Cellular and Molecular Biology

Vishnoi et al., Cell Mol Biol 2016, 62:3

Review Article Open Access

Differential Pattern of Arsenic Binding by the Cell Wall in Two Arsenite Tolerant *Bacillus* Strains Isolated from Arsenic Contaminated Soil

Vishnoi N, Dixit S and Singh D P*

Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raibareilly Road, Lucknow, Uttar Pradesh, India

Abstract

Arsenite binding was evaluated in two *Bacillus* strains i.e., *B. megaterium* and *B. pumilus*, isolated from arsenic contaminated soil of Unnao district of Uttar Pradesh (India). Initial results showed that more than 90% of arsenite was removed by surface binding by the cell wall component in both the tested species of bacteria. Results on the concentration dependent arsenic binding in bacterial strains exhibited higher efficiency of arsenite binding in *B. megaterium* (q_{max} - 1000 mg g^{-1} protein) than *B. pumilus* (q_{max} - 666.7 mg g^{-1} protein). The pH optima for arsenic (As) binding in both *B. megaterium* (pH 6.0) and *B. pumilis* (pH 8.0) were found to be different. Results on temperature dependent arsenite binding by *B. megaterium* showed maximum binding at 30ºC, while arsenic binding maxima in *B. pumilus* showed a broad temperature range (25ºC to 35ºC). The kinetic parameters on arsenite binding revealed that both the bacterial strains followed pseudo-second order kinetics. The As adsorption behavior of the bacterial strains was better explained by Langmuir isotherm rather than Freundlich model. Results of FTIR spectra on surface binding of As revealed major spectral changes in the band region of 1600 cm^{-1} to 800 cm^{-1} in case of *B. megaterium*, indicating involvement of mainly amines, alkenes and C-N functional groups. Whereas FTIR spectrum of *B. pumilus* showed changes in the band region of 3433 cm^{-1} to 2924 cm^{-1} indicating the involvement of hydroxyl, alkanes, alkenes, amides and aromatic functional groups in the arsenic binding. A corollary of these results indicated differential binding of arsenite in both the *Bacillus* strains was on account of different arsenite binding ligands on cell surface as evident from the FTIR results as well as different pH and temperature optima.

Keywords: Arsenate; *Bacillus megaterium; Bacillus pumilus*; Isotherm; Surface binding

Introduction

Among the various heavy metals, arsenic is well-known toxic metalloid that is considered as priority pollutant. Arsenic, predominantly from natural sources, has also been found to be contaminating groundwater in parts of the Indian sub-continent. Arsenic is generally toxic to life but it has been demonstrated that microorganisms can use arsenic compounds as electron donors or electron acceptors, and they can possess arsenic detoxification mechanisms [1-3]. The ubiquity of arsenic in the environment has forced the microorganisms to evolve an arsenic defense mechanism [4]. Certain microorganisms are known to have developed the necessary genetic components which confer arsenic-tolerance mechanisms, allowing them to survive and grow in environment containing high levels of arsenic that would be toxic to most of the other organisms [5]. The adverse health effects of As are well documented [6]. Chronic arsenic poisoning can cause a lot of human health problems through either contaminated drinking water or agriculture products irrigated by contaminated water [7-9]. Arsenic occurrence in the environment, its toxicity, health hazards, and the techniques used for speciation analysis are well known and has been reviewed [10-12]. Metal adsorption is generally recognized as fast interaction between metal cations and electronegative surface of the cell membrane [13]. Since the arsenite in aqueous solution is present as negatively charged anion, the zeta potential of membrane surface, apparently unfavourable for negatively charged anions, makes their removal difficult from the arsenic contaminated sites [14,15]. The use of microbes for arsenite removal has so far not yielded satisfactory results as the membrane surface requires preprocessing to change the surface charge. However, genetic modification of cell surface of bacterium *E. coli* is reported to be favourable for the arsenite removal [16]. Since the arsenite is more toxic than arsenate, the natural selection pressure might have resulted into arsenite tolerance in some bacteria in contaminated sites by bringing about required changes in the gene pool. For successful bioremediation of arsenite by using microorganisms, an effort is required to target isolation of specifically arsenite-tolerant bacteria with enhanced efficacy of arsenite adsorption.

In view of the above, the main objective of the present study was aimed to isolate arsenite tolerant bacterial strains with efficient arsenite adsorption efficiency, which can be exploited for bioremediation of arsenic contaminated sites. Further, attempts have been made to study the nature of arsenite interaction with membrane cell surface by employing kinetic studies and isotherm studies.

Materials and Methods

Chemicals and equipment

Stock of As (III) was prepared by dissolving sodium arsenite in sterile deionized Milli Q water, stored at 4ºC in dark. For pH adjustment 0.1N HCl and 0.1N NaOH solutions were used. The arsenic concentration were analyzed by using an atomic absorption spectrophotometer (AAS) (Varian AA 240 FS, Australia) at a wavelength of 193.7 nm. All chemicals used are of analytical reagent (AR) grade and were product of Loba Chemicals Pvt. Ltd.

Isolation and identification of bacterial isolates

The arsenite resistant bacterial strains were isolated from arsenic

***Corresponding author:** Singh DP, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raibareilly Road, Lucknow-226025, Uttar Pradesh, India, Tel: 09415575735; Fax: 0522-2441888; E-mail: dpsingh_lko@yahoo.com

Received March 01, 2016; **Accepted** October 25, 2016; **Published** October 30, 2016

Citation: Vishnoi N, Dixit S, Singh DP (2016) Differential Pattern of Arsenic Binding by the Cell Wall in Two Arsenite Tolerant *Bacillus* Strains Isolated from Arsenic Contaminated Soil. Cell Mol Biol 62: 138.

contaminated soil of Gaja Khera, Shukla Ganj and Murtaza Nagar village located in Unnao district (27° 40' N, 80° 00' E) of Uttar Pradesh, India. As per survey of U.P. Jal Nigam (a statutory body of State Government which looks over the development and regulation of water supply and sewerage systems of state), these areas have been declared as arsenic affected areas. The soil samples were collected from a depth of 15 cm in sterile plastic bags. The arsenite tolerant colonies of bacteria were isolated and screened on nutrient agar plates containing 40 mM of sodium arsenite. The standard microbiological techniques were employed to screen the microorganism. Discrete arsenic tolerant bacterial colonies were picked up and maintained in nutrient broth containing 40 mM of arsenite.

