Biosorption of As(V) from aqueous solutions by living cells of Bacillus cereus

ABSTRACT
In this work, the biosorption of As(V) from aqueous solutions by living cells of Bacillus cereus has been reported. The batch biosorption experiments were conducted with respect to biosorbent dosage 0.5 to 15 g/L, pH 2 to 9, contact time 5 to 90 min, initial concentration 1 to 10 mg/L and temperature 10 to 40 °C. The maximum biosorption capacity of B. cereus for As(V) was found to be 30.04 at pH 7.0, at optimum conditions of contact time of 30 min, biomass dosage of 6 g/L, and temperature of 30 ± 2 °C. Biosorption data were fitted to linearly transformed Langmuir isotherms with \( R^2 \) (correlation coefficient) >0.99. Bacillus cereus cell surface was characterized using AFM and FTIR. The metal ions were desorbed from B. cereus using both 1 M HCl and 1 M HNO₃. The pseudo-second-order model was successfully applied to predict the rate constant of biosorption.

Key words | atomic force microscopy, As(V), Bacillus cereus, biosorption isotherm, biosorption kinetics

INTRODUCTION
The bioremediation technique, which involves the use of microbes to detoxify and degrade environmental contaminants, has received increasing attention in recent times to clean up a polluted environment (Iwamoto & Nasu 2001; Sari et al. 2011). Biosorption of metal ions is an example of the wide variety of potential and actual applications of bioremediation techniques in wastewater treatment (Hansen et al. 2006; Tuzen et al. 2009a). Arsenic is one of the most significant and highest priority metals found in natural and anthropogenic processes such as weathering, biological activity, geochemical reactions, volcanic eruption, smelting of non-ferrous metals and highest burning of fossil fuels (Smedley & Kinniburgh 2002; Smith et al. 2002). The most common species of arsenic in groundwater are in inorganic forms: arsenite (H₃AsO₃) and arsenate (H₂AsO₄), which can be potentially toxic and cause serious damage to biological systems (Thomas et al. 2007). Therefore, the World Health Organization (WHO) has recommended the standard concentration of arsenic in drinking water as 10 μg/L (Smith et al. 2002). Most removal methods suffer from some disadvantages, such as high capital and operational cost, limited tolerance to pH change, incomplete metal removal, high cost of reagent, and energy requirements. Biosorption plays an important role in elimination of metal ions from aqueous solutions in water pollution control (Veglio & Beolchini 1997; Murugesan et al. 2006). The main advantages of the technique used here are the reusability of biomaterial, low operating cost, improved selectivity for specific metals of interest, removal of heavy metals from effluent irrespective of toxicity and short operation time. The biosorption is a passive process which utilizes the cell wall of biomass to sequester the metal ions from aqueous solutions. Mechanisms of cell surface sorption are independent of cell metabolism which is based on physico-chemical interactions between metal and functional groups of the cell wall. The cell walls of microorganisms mainly consist of polysaccharides, lipids and proteins that serve as binding sites for metals (Dursun 2003; Seki et al. 2005; Sari & Tuzen 2010). The surface properties of B. cereus cells change during biofilm formation and the extracellular polymeric substance proteins function as non-specific adhesions during biofilm formation. The extracellular polymeric substances (EPS) are implicated in imparting biofilms with structural stability and resistance to cleaning heavy metals. In previous studies, it has been reported that B. cereus biomass is capable of removing heavy metal ions from aqueous solution (Ray et al. 2005; Giri et al. 2011).
The objective of the present work was to investigate the biosorption of living cells of B. cereus biomass in the removal of As(V) from aqueous solution. Optimum biosorption conditions were determined as a function of pH, biomass dosage, contact time, initial concentration and temperature. The Langmuir models were used to describe equilibrium isotherms. Biosorption mechanisms of As(V) onto B. cereus biomass were also evaluated in terms of kinetics. B. cereus biomass was characterized by atomic force microscopy and Fourier Transfer Infrared Spectroscopy techniques to evaluate the biosorption of As(V).

