobilized and dead form of *N. muscorum* cells was studied (Fig.5). Sorption of Pb and Cd by all the three physiological states of *N.muscorum* were highly dependent on the external pH condition. The results showed that removal of both the metals increased with increase in pH of the medium from 3.0 to 6.0 and was maximum at pH 5.1 and 6 for Pb and Cd, respectively (96.2 % Pb and 90.3 % Cd), by all the three forms of *N. muscorum*. Immobilized cells of *N. muscorum* was found more efficient in removal of both the metals as compare to free living and dead cells. At acidic pH range removal of Pb was always more than Cd however, at alkaline pH Cd was removed more as compared to Pb by all the three forms of *N. muscorum*.

### *Biosorption Kinetics*

The Lagergren first-order and pseudo-second order kinetic models were used to test the data on Pb and Cd adsorption by *N. muscorum*. Both the kinetic plots were constructed for the adsorption of Pb and Cd at three initial concentrations (25, 50 and 100 µg ml$^{-1}$). The t/q *vs* t plot for pseudo second order kinetics for both the metal gave a straight line(Fig. 6), whereas plot of log (qe-qt) *vs*t, used to verify the Lagergren first order kinetics, did not fairly follow a linear relationship (result not shown). This suggested that the pseudo-second order kinetics is applicable to adsorption of Pb and Cd by free living,

**Table 1.** Langmuir and Freundlich isotherm constants for the adsorption of Pb and Cd by free living, immobilized and dead biomass of *Nostocmuscorum.*

| Metal | Biomass State | Langmuir Constant: Max.adsorption capacity '$q_{max}$' (mg g$^{-1}$) | Adsorption affinity '$b$' (L mg$^{-1}$) | $R^2$ | Freundlich Constant: Adsorption coefficient '$K_f$' (mg g$^{-1}$) | Adsorption intensity '$n$' | $R^2$ |
|---|---|---|---|---|---|---|---|
| | Free Living | 833.3 | 0.016 | 0.997 | 0.04 | 1.01 | 0.998 |
| | Dead | 555.6 | 0.016 | 0.993 | 0.01 | 1.10 | 0.991 |
| Pb | Immobilized | 1000.0 | 0.017 | 0.996 | 0.08 | 1.00 | 0.998 |
| | Free Living | 666.7 | 0.013 | 0.991 | 0.01 | 1.07 | 0.989 |
| Cd | Dead | 250.0 | 0.035 | 0.997 | 0.02 | 1.40 | 0.991 |
| | Immobilized | 769.2 | 0.013 | 0.988 | 0.01 | 1.00 | 0.993 |

**Table 2.** Separation factor ($R_L$) values for adsorption of Pb and Cd on free living, immobilized and dead biomass of *Nostocmuscorum*(if RL>1=unfavourable adsorption, RL is equal to1=linear adsorption, 0<RL<1=favourable adsorption, RL is equal to 0=irreversible adsorption).

| Initial Conc. (µg ml$^{-1}$) | Pb Living | Pb Dead | Pb Immobilized | Cd Living | Cd Dead | Cd Immobilized |
|---|---|---|---|---|---|---|
| 10 | 0.87 | 0.86 | 0.86 | 0.88 | 0.74 | 0.88 |
| 20 | 0.76 | 0.76 | 0.75 | 0.79 | 0.59 | 0.79 |
| 40 | 0.62 | 0.61 | 0.6 | 0.65 | 0.42 | 0.65 |
| 60 | 0.52 | 0.51 | 0.5 | 0.55 | 0.32 | 0.55 |
| 80 | 0.45 | 0.44 | 0.43 | 0.48 | 0.26 | 0.48 |
| 100 | 0.39 | 0.39 | 0.38 | 0.43 | 0.22 | 0.43 |