The bacterial isolates were further identified by using standard morphological and biochemical tests [17] and then send for 16S rDNA sequencing at Genetech, Biotech Park, Lucknow (U.P., India). The gene sequences of these strains were submitted at to NCBI. The identified bacterial strains are *Bacillus megaterium* (Accession no. KC633281) and *Bacillus pumilus* (Accession no. KC633283).

Arsenic biosorption experiment

Biosorption of arsenite was measured in 100 ml Erlenmeyer's flask containing 20 ml BSMY II. Exponentially growing culture was harvested by centrifugation (3000 X, 15 min), washed with distilled water and the pellets were suspended in the flasks containing different concentrations (0-100 µg ml^{-1}) of arsenite. They were kept in shaking incubator at 30°C temperature for 120 minutes. At the end of incubation period, 10 ml of sample was taken and centrifuged (1500 X, 10 min). Supernatant was collected in test tubes for the measurement of remaining as concentration in the medium. The cell pellets were washed twice with distilled water to remove any unbound metal. Thereafter, cells were washed with 2 ml of 10 mM EDTA in order to measure the membrane bound metal concentration. The EDTA washed cell pellets were washed with distilled water then dried at 60°C and were digested in acid solution containing 10:1 mixture of HNO_3 and $HClO_4$ acids as described by [18]. Total EDTA washed fraction was also treated in the same way. The substraction of membrane bound as from the total as removed from the medium gave the concentration of intracellular uptake of arsenite. Surface bound arsenite concentration was calculated from EDTA washable fraction.

To study the effect of pH on biosorption of arsenite (III) on selected bacterial strains, experiments were conducted at initial pH 4, 5, 6, 7, 8 and 9. Effect of temperature was determined in temperature range of 25°C, 30°C, 35°C, 40°C, 45°C. The initial concentration of as was fixed at 50 µg ml^{-1} for both the above experiments. Rest of the procedure was same as described for adsorption experiment.

Determination of adsorption isotherms

An isotherm is a plot of amount of solute adsorbed per unit amount of adsorbent against the corresponding equilibrium concentration in the solution phase keeping temperature constant. Vital conclusion can be drawn from these isotherms, which are useful in designing of adsorption systems. Langmuir and Freundlich isotherms have been used in this study.

Langmuir isotherm

Langmuir isotherm is obtained from a kinetic derivation of equilibrium between adsorbed and desorbed molecules [19]. This gives the following equation-

$$q_e = q_m.K_A.C_e + K_A.C_e$$

where, q_e is the amount of adsorbate adsorbed per unit amount of adsorbent at equilibrium; K_A is 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-

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

Where, C_e is equilibrium concentration (mg l^{-1}), q_e is amount adsorbed per gram of adsorbent at equilibrium (mg g^{-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.

Freundlich isotherm

This isotherm is derived from empirical consideration and expressed as-

$$qe = K_f Ce^{1/n}$$

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 [20].

The model can be linearised logarithmically as below,

$$\textbf{Log } q_e = \textbf{(1/n) log } C_e + \textbf{log } K_f$$

Thus a plot between log q_e and log C_e is a straight line. Values of K_f and 1/n are 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.

Determination of adsorption kinetics

Kinetic study of metal adsorption by the selected bacterial strains was carried out at 50 µg ml^{-1} initial concentration of arsenic at room temperature, wherein the extent of adsorption was analyzed at regular time intervals (5, 15, 30, 45, 60, 90,180 and 240 minutes) until as removal attains a saturation level. The Lagergren first order and pseudo-second-order models were used to test adsorption kinetics data to investigate the mechanism of biosorption.

The Lagergren rate equation is most widely used model for the sorption of a solute from a liquid solution and the first order rate expression is given as [21].

$$\log(q_e - q_t) = \log Q_e - \frac{k_1}{2.303}t$$

Where, qe and qt (µg mg^{-1}) are the amounts of metal adsorbed on the algal surface at equilibrium and time t, and k_1 (min^{-1}) is the rate constant of first order adsorption. The slope and intercept of the plot of log (qe-qt) versus t were used to determine the value of Q_e and k_1.

The pseudo-second order kinetic model in its integrated and linearized form has been used and is given as [22].

$$\frac{t}{q} = \frac{1}{k_2 q_e^{\,2}} + \frac{1}{q_e}t$$

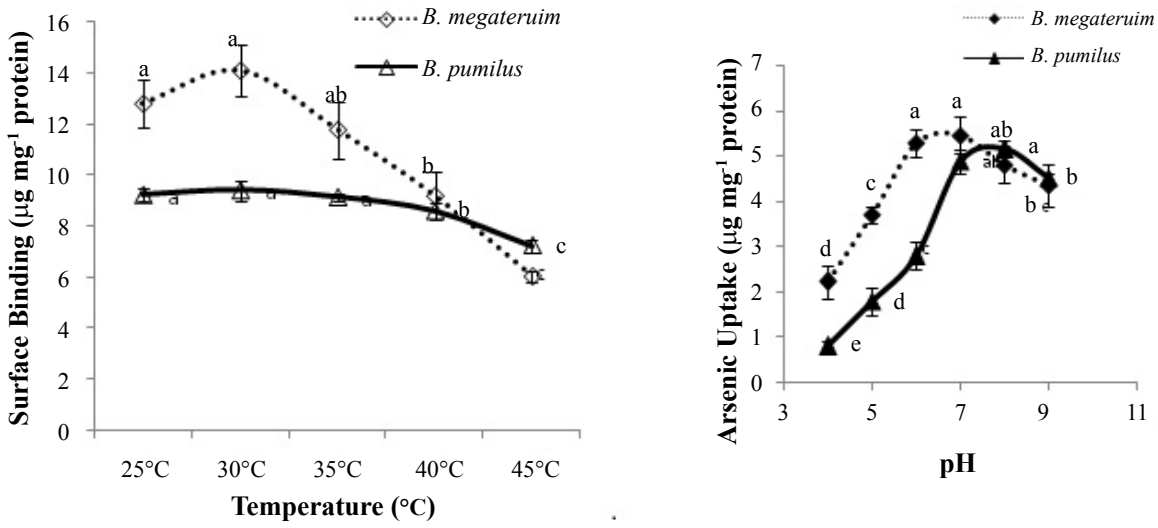

Figure 1: Comparison between extracellular and intracellular biosorption by *Bacillus megaterium* and *Bacillus pumilus* at different pH (4-9). Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at p<0.05. Different alphabets show significant differences between the treatments.

where k_2 (g mg^{-1} min^{-1}) is the rate constant of second-order adsorption. The slope and intercept of the plot of t/q versus t were used to determine the value of q_e and k_2. It is important to notice that for the application of this model the experimental estimation of q_e is not necessary.