MATERIALS AND METHODS

Preparation of standards and reagents

All the chemicals used in the studies were of analytical grade (Merck Chemicals, Germany) and used without further purification. In all experiments, double distilled water (Milli-Q Millipore 18.2 MΩ cm⁻¹ conductivity) was used for the preparation, dilution and analytical purposes of the solutions. A stock As(V) solution of 1,000 mg/L was prepared by dissolving 4.164 g of sodium arsenate (Na₂HAsO₄·7H₂O) in a 1,000 mL of deionized water. The stock solutions were preserved with 1% trace metal grade nitric acid. The 500-mL NaBH₄ solution was prepared by dissolving 2.5 g NaOH and 2.0 g NaBH₄, in double-distilled water and diluting up to the mark. The NaBH₄ reagent was always prepared immediately before use (Greenberg et al. 2005). Subsequently, different working solutions of required concentrations were prepared by proper dilution. The pH of the test solutions was adjusted using reagent grade dilute 1 M HNO₃ and 1 M NaOH solution. Test solutions of As(V) with concentration 1–10 mg L⁻¹ were prepared from stock solution by proper dilution. The stock solutions were preserved with 1% trace metal grade nitric acid. The 500 mL NaBH₄ solutions were prepared by dissolving 2.5 g NaOH and 2.0 g NaBH₄ in double-distilled water and diluting up to mark. The NaBH₄ reagent was always prepared immediately before use. Sodium tetrahydroborate solution was dispensed into the acidic test sample solution. The reaction of sodium tetrahydroborate in acidic solution and the simultaneous reduction of the hydride-forming element can be described simply as follows:

$$3BH_4^- + 3H^+ + 4H_2O \rightarrow 4AsH_3 \uparrow + 3H_2O + 3H_3BO_3$$

and

Bacterial growth and preparation

Bacillus cereus (MTCC NO: 1305) of microbial type culture and collection was obtained from the Institute of Microbial Technology, Chandigarh, India, to undertake the study. The identified species of B. cereus is a gram-positive, rod shaped bacterium. The bacterial strain was first grown on a Petri dish containing agar medium, which consists of beef extract (3.0 g), peptone (5.0 g), agar (20.0 g), NaCl (5.0 g) in 1 L double distilled water, and the pH was adjusted to 7.2 ± 0.3 with 10% (w/v) NaOH and 10% (w/v) HCl. After the incubation of cultures at 30 °C for 24 h in agar plates, the bacteria were inoculated from the plates onto the agar slants and stored at 4 °C until needed for further experiments.

Before the beginning of each experiment, strains were enriched by transferring one loop of cells from the agar slants to 100 mL of previously sterilized liquid nutrient medium in 250 mL flasks and incubated at 50°C for 24 h by shaking at 160 rpm in an orbital incubator. The liquid medium contains the same components described above in agar medium except agar, and the pH value was also adjusted to 7.2 ± 0.3 in the way mentioned above. The cells grown in liquid nutrient medium were centrifuged at 7,000 rpm for 30 min at 4 °C. The supernatant was discarded and the cell pellets were washed six times and suspended in phosphate buffer (1/15 mol/L Na₂HPO₄, 1/15 mol/L Na₂HPO₄, pH 7) before use in the experiments. The components of the mineral liquid medium were KH₂PO₄ 0.5 g/L, K₂HPO₄ 0.5 g/L, MgSO₄·7H₂O 0.2 g/L, CaCl₂ 0.1 g/L, NaCl 0.2 g/L, MnSO₄·H₂O 0.01 g/L, NH₄NO₃ 1.0 g/L, and arsenate was added to the required concentration.