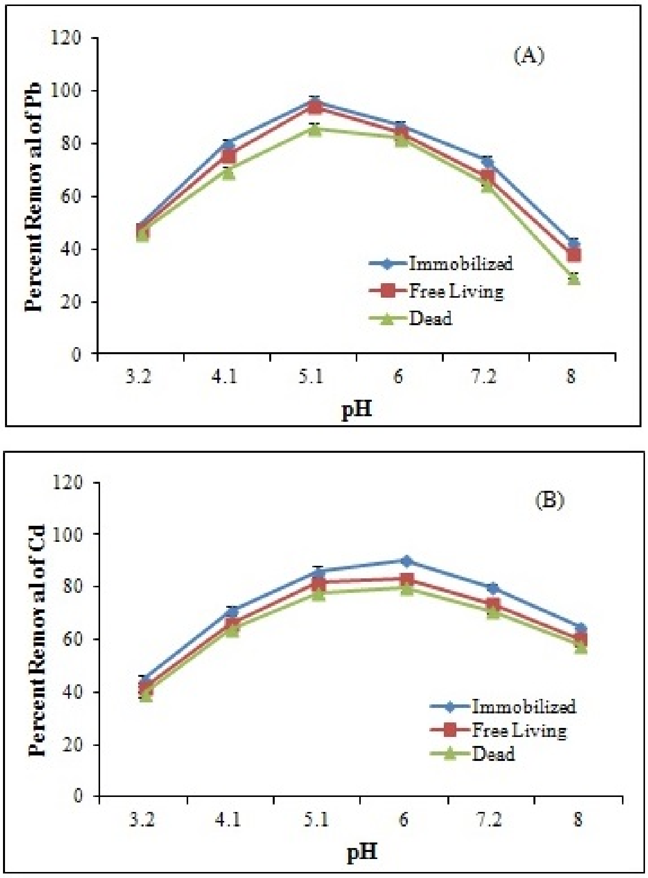



**Figure 5.** Effect of different pH range (3-8) on total percent removal of Lead (A) and Cadmium (B) (conc. 100 µgml$^{-1}$) by free living, dead and immobilized living biomass of *Nostocmuscorum.*All the values are mean ± SE of three replicates.

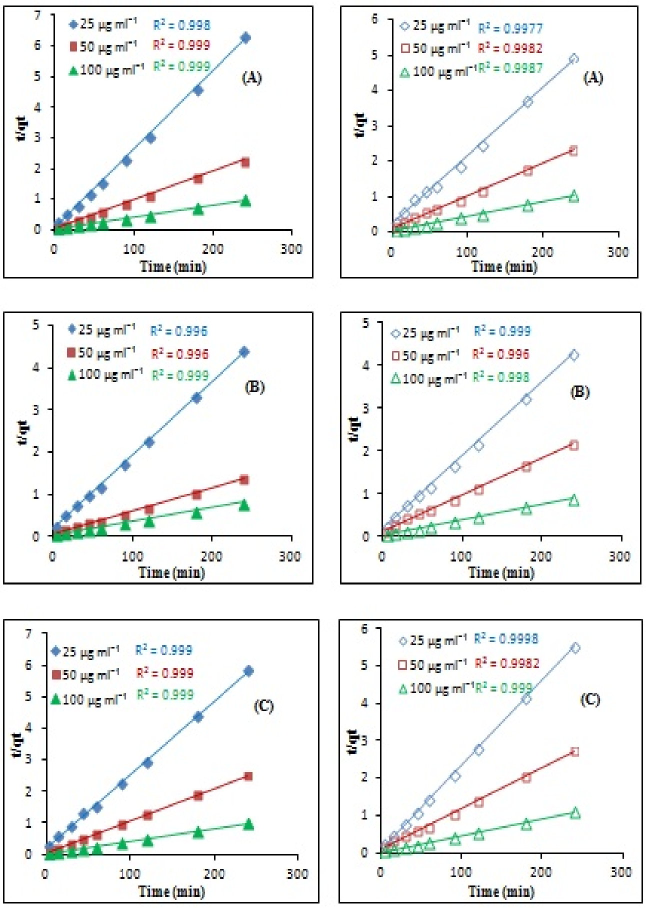



**Figure 6.** Lagergrensecond-order kinetic modelling for adsorption of Pb(Closed symbols) and Cd (Open symbols) by free living (A), immobilized (B) and dead (C) biomass of *Nostocmuscorum.*

immobilized and dead form of *N. muscorum,* not the first order kinetics. The results on kinetics revealed that an increase in metal concentration from 25 to 100 µg ml$^{-1}$ led to corresponding increase in the equilibrium sorption capacity (qe) for both the metals in all the three forms of *N. muscorum*. The rate constants for both Pb and Cd sorption by free living, immobilized and dead *N. muscorum* calculated from the Langergren first-order and pseudo-second-order models were shown in(Table 3). The results depicted that value of regression coefficient ($R^2$) for the second order adsorption model is relatively high. However the values of $R^2$ for pseudo first order reaction is not satisfactory. The calculated values of qe agree fairly well with the experimental qe values of second order equation. Therefore, it was concluded that the pseudo second order adsorption model is more suitable to describe the adsorption kinetics of Pb and Cd by *N. muscorum.*