Fourier transforms infrared (FTIR) spectroscopy

FTIR spectroscopy was used to detect the changes in the membrane surface moieties in bacteria which are responsible for biosorption of arsenic. The bacterial pellet treated with fixed concentration of arsenic for 2 hours were collected and oven dried for 12 hours at 50°C. A measured amount of biomass was mixed with KBr (1:100). Then the mixture was grounded into fine particles with a pestle and mortar and was compressed into translucent sample disk by a manual hydraulic pressure. The spectra for both control and treated cells were measured within the range of 400-4000 cm^{-1} using a KBr window by Fourier Transform Spectrophotometer (Nicolet 6700, Thermoscientific USA). The background obtained from the scan of pure KBr was automatically subtracted from the sample spectra.

Statistical analysis

All the experiments were conducted in triplicates and the reported value for each studied parameter are means ±SD. Data were analyzed by one way analysis of variance (ANOVA) at p<0.05. Statistically significant data are shown by different alphabets by using Duncan multiple range test (DMRT) using the SPSS software (Version 7).

Results and Discussion

Effect of pH on surface binding and intracellular uptake

Many studies have shown that pH of the surrounding medium was an important factor in influencing the biosorption of metal ions [23-25]. The effect of pH (pH 4-pH 9) on arsenic biosorption was studied for both surface binding and intracellular uptake of arsenic and it was observed that biosorption was highly dependent on the pH of the external medium. Results (Figure 1) revealed that maximum surface binding and intracellular uptake of arsenic occurred at pH 8 and pH 6 for *B. pumilus* and *B. megaterium,* respectively. Both the species are of same genus but *B. pumilus* binds arsenite in alkaline region whereas *B. megaterium* binds it in acidic region. The reason might be change in the cell wall components of both the species. These results were also confirmed by FTIR spectra which showed presence of different functional groups on the cell surface of both the species.

As pH of the aqueous metal solution increases the absorption capacity of *B. pumilus* for arsenic also increases up to pH 8 and then it decreases. These results were in agreement with studies of Wu et al. and Miyatake [16, 26] who demonstrated reduced metal uptake with a decrease in pH perhaps due to suitable ionic state of binding ligands and metal ion species for enhanced interaction. An increase in number of negatively charged active sites (functional groups such as carboxyl, hydroxyl, amine and phosphate groups) with increasing pH facilitates a higher electrical attraction to positively charged metal ions [27,28]. For *B. megaterium* the maximum absorption of arsenic occurred at pH 6. The possible reason might be that Arsenic oxides with negative charge preferred more positive charge on the membrane surface, which was possible only at acidic pH value. Loukidou et al. and Seki et al. [15,29] identified availability of different functional groups at different pH values for binding of metals. A reduced metal uptake rate at pH values higher than 8 was ascribed to metal-hydroxylation yielding metal hydroxides or hydrated oxides, which leads to metal passivation.

Effect of temperature on surface binding and intracellular uptake

The temperature could be an important factor for the energy dependent metal biosorption onto the microbial cell surface. The effect of temperature on the surface binding and intracellular uptake of arsenic was investigated at five different temperature ranges (25°C to 45°C) as shown in Figure 2. An increase in the temperature from 25°C to 30°C lead to an increase in binding of arsenic by both the bacterial strains. The intracellular uptake was maximum at temperature of 35°C in both the strains. An increase in the absorption of metals with increasing temperature could be correlated with endothermic nature of the absorption process [23,30]. Surface binding was about 80% higher throughout the tested range of temperature as compare to intracellular uptake in both the species. This shows that it is the cell surface and not the metabolic activity which mainly contributes in the removal of

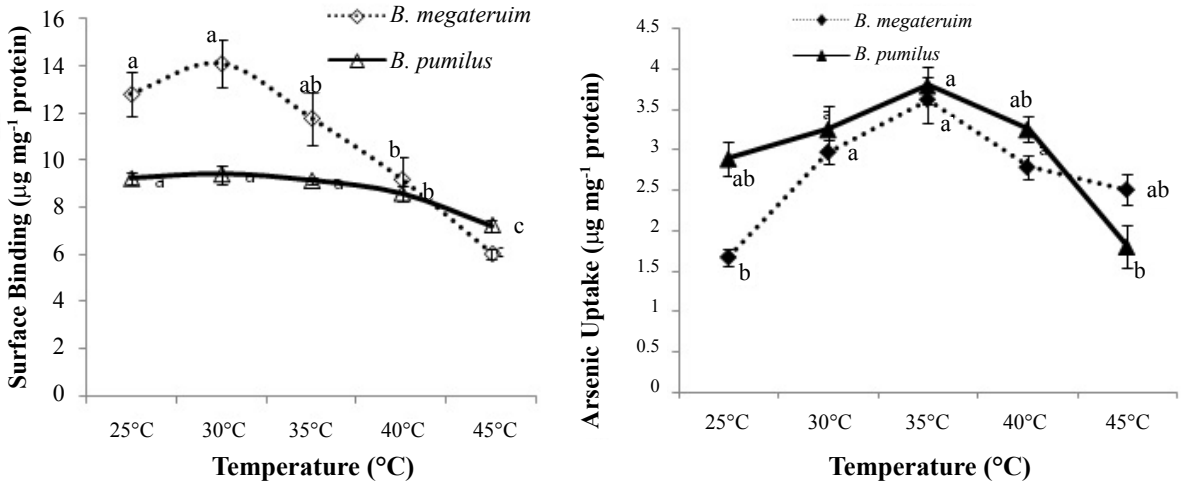

Figure 2: Comparison between Extracellular and Intracellular biosorption by *Bacillus megaterium* and *Bacillus pumilus* at different Temperature (25°C to 45°C). Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at p<0.05. Different alphabets show significant differences between the treatments.