Batch biosorption experiment

The biosorption experiments were optimized out at the desired pH value, contact time and biomass dosage level using the necessary biomass in a 100 mL stoppered conical flask containing 50 mL of test solution. The necessary amount of the biomass was then added and the contents in the flask were shaken for the desired contact time in an electrically thermostatic reciprocating shaker at 120 rpm. The experiments were repeated at 10°C to 40°C. The time required for reaching the equilibrium condition was estimated by drawing samples at regular intervals of time until
equilibrium was reached. The contents of the flask were filtered through filter paper and the filtrate was analyzed for total arsenic concentration by using the standard method given in the users guide of the (Perkin-Elmer 200) hydride generated system (HG-AAS). The filtrate was analyzed for arsenite concentration by using the same standard method given the users manual of the (Perkin-Elmer 200) hydride generated system (HG-AAS). Arsenate (mg/g) = Total arsenic (mg/g) – Arsenite (mg/g). Biosorption experiments for the effect of pH were conducted by using a solution having 1 mg/L of arsenate concentration with a biomass dosage of 6 g/L. Throughout the study, the contact time was varied from 5 to 90 min, the pH from 2.0 to 9.0, the initial metal concentration from 1 to 10 mg/L, and the biosorbent dosage from 0.5 to 15 g/L. The biosorption capacity, expressed as the As(V) sorbed per gram of sorbent (mg g⁻¹), was calculated as follows:

\[ q_e = \frac{(C_i - C_f)V}{M} \] (1)

The percentage of arsenic (III) ions sorbed was calculated as per the equation:

\[ \text{Sorption(\%)} = \left( \frac{C_i - C_f}{C_i} \right) \times 100 \] (2)

where \( C_i \) and \( C_f \) are the initial and final concentrations of the As(V) in the aqueous solution (mg L⁻¹), respectively. \( V \) is the volume (L); and \( M \) is the mass of sorbent (g) used. (Perkin-Elmer 200).

**Desorption procedure**

The sample volume of 50 mL, containing 1 mg/L of As(V), was transferred separately into a beaker; 10 mL of buffer solution was added. After a fast shaking, 6 g/L of \( B. \) cereus was added and the mixture was shaken again for 90 min at 150 rpm. The system was filtered with blue band filter paper. Then the filter and constituents were washed with distilled water. In order to elute the sorbed analytes on \( B. \) cereus, 10 mL of 1 M HCl and 10 mL of 1 M HNO₃ was used separately. Analyte contents of the final solution were determined by the hydride generated system (HG-AAS).

**RESULTS AND DISCUSSION**

**Characterization of the biosorbent**

The surface morphology of \( B. \) cereus cells without and with sorption of As(V) during the biosorption process was observed with the help of Atomic Force Microscopy (Digital Instruments, Santa Barbara, CA, USA). The standard V-shaped silicon nitride (Si₃N₄) cantilevers (with integral tips, Model OTR8-35) of different stiffness and tip sharpness were used for imaging. \( B. \) cereus bacteria without As(V) ion exposure in the control blank were rod-like in shape with a smooth surface (the dimension of these cells was about 4.0 μm long and 1.0 μm wide, on average, as shown in Figure 1(a)). After the As(V) ion exposure and the ultra-structures mostly disconnected with the cells adhering to each other randomly, it can be clearly observed that the biomass shape has changed into a spindle-like structure after As(V) sorption, as shown in Figure 1(b). The morphological changes of the sample can be attributed to the interactions between heavy metal and the surface of \( B. \) cereus cells. These agree with the results of FTIR spectra analysis.

The Fourier Transfer Infrared Spectroscopy (PerkinElmer SPECTRUM RX-I, USA) spectra of the \( B. \) cereus biomass without As(V) loaded displays a number of absorption peaks, indicating the complex nature of the bacterial
biomass, as shown in Figure 2(a). The spectra of loaded with As(V) and without were compared and the shift shown in Figure 2(b) was found. The spectra of sorbent exhibits a broad absorption band at 3,448.94 cm\(^{-1}\) due to the bonded –OH stretching vibration which is shifted to 3,460.29 cm\(^{-1}\) and may be due to complexation of –OH groups (Doshi et al. 2007). The next absorption peak at 1,655.83 cm\(^{-1}\) may be due to the presence of amide group (N-H stretching and C=O stretching vibration) is shifted to lower frequency and appearing at 1,652.62 cm\(^{-1}\) may be due to the complexation of amide group with As(V) (Pangnanelli et al. 2000). The absorbance peaks at 1,407.37 cm\(^{-1}\) may be attributed to N-H stretching vibration, \(-\text{CH}_2\) scissoring or \(-\text{CH}_3\) antisymmetrical bending vibration and O-H deformation shifted to higher frequency and appearing at 1,450.38 cm\(^{-1}\) may be due to the complexation. The peaks at 1,074.06 cm\(^{-1}\) which may be attributed to C-N stretching vibrations of amino groups are shifted to higher frequency and appear at 1,116.26 cm\(^{-1}\) due to the interaction of nitrogen from the amino group with As(V) (Sari & Tuzen 2013; Ghimire et al. 2002). The above changes in the spectra may be attributed to the interaction of As(V) with the amide, amino groups and hydroxyl present on the surface of the \(B.\ cereus\) biomass.