## Discussion

The present study showed that the total metal accumulation potential of immobilized-living cells of *N. muscorum* was always higher than the free living and dead cell biomass for both the metals. Immobilization generally tends to increase metal accumulation by biomass(26, 27). Immobilized cells accumulate more metals than free cells due to (i) enhanced photosynthetic capacity (28), and (ii) increased cell wall permeability (29). Immobilization of living biomass also provides protection to cells from metal toxicity (30).On the contrary, some reports show a higher metal sorbing efficiency of free cells compared to immobilized cells (31, 32). A change in the structure of cell wall during immobilization process has been considered to be responsible for decreased metal sorption capacity of the immobilized biomass (19). Other workers suggested that part of cell surface might be shielded by the gel matrix making the sites unavailable for metal binding (18). On comparing metal binding by live and dead biomass, the live cells are found to be more potent for biosorption, in the presnt study. Similar results are obtained by Doshi*et al.*(33) who have found that live cells of *Spirulina* sp. could remove more Cd in comparison to dead cells. The metabolic activities in live cell biomass possibly helped in higher uptake of metal ions as compared to dead biomass (34). Further, intracellular polyphosphates as well as extracellular polysaccharides in live algal cells might be helping in metal sequestration (35, 36). On the contrary some workers suggested that dead cells could remove more metal than live cells (18).

The main advantage of Langmuir model is the evaluation of $q_{max}$- maximum possible quantity of metal ion adsorbed per gram of adsorbent, and *b* – parameter related to the affinity of binding sites for a metal ion (37). In general, for good biosorbents, a higher $q_{max}$and *b* values are desirable (38). From the present study it was observed that dead cells of *N. muscorum* have least adsorption capacity ($q_{max}$) for both the metals in comparison to free living and immobilized biomass. However, the dead biomass exhibits about three times greater affinity (*b*) towards Cd when compared with that of free living and immobilized biomass. Hasim and Chu (39) have suggested that a biosorbent with low$q_{max}$ and high

**Table 3.** Lagergren pseudo-first-order and Pseudo-second-order kinetics rate constants for adsorption of Pb and Cd by free living, immobilized and dead biomass of *Nostocmuscorum.*

| Metal | Biomass State | Initial Metal Conc. (µg mL$^{-1}$) | Qe Experimental (µg mg$^{-1}$) | Pseudo-second Order Kinetics | | | First Order Kinetics | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | $q_e$Calculated (µg mg$^{-1}$) | $K_2$ | $R^2$ | $q_e$ Calculated (µg mg$^{-1}$) | $K_1$ | $R^2$ |
| Pb | Free Living | 25 | 52.3 | 39.21 | 0.0082 | 0.998 | -0.165 | 0.011 | 0.347 |
| | | 50 | 117.6 | 106.38 | 0.0037 | 0.999 | 0.051 | 0.015 | 0.723 |
| | | 100 | 282 | 250 | 0.0039 | 0.999 | -0.162 | 0.011 | 0.279 |
| | Immobil-ized | 25 | 53.8 | 57.14 | 0.0017 | 0.998 | 0.115 | 0.020 | 0.882 |
| | | 50 | 120 | 181.81 | 0.0005 | 0.996 | 0.221 | 0.021 | 0.632 |
| | | 100 | 290 | 303.03 | 0.0004 | 0.999 | 0.309 | 0.024 | 0.950 |
| | Dead | 25 | 47 | 42.91 | 0.0029 | 0.999 | 0.043 | 0.018 | 0.839 |
| | | 50 | 109 | 97.08 | 0.0032 | 0.999 | 0.078 | 0.015 | 0.837 |
| | | 100 | 257 | 250 | 0.0006 | 0.999 | 0.269 | 0.020 | 0.937 |
| Cd | Free Living | 25 | 47.56 | 52.08 | 0.0014 | 0.997 | 0.114 | 0.014 | 0.801 |
| | | 50 | 110 | 108.69 | 0.0007 | 0.998 | 0.219 | 0.021 | 0.887 |
| | | 100 | 256 | 238.09 | 0.0011 | 0.998 | 0.044 | 0.017 | 0.414 |
| | Immobil-ized | 25 | 49.5 | 58.47 | 0.0015 | 0.999 | 0.143 | 0.018 | 0.932 |
| | | 50 | 114 | 117.64 | 0.0005 | 0.996 | 0.264 | 0.024 | 0.958 |
| | | 100 | 250 | 270.27 | 0.0007 | 0.998 | 0.211 | 0.211 | 0.769 |
| | Dead | 25 | 44.6 | 44.44 | 0.0045 | 0.999 | -0.014 | 0.014 | 0.782 |
| | | 50 | 93 | 92.59 | 0.0010 | 0.998 | 0.182 | 0.023 | 0.880 |
| | | 100 | 175 | 227.27 | 0.0007 | 0.999 | 0.215 | 0.021 | 0.729 |