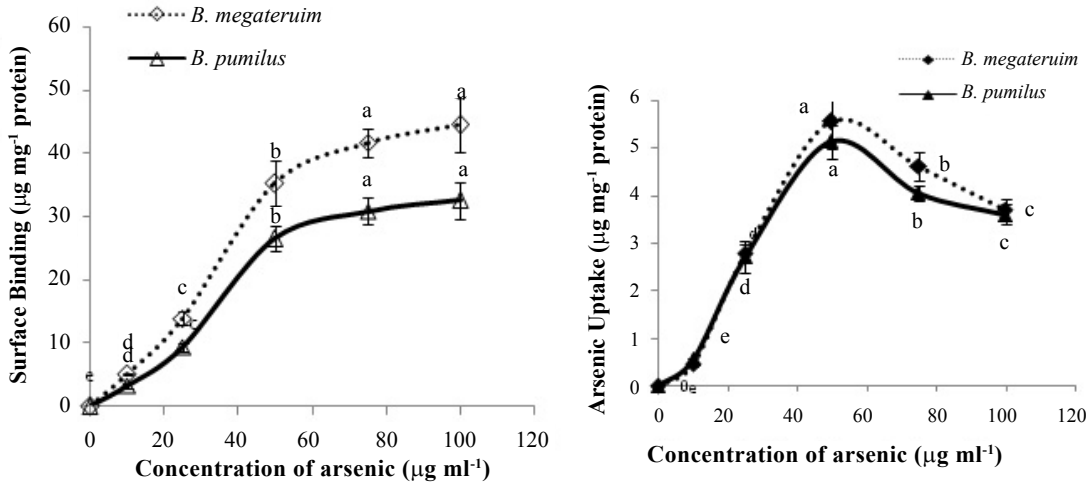

Figure 3: Comparison between Surface Binding and Intracellular uptake by *Bacillus megaterium* and *Bacillus pumilus* at different concentration of Arsenic (0 µg ml^{-1} to 100 µg ml^{-1}). Data are the mean of three replicates ± SD. Data was analyzed by one way analysis of variance (Duncan Multiple Range Test) at p<0.05. Different alphabets show significant differences between the treatments.

arsenite by *Bacillus* species. A further increase in temperature beyond 30°C to 35°C showed decline in surface binding as well as intracellular uptake of arsenic. Meena et al. [31] reported that an increase in metal sorption with temperature might be attributed to either increase in the number of active surface sites available for sorption on the adsorbent or due to decrease in the boundary layer thickness surrounding the sorbent, which resulted into reduced mass transfer resistance of adsorbate.

Effect of As concentration

Effect of different concentrations (10 µg ml^{-1} to 100 µg ml^{-1}) of arsenic on surface binding and intracellular uptake efficiency of a known amount of bacterial biomass at optimum pH and temperature is shown in Figure 3. Continuous increase in surface binding and intracellular uptake was observed with increasing concentrations of arsenic up to 50 µg ml^{-1}. Later on, the rate of As adsorption became more or less constant in both the strains. These results were in agreement with the findings of several other workers who suggested that metal sorption increases with increasing metal ion concentration, and then become saturated after reaching to a certain metal concentration [32,33]. At higher concentration, the number of As species available for binding onto the cell surface of bacteria was enhanced, which ultimately increased the biosorption of As [34]. Maximum surface binding (76%) and intracellular uptake (12%) of arsenic was observed in case of *B. megaterium*. The surface binding in both the bacterial strains contributed about 6 times more in total removal of arsenite when compared with the intracellular uptake of As. These results were in conformity with the previous reports that a large proportion of metals remained adsorbed onto the cell surface, and a very small fraction of metals entered into the intracellular compartment [26,35-37]. A higher contribution of cell surface binding of metal could be an advantageous point in the sense that the adsorbed metals can be recovered by using a suitable desorbing agents, especially in the case of precious metals. The binding/chelation of metal ions onto the extracellular surface is the main defense strategy adopted by the microorganisms in mitigation of metal toxicity [38,39].

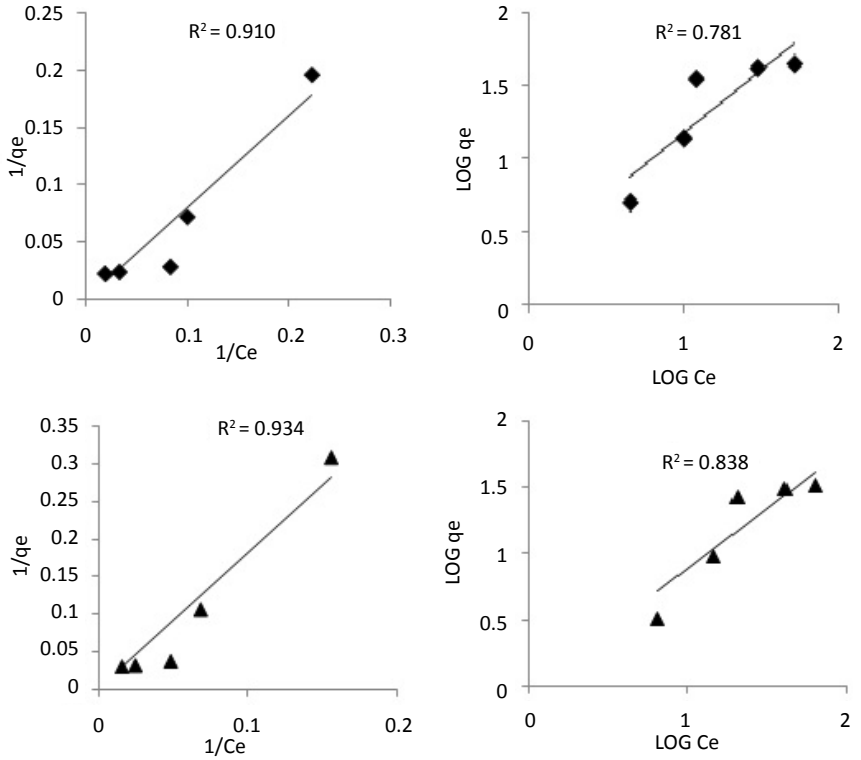

Figure 4: Langmuir and Freundlich isotherm for the adsorption of Arsenic by *Bacillus megaterium* (A) and *Bacillus pumilus* (B).