**Effect of biosorbent dose and pH**

Biosorbent dose is an important parameter which determines the capacity of biosorbent for an initial concentration of the sorbate. The effect of biosorbent dose...
The biosorption of As(V) ions was studied at pH 7.0, at ambient temperature 30 ± 2 °C and a contact time of 60 min for initial arsenate concentrations of 1, 5 and 10 mg/L. Experimental results generally showed that the biosorbent increased from 1 g/L to 6 g/L, the percentage removal of As(V) increased from 60% to 86%, 55% to 83% and 51% to 80%, for 0.05–0.5 g/50 mL of living cells of *B. cereus* biomass and initial As(V) concentration of 1, 5 and 10 mg/L, as shown in Figure 3(a). It is observed that after dosage of 0.30 g/50 mL, there is no significant change in percentage removal of As(V) ions. This may be due to the higher dosage producing a 'screen effect' on the cell wall which could protect the binding sites, thus resulting in lower arsenate sorption. So, 0.30 g/50 mL of initial arsenate concentration 1 mg/L is considered the optimum dose and was used for further study.

The pH is one of the important factors affecting the sorption of As(V) ions in the solution. Percentage removal of As(V) at pH 2.0–9.0 was studied in batch experiments using 0.30 g of *B. cereus* biomass in 50 mL synthetic solution, at ambient temperature (30 ± 2 °C) and contact time of 60 min for initial As(V) concentrations of 1, 5 and 10 mg/L and the results are presented in Figure 3(b). The results in Figure 3(b) clearly indicate that the sorption efficiency was increased with the increase of pH from 2.0 to 7.0 then decreased with further increase in pH up to 9.0. At low pH (2.0–6.0) the surface of living *B. cereus* biomass is highly protonated and as a result, a strong attraction exists between the oxyanion and the positively charged surface of the biomass (Boddu *et al*. 2008). The further decreases in As(V) uptake with increase in pH (7.0–9.0) may be due to the fact that at higher pH, the substrate may be negatively charged by adsorbing hydroxyl ions on the surface or by ionization of very weak acidic functional groups of the living *B. cereus* biomass. A repulsive force may develop between the negatively charged surface and the anions. At lower pH, the process of regeneration predominates over the process of removal. The process of sorption and regeneration is demonstrated in Figure 4. And hence the process of conversion of biosorbent into its H⁺ form plays an important role leaving behind As(V) ions in the aqueous solution (Costa *et al*. 2001; Zhu & Jyo 2001).

**Biosorption mechanism of As(V) ions with biosorbent**

The mechanism of any biosorption process is an important component in understanding the process as well as to know the characteristics of the material which help to design a new biosorbent for future applications. A mechanism for the biosorption of As(V) ions by ion-exchanger *B. cereus* biomass has been proposed by taking the results obtained from the experimental investigations (Figure 4). At lower pH of the medium, surface sites were positively charged and, therefore, attracted negatively charged arsenate, by an electrostatic interaction process (Mashitah *et al*. 1999; Hansen *et al*. 2006). The materials under hydration, the *B. cereus* biomass surface completes the coordination shells with the available OH group. On the variation of pH, these

![Figure 3](https://iwaponline.com/wst/article-pdf/66/8/1699/441927/1699.pdf)

**Figure 3** | (a) Effect of biosorbent dose and (b) pH on the biosorption of As(V) ions with initial concentrations of 1, 5 and 10 mg/L.