*b* could outperform a biosorbent with high $q_{max}$ and low *b*, especially in cases where metal ions to be removed are present in traces. Therefore higher *b* values obtained for dead cells of *N. muscorum*indicated its higher affinity and suitability for adsorption where Cd is present in traces.The magnitudes of *Kf* and *n* values showed separation of metal ions from aqueous medium. It has been suggested that *n* values between 1-10 represent beneficial adsorption. Results of the present study indicates the favourable adsorption of both Pb and Cd ions by *N. muscorum*.

Results suggest that adsorption of Pb and Cd can be described by using both Langmuir and Freundlich model. Similar results were obtained by Shen *et al.*(32) who found that adsorption of Pb (II) by *Synechococcus* species obeys both Langmuir and Freundlich model, while the adsorption of Cr (VI) obeys only Freundlich model. Anjana*et al.*(4) have also observed that biosorption of Cr (VI) by immobilized biomass of *Chroococcus*sp HH-11 fit in well with both the models. The metal biosorption process fitting better toFreundlich isotherm, indicatesheterogenecity of algal surface and significant influence of one occupied site on the other biosorption site.A large variation has been found by several authors among various biomass treated with different metals for applicability of both the isotherms (33, 40, 41).

pH of the surrounding medium plays an important role in the ionization of functional moieties on the adsorbent surface, which facilitates the metal binding in aqueous medium (42). Results of the present investigation revealed that an increase in pH from 3 to 8 increased the absorption capacity of the biomass and reached a plateau around pH 5 and 6 for Pb and Cd, respectively. These results are in agreement with studies of several other workers (44, 44, 45) who have suggested that reduced metal uptake with a decrease in pH is perhaps due to enhanced competition between metal cation and protons for binding on the algal surface. A reduced metal uptake as well as surface binding at pH values greater than 7 may be ascribed to metal-hydroxylation, yielding the metal hydroxides or hydrated oxides, which leads to metal passivation (46).

Kinetic study on adsorption of both Pb and Cd was performed by using both Lagergren first-order and pseudo-second-order kinetic models. Results showed that adsorption of Pb and Cd by all the three forms of *N. muscorum*follows pseudo-second order kinetics. The increasing value of qe with increase in metal concentration suggested that Pb and Cd are likely to bind the adsorption sites on biomass by displacing other cations linked through energetically weaker bonds. Other investigators (33, 47, 48) have also reported that adsorption of metals by algae follows second order kinetics with few exceptions (42, 49).

It might be suggested that Pb and Cd metal binding by *N. muscorum* cells is mediated by displacement of other ions, as evident from its dependence on pH of the medium as well as suitability of both Langmuir and Freundlich isotherms for metal sorption. A higher $q_{max}$ and $K_f$ values for immobilized living cells was indicative of its better metal removal efficiency than the dead or free living biomass of *N. muscorum*.In industrial operations, immobilized microbial cellscould also provide additional advantages over freelysuspended cells, e.g., ease of regeneration and reuse ofthe biomass, easier solid–liquid separation, and minimalclogging in continuous flow systems.

Other articles in this theme issue include references (50-65).

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