Bacterial Strains	Langmuir Constant			Freundlich Constant		
	Max. adsorption capacity 'q_{max}' (mg g $^{-1}$protein)	Adsorption affinity '*b*' (L mg $^{-1}$)	Regression Coefficient 'R²'	Adsorption coefficient 'K_f' (mg g^{-1} protein)	Adsorption intensity '*n*'	Regression Coefficient 'R²'
B. megaterium	1000.0	0.0013	0.910	-0.499	1.17	0.781
B. pumilus	666.7	0.0008	0.934	-3.0	1.12	0.838

Table 1: Langmuir and Freundlich isotherm constants for the adsorption of Arsenic by *B. megaterium* and *B. pumilus*.

Bacterial Isolates	Q (Experimental)	Pseudo Second Order Kinetic Constants			Pseudo First Order Kinetic Constants		
		Q_e(Calculated) (µg/mg) mg)	K_2(gmg^{-1} min^{-1})	R^2	Q_e(Calculated) (µg/mg)	K (min^{-1})	R^2
B. megaterium	35.62	38.46154	0.006318	0.999	-0.08197	0.006909	0.591
B. pumilus	26.76	28.57143	0.007206	0.999	-0.0846	0.009212	0.702

Table 2: Lagergren pseudo first order and Pseudo- second-order kinetic rate constants of Arsenic adsorption by *B. megaterium* and *B. pumilus*.

Biosorption isotherms

Adsorption of arsenic was studied to understand the nature of adsorption by fitting the experimental data to Langmuir and Freundlich isotherm models. During modeling of the adsorption of arsenic by bacterial strains, 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. The Langmuir model assumes monolayer biosorption onto a surface with a finite number of identical sites. Langmuir isotherm showed linear plots of 1/qe versus 1/Ce (Figure 4). Langmuir constants q_{max} defined the total adsorption capacity and b denotes the metal binding affinity of cell. The q_{max} values of the bacterial strains were found to be in the sequence of *B. megaterium* > *B. pumilus* (Table 1). Based on q_{max}, it was revealed that *B. megaterium* showed higher biosorption capacity for arsenic as compare to *B. pumius*. Hasim and Chu [29] have suggested that a biosorbent with low q_{max} and high 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.

In the case of Freundlich isotherm, K_F represents the 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. Plots of Log qe and Log Ce (Figure 4) for the adsorption of arsenic by both the bacterial strain were found to be non-linear throughout the concentration range studied. The adsorption coefficient (K_F) was found negative in both the strains which show that none of them follow Freundlich adsorption isotherm. Based on the R^2 values, it was observed that the nature of adsorption of arsenic in both the strains could be better described only by Langmuir model and in no case adsorption data fit the Freundlich model. These results are in agreement with Wu et al. [16], who studied the adsorption of arsenite in *E. coli* and showed that arsenite binding followed only Langmuir isotherm.

Biosorption kinetics

Lagergren first order and pseudo second order plots were constructed for the adsorption of arsenic by both the *Bacillus* strains at

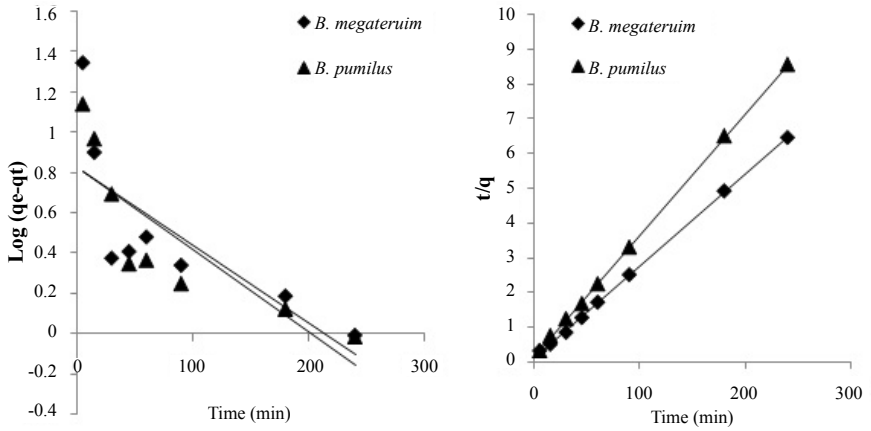

Figure 5: Lagergren first-order (A) and second order (B) kinetics modeling of Arsenic adsorption by *Bacillus megaterium* and *Bacillus pumilus*.