![Figure 4](https://iwaponline.com/wst/article-pdf/66/8/1699/441927/1699.pdf)

**Figure 4** | Biosorption and regeneration process of living cells of *Bacillus cereus* with As(V) ions.
surface active OH groups may further bind or release H⁺ where the surface remains positive due to the reaction:

\[ \text{MOH} + \text{H}_3\text{O}^+ \rightarrow \text{MOH}_2^+ + \text{H}_2\text{O} \]

Thus, when pH < 7.00, the overall arsenate, sorption mechanism can be represented in three different forms: (i) electrostatic interaction between positively charged center (nitrogen, OH) and negatively charged arsenate, in solution, (ii) electrostatic attraction between positively charged surface hydroxyl group and AsO\(_4^{3-}\),

\[ \text{MOH} + \text{H}_3\text{O}^+ + \text{AsO}_4^{3-} \rightarrow \text{MOH}_2\text{AsO}_4^{2-} + \text{H}_2\text{O} \]

(electrostatic attraction) and (iii) ion-exchange reaction between positively charged metal center and AsO\(_4^{3-}\).

\[ \text{MOH} + \text{H}_3\text{O}^+ + \text{AsO}_4^{3-} \rightarrow \text{M}^+\text{AsO}_4^{2-} + 2\text{H}_2\text{O} \text{(ion-exchange)} \]

Further, when the pH of the medium remains relatively in a neutral range, (pH = 7.00), the As(V), sorption onto the neutral biosorbent surface can be described by a ligand or ion exchange reaction mechanism, which is represented as:

\[ \text{MOH} + \text{AsO}_4^{3-} \rightarrow \text{M}^+\text{AsO}_4^{2-} + \text{OH}^- \]

The modeling of the specific sorption of AsO\(_4^{3-}\) on any material surface depends on a number of external factors such as temperature, pH, AsO\(_4^{3-}\) concentration, as well as the density of surface functional groups available for coordination. In light of the above mentioned mechanism of biosorption, it may be further noted that \textit{B. cereus} biomass showed biosorption capacity at a wide pH of neutral, which could be useful for commercial exploitation purposes.

**Effect of contact time and temperature**

Contact time is one of the important parameters for successful use of the biosorbents for practical application and rapid sorption is among the desirable parameters. Biosorption of As(V) ions at different contact times was studied for initial As(V) concentrations of 1, 5 and 10 mg/L at pH 7.0, keeping all other parameters constant and the results are presented in Figure 5(a). It is clear from the figure that more than 80% removal takes place within 30 min and equilibrium is reached after 60 min. The change in the rate of removal might be due to the fact that initially all sorbent sites were vacant and also the solute concentration gradient was high. At higher concentrations, metals need to diffuse to the sorbent surface by intraparticle diffusion and greatly hydrolyzed ions will diffuse at a slower rate. This indicates the possible monolayer formation of As(V) ions on the outer surface.

The effect of different temperature (10–40 °C) on the biosorption of As(V) with initial concentrations 1, 5 and 10 mg/L was studied using optimum sorbent dose (0.30 g /50 mL) and the results are presented in (Figure 5(b)). It is clear from the figure that the biosorption increased considerably with increasing contact time up to 30 min and after that, it is nearly constant. Therefore, the optimum contact time was selected as 30 min for further experiments. The increase in sorption capacity with the increases in temperature indicates that the sorption process is endothermic in nature.

**Biosorption kinetics**

The prediction of sorption rate gives important information for designing batch biosorption systems. The experimental
data were applied to pseudo-second-order models to clarify the sorption kinetics of As(V) onto *B. cereus* biomass. Sorption of metal ions was rapid for the first 30 min and its rate slowed down as it approached equilibrium (Figure 6).

Experimental data were also tested by the pseudo-second-order kinetic model which is given in the following form (Ho & McKay 1999; Pattanayk et al. 2000):

\[
\frac{t}{q_t} = \frac{1}{K_2q_e^2} + \frac{t}{q_e}
\]

where \( k_2 \) (g/mg min) is the rate constant of the second-order equation, \( q_t \) (mg/g) is the amount of biosorption time \( t \) (min) and \( q_e \) is the amount of biosorption equilibrium (mg/g). This model is more likely to predict kinetic behavior of biosorption with chemical sorption being the rate-controlling step (Dursun 2003; Anirudhan & Unnithan 2007). The linear plots of \( t/q_t \) vs \( t \) for the pseudo-second-order model for the biosorption of As(V) ions onto *B. cereus* at 10–40°C are shown in Figure 6. The rate constants \( k_2 \) (min\(^{-1}\) × 10\(^2\)) and \( R^2 \) values are 0.0138, 0.993 for 10°C, 0.0114, 0.996 for 20°C, 0.0092, 0.998 for 30°C and 0.0076, 0.997 for 40°C. It is clear from these results that the \( R^2 \) values are very high range (0.993–0.998) for the As(V) biosorption. In the view of these results, it can be said that the pseudo-second-order kinetic model provided a good correlation for the biosorption of As(V) ions onto living cells of *B. cereus*.