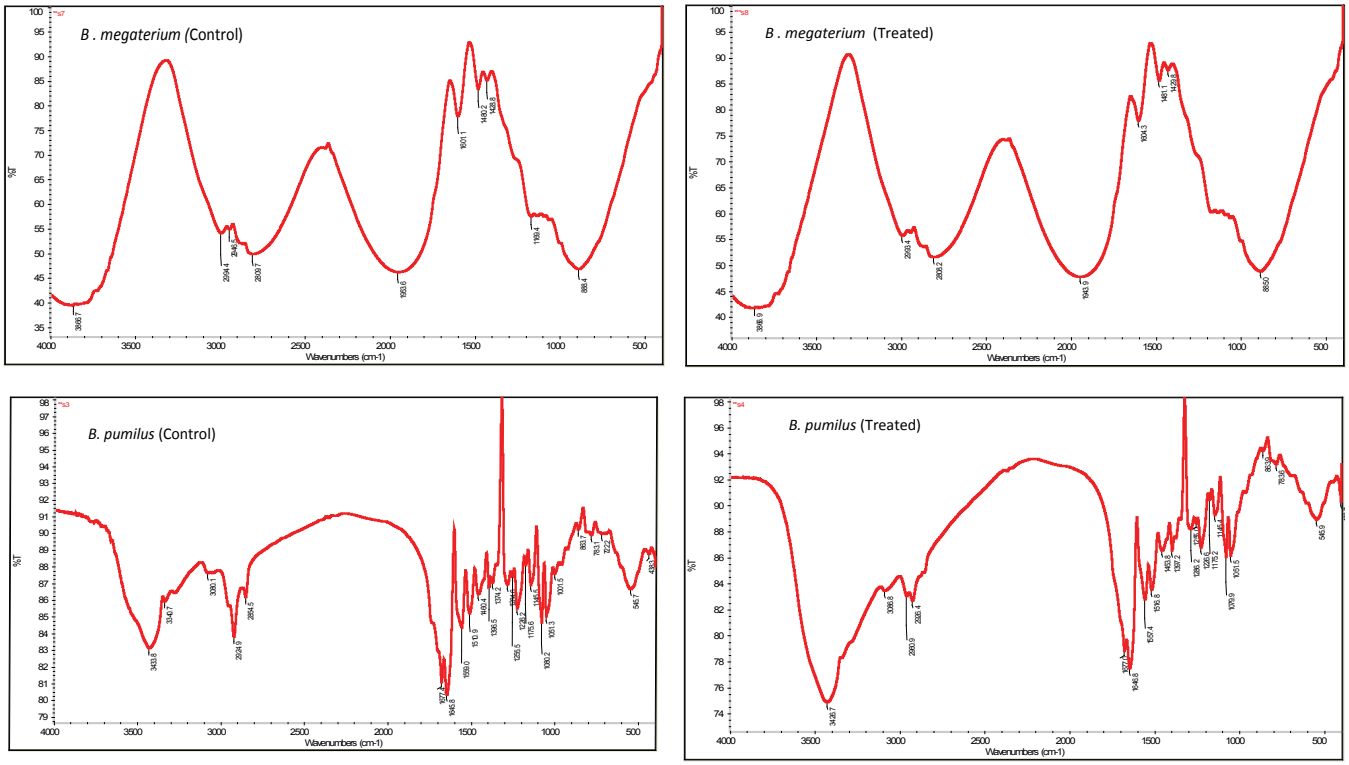

Figure 6: FTIR spectra of *Bacillus megaterium* and *Bacillus pumilus*.

concentration of 50 µg ml^{-1} (Figure 5) to test the adsorption kinetics and nature of biosorption. The t/q *vs* t plot for pseudo second order kinetics gave a straight line whereas plot of first order kinetics log (qe-qt) *vs* t did not fairly follow a linear relationship. The results suggested that the second order kinetics is applicable in the case of arsenite biosorption by the bacterial strains. The rate constants for both the bacterial strain were calculated from the Lagergren first-order and pseudo-second-order models as shown in Table 2. The 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 q_e value of second –order kinetics fairly agreed with the experimental q_e value. Similarly, several workers observed that adsorption of metals (As, Cd, Cu, Pb) by bacteria and algae follow Pseudo second-order kinetics [40-44].

Surface characterization of bacterial strains by FTIR

In order to understand the nature of surface binding, it is essential to identify the functional groups involved in arsenite binding on the membrane cell surface. The metal binding sites were identified by FTIR spectral analysis (wave numbers 400 cm^{-1} to 4000 cm^{-1}) by comparing it with the control (Table 3). Conspicuous changes in the FTIR spectrum were observed after adsorption of arsenic on bacterial biomass. The results showed that the cell surface of bacterial strains contain several functional groups such as amide, N-H, alkanes, alkenes, phosphines, amino, amines and COO^- groups which contribute to As binding by the cell surface of all Arsenite tolerant bacterial strains (Figure 6).

A shifting of the FTIR peak of *B. megaterium* in the range of 1601 cm^{-1} were attributed to nitro groups which after adsorption of arsenite

B.megaterium Control (cm^{-1})	*B.megaterium* Treated (cm^{-1})	*B.pumilus* Control (cm^{-1})	*B.pumilus* Treated (cm^{-1})	Band Assignment
-	-	3433	3426	N-H stretch of Amides & O-H stretch of hydroxyl groups
-	-	3080	3066	C—H stretching of fatty acid
2946	-	-	2960	CH of CH_3 stretching mainly lipids
-	-	2924	2926	C-H of CH_3 stretching mainly proteins
1601	1604	-	-	N-H bending & C-N stretch
-	-	1460	1453	-NO_2 aromatics
1428	1429	-	-	Alkenes, Carboxylic group
-	-	-	970	S-O stretch sulphonates, alkenes
888	885	-	-	C-H bend (Alkenes)

Table 3: IR absorption peaks indicating different functional groups on the surface of *B. megaterium* and *B. pumilus* (cm^{-1}).

showed a shift towards the left at 1604 cm^{-1} position. A shift in the peaks from 1428 cm^{-1} to 1429 cm^{-1} could be attributed to carboxylic acid and alkenes. Some peaks in the band region of 888 cm^{-1} ascribed to alkenes which changes to 885 cm^{-1} after adsorption of arsenic (Table 3).

Shift in the FTIR peak from 3433 cm^{-1} to 3426 cm^{-1} in *B. pumilus* showed stretching of amide and hydroxyl groups. A shift in the peaks from 3080 cm^{-1} to 3066 cm^{-1} can be attributed to C—H stretching of fatty acid. Peaks at 2924 cm^{-1} was assigned to C-H of CH_3 stretching of proteins, which exhibited a shift towards the wavelength of 2926 cm^{-1} after interaction with arsenite. Some new peaks (2960 cm^{-1} and 970 cm^{-1}), not present in the control set, appeared in the arsenite treated cells. These changes indicated that alkanes, alkenes and sulphonates moieties present on to the bacterial cell surface were involved in the arsenite binding. Some peaks in the band region of 1460 cm^{-1} ascribed to nitro groups also shifted to 1453 cm^{-1} after interaction with arsenite (3).

The cell surfaces of many microorganisms consist of polysaccharides, proteins and lipids, these macromolecules confer several functional groups capable of binding with toxic metal ions [45]. The present results showed a shift in the IR peaks for Carboxylic, C-H of alkane, alkene, PO_2 moeity, amines, amide and sulphonate groups when treated with arsenite, indicating involvement of these functional moieties in the arsenite binding. Many workers have also used the FTIR spectra for qualitative and preliminary analysis of the chemical functional groups present in the cell wall of microorganisms. The IR signature of cell surface moieties yields basic information on the nature of the possible cell surface and metal ion interaction [46-49]. The carboxyl, hydroxyl, amino, phosphate and sulphate groups on microbial cell surface have been implicated in the binding of various charged molecules [50,51]. Carboxylic, C-H of alkane, amine, amide and amino group were also responsible for the binding of arsenite (III) in *E. coli* [16].

Conclusion

Based on the foregoing evidences, it may be concluded that surface binding of arsenite (about 90%) on the bacterial cell surface might be the primary mode of arsenite removal and arsenite tolerance in the isolated bacterial strains. However, pH and temperature optima of arsenite adsorption including the FTIR results clearly demonstrated that both the *Bacillus* strains involve different functional groups in the arsenite binding, depending upon the cell wall characteristics of each genotype.

Acknowledgement

The authors are grateful to the Head of the Department of Environment Science, Babasaheb Bhimrao Ambedkar University, Lucknow, for providing all the facilities required to carry out the present investigation.

References

1. Ahmann D., Roberts A.L., Krumholz L.R. & Morel F.M.
Microbe grows by reducing arsenic.
Nature. 1994, **371**: 750.

2. Cervantes C., Ji G., Ramirez J.L. & Silver S.
Resistance to arsenic compounds in microorganisms.
FEMS Microbiol. Rev. 1994, **15**: 355-367.

3. Patel P.C., Goulhen F., Boothman C., Gault A.G., Charnock J.M., Kalia K. & Lloyd J.R.
Arsenate detoxification in a *Pseudomonad* hypertolerant to arsenic.
Arc. Microbiol. 2007, **187**: 171-183.

4. Rehman A., Ali A., Muneer B. & Shakoori A.R.
Resistance and biosorption of mercury by bacteria isolated from industrial effluents.
Pakistan J. Zool. 2007, **39**: 137-146.

5. Liao V.H.C., Chu Y.J., Su Y.C., Hasiao S.Y., Wei C.C., Liu C.W., Lia C.M., Shen W.C. & Chang F.J.
Arsenite oxidizing and arsenate reducing bacteria associate with arsenic-rich ground water in Taiwan.
J. Contamin. Hydrol. 2011, **123**: 20-29.

6. National Research Council (NRC).
National Research Council: Arsenic in the drinking water.
National Academy Press. Washington, 1999.

7. Guha Mazumder D.N.
Arsenic and non-malignant lung diseases.
J. Environ. Sci. Health Part A. 2007, **42**: 1859-1867.

8. Wang T.C., Jan K.Y., Wang A.S. & Gurr J.R.
Trivalent arsenicals include lipid peroxidation, protein carbonylation, and oxidative DNA damage in human urothelial cells.
Mutat. Res. 2007, **615**: 75-86.

9. Zhao Y., Toselli P. & Li W.
Microtubules as a critical target for arsenic toxicity in lung cells *in Vitro* and *in Vivo*.
Int. J. Environ. Res. Public Health. 2012, **9**: 474-495.

10. Bissen M. & Frimmel F.H.
Arsenic: A review. Part I: Occurrence, toxicity, speciation and mobility.
Acta Hydrochim. Hydrobiol. 2003, **31**: 9-18.

11. Jain C.K. & Ali I.
Arsenic: Occurrence, toxicity and speciation techniques.
Water Res. 2000, **34**: 4304-4312.

12. WHO (World Health Organisation)
Environmental Health Criteria, Arsenic.
World Health Organisation, Geneva. 1981.

13. Bates, S.S., Tessier A., Campbell P.G.C. & Buffle J.
Zinc adsorption and transport by *Chlamydomonas variabilis* and *Scenedesmus subspicatus* (Chlorophyceae) grown in semi continuous culture.
J. Phycol. 1982, **18**: 521-529.

14. Cullen W.R. & Reimer K.J.
Arsenic speciation in the environment.
Chemical Reviews. 1989, **89**: 713-764.

15. Loukidou M.X., Matis K.A., Zouboulis A.I. & Liakopoulou-Kyriakidou M.

Removal of As (V) from waste-waters by chemically modified fungal biomass. Water Res. 2003, **37**: 4544-4552.

16. Wu Y.H., Feng S.X., Li B. & Mi X.M.
The characteristics of *Escherichia coli* adsorption of arsenic (III) from aqueous solution.
W. J. Microbiol. Biotechnol. 2010, **26**: 249-256.

17. Lechevalier H.A.
The Actinomycetes III, a practical guide to generic identification of actinomycetes. In: Bergey's Manual of Systematic Bacteriology, Williams, S.T., Sharpe, M.E. & Holt, J.G. (eds).
Williams & Wilkins Company, Baltimore, Maryland, 1989 pp. 2344-2347.

18. Martin J.H.
Bioaccumulation of heavy metals by littoral and pelagic marine organisms.
USEPA. 1979, 600/3–77–038.

19. Langmuir I.
The adsorption of gases on plane surface of glass, mica and platinum.
J. Am. Chem. Soc. 1918, **40**: 1361-1403.

20. Freundlich H. & Helle W.J.
Rubber dye adsorption in lusungen.
J. Am. Chem. Soc. 1939, **61**: 2-28.

21. Ho Y.S. & McKay G.
The sorption of lead (II) ions on peat.
Water Res. 1999a, **33**: 578-584.

22. Ho Y.S. & McKay G.
Pseudo-second order model for sorption processes.
Process Biochem. 1999b, **34**: 451-465.

23. Huang J.P., Huang C.P. & Morehart A.L.
The removal of Cu (II) from diluted aqueous solution by *Saccharomyces cerevisiae*.
Water Res. 1990, **24**: 433-439.

24. Matheickal J.T., Yu Q. & Woodburn G.M.
Biosorption of cadmium of marine algae *Durivillaea potatorum*.
Water Res. 1999, **33**: 335-342.

25. Sanchez A., Balleste A., Blazquez M.L., González F., Muñoz J. & Hammaini, A.
Biosorption of copper and zinc by *Cymodocea nodosa.*
FEMS Microbiol. Rev. 1999, **23**: 527-536.

26. Miyatake M. & Hayashi S.
Characteristics of arsenic removal from aqueous solution by *Bacillus megaterium* Strain UM-123.
J. of Environ. Biol. 2009, **9**: 123-129.

27. Klimmek S., Stan H.J., Wilke A., Bunke G. & Buchholz R.
Comparative analysis of the biosorption of cadmium, lead, nickel and zinc by algae.
Environ. Sci. Technol. 2001, **35**: 4283-4288.

28. Romera E., Gonzalez F., Ballester A., Blazquez M.L. & Munoz J.A.
Biosorption with algae: statistical review.
Crit. Rev. Biotechnol. 2007, **26**: 223-235.

29. Seki H., Suzuki A. & Hideo M.
Biosorption of chromium (VI) and arsenic (V) onto methylated yeast biomass.
J. Colloid Interf. Sci. 2005, **281**: 261-266.

30. Bayramoglu G., Denizli A., Bektas S. & Arica M.Y.
Entrapment of *Lentinus sajor-caju* into Ca-alginate gel beads for removal of Cd (II) ions from aqueous solution: preparation and biosorption kinetics analysis
Microchem. J. 2002, **72**: 63-76.

31. Meena A.K., Mishra G.K., Rai P.K., Rajgopal C. & Nagar P.N.
Removal of heavy metals ions from aqueous solution using carbon aerogel as an adsorbent.
J. Hazard. Mater. 2005, **122**: 169-170.

32. Joshi D.N., Patel J.S., Flora S.J.S. & Kalia K.
Arsenic accumulation by *Pseudomonas stutzeri* and its response to some thiol chelators.
Environ. Health Prev. Med. 2008, **13**: 257-263.

33. Kostal J., Yang R., Wu C.H., Mulchandani A. & Chen W.
Enhanced arsenic accumulation in engineered bacterial cells expressing ArsR.
Appl. Environ. Microbiol. 2004, **70**: 4582-4587.

34. De Philippis R. & Vincenzini M.
Exocellular polysaccharides from cyanobacteria and their possible application.
FEMS Microbiol. Rev.1998, **22**: 151-175.

35. Ghodsi H., Hoodaji M., Tahmourespour A. & Gheisari M.M.
Investigation of bioremediation of arsenic by bacteria isolated from contaminated soil.
African J. Microbiol. Research. 2011, **5**: 5889-5895.

36. Guzman M.C. & Cao E.P.
Cadmium binding ability of the blue green alga *Hapalosiphon welwitschii Nägel* under controlled conditions.
Phillipp. Sci. Lett. 2010, **3**: 76-86.

37. 37. Takeuchi M., Kawahata H., Gupta L.P., Kita N., Morishita Y., Ono Y. & Komai T.
Arsenic resistance and removal by marine and non-marine bacteria.
J. Biotechnol. 2007, **127**: 434-442.

38. Macfie S.M. & Welbourn P.M.
The cell wall as a barrier to uptake of metal ions in the unicellular alga *Chlamydomonas reinhardti* (Chlorophyceae).
Arch. Environ. Contam. Toxicol. 2000, **39**: 413-419.

39. Perez-Rama M., Alonso J.A., Lopez C.H. & Vaamonde E.T.
Cadmium removal by living cells of the marine microalga *Tetraselmis suecica*.
Bioresour. Technol. 2002, **84**: 265-270.

40. Dixit S. & Singh D.P.
An evaluation of phycoremediation potential of cyanobacterium *Nostoc muscorum*: Characterization of heavy metal removal efficiency
J. Appl. Phycol. 2014, **26**: 1331-1342.

41. Doshi H., Ray A. & Kothari I.L.
Biosorption of cadmium by live and dead *Spirulina*: IR Spectroscopic, Kinetics and SEM studies.
Curr. Microbiol. 2007, **54**: 213-218.

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

43. Hashim M.A. & Chu K.H.
Biosorption of cadmium by brown, green and red seaweeds.
Chem. Eng. J. 2004, **97**: 249-255.

44. Liping D., Yingying S., Hua S., Xinting W. & Xiaobin Z.
Biosorption of copper (II) and lead (II) from aqueous solutions by nonliving green algae *Cladophora fascicularis*: Equilibrium, kinetics and environmental effects.
Adsorption. 2006, **12**: 267-277.

45. Yan G. & Viraraghavan T.
Heavy metals removal from aqueous solution by fungus *Mucor rouxii.*
Water Res. 2003, **37**: 4486-4496.

46. Arica M.Y., Tuzun I., Yalcin E., Ince O. & Bayramoglu G.
Utilization of native, heat and acid-treated microalgae *Chlamydomonas reinhardtii* preparations for biosorption of Cr (VI) ions.
Process Biochem. 2005, **40**: 2351-2358.

47. Doshi H., Ray A. & Kothari I.L.
Biosorption of cadmium by live and dead *Spirulina*: IR Spectroscopic, Kinetics and SEM studies.
Curr. Microbiol. 2007, **54**: 213-218.

48. Singh R., Chadetrik R., Kumar R., Bishnoi K., Bhatia D., Kumar A., Bishnoi N.R. & Singh N.
Biosorption optimization of lead (II), cadmium (II) and copper (II) using response surface methodology and applicability in isotherms and thermodynamics modeling.
J. Hazard. Mater. 2010, **174**: 623-634.

49. Tunali S., Kiran I. & Akar T.
Chromium (VI) biosorption characteristics of *Neurospora crassa* fungal biomass.
Miner. Eng. 2005, **18**: 681-689.

50. Sheng P.X., Ting Y.P., Chen J.P. & Hong L.
Sorption of lead, copper, cadmium, zinc, and nickel by marine algal biomass: Characterization of bio-sorptive capacity and investigation of mechanisms.
Colloid Interface Sci. 2004, **275**: 131-141.

51. Veglio F. & Beolchini F.
Removal of metals by biosorption: A review.
Hydrometallurgy. 1997, **44**: 301-316.