**Effect of initial As(V) ion concentration**

The biosorption of As(V) ions onto living cells of *B. cereus* biomass was studied by varying arsenate concentration using optimum biosorbent dose (0.5 g/50 mL) at ambient temperature (30°C) and contact time of 60 min. The initial arsenate concentration was decreased from 1 mg/L to 10 mg/L and the corresponding removal gradually decreases from 86% to 73.24% at pH 7.0, respectively. It is clear from the results that more than 80% sorption of As(V) ions took place in first 30 min and equilibrium was established after 60 min. At higher concentrations, metals need to diffuse to the biosorbent surface by intraparticle diffusion and greatly hydrolyzed ions will diffuse at a slower rate. This indicates the possible monolayer formation of As(V) ions on the outer surface.

**Biosorption isotherm models**

The capacity of a biomass can be described by an equilibrium sorption isotherm, which is characterized by certain constants whose values express the surface properties and affinity of the biomass. The biosorption isotherms were investigated using Langmuir equilibrium models. Langmuir models are the sorption equilibria between the concentrations of sorbed metal ions and solid biosorbent. A basic assumption of the Langmuir theory is that sorption takes place at specifically homogeneous sites within the sorbent. This model can be written in linear form (Langmuir 1918; Hlavay & Polyak 2005):

\[
\frac{1}{q_e} = \frac{1}{q_0bC_e} + \frac{1}{q_0}
\]

where \( q_e \) is the equilibrium metal ion concentration on the biosorbent (mg/g), \( C_e \) is the equilibrium metal ion concentration in the solution (mg/L), \( q_0 \) is the monolayer biosorption capacity of the biosorbent (mg/g), and \( b \) is the Langmuir biosorption constant (L/mg) related with the free energy of biosorption. The linear plot of \( 1/C_e \) versus \( 1/q_e \) indicates the applicability of the Langmuir adsorption isotherm presented in Figure 7. The Langmuir parameters...
(q₀, b and R²) for the As(V) biosorption onto living cells of B. cereus were 30.04 mg/g, 0.018 L/mg and 0.99. From this data it can be concluded that the maximum sorption corresponds to a saturated monolayer of sorbate molecules on the biosorbent surface. The comparison of biosorption capacity (q₀: mg/g) of B. cereus biomass for As(V) ions with that of various biomasses reported in the literature is presented in Table 1.

**Desorption efficiency**

Desorption of adsorbed analyte ions onto B. cereus was also studied by using HCl and HNO₃ at various concentrations in Table 2. For these studies, 10 mL of each eluent was used. Analyte ions were desorbed from B. cereus with both 1 M HCl and 1 M HNO₃. The highest recovery for arsenate ions was found to be 90% using 1 M HNO₃ and 80% using 1 M HCl. The effects of volume of 1 M HNO₃ as eluent are also investigated in the range of 5.0–10.0 mL. The highest recovery values (90%) were obtained for arsenate ions after 8.0 mL of 1 M HNO₃. Subsequent elution with 10 mL 1M HNO₃ readily stripped the sorbed arsenate ions from B. cereus.

**CONCLUSIONS**

The operating parameters, pH of solution, biomass dosage, contact time, initial arsenate concentration and temperature, were effective in the biosorption efficiency of As(V). The biosorption capacity of B. cereus biomass was found to be 30.04 mg/g, at optimum conditions of pH 7.0, contact time of 30 min and temperature of 30 ± 2°C. The kinetic data signified that the biosorption of As(V) onto B. cereus followed well the pseudo-second-order kinetic model. The AFM imaging of B. cereus biomass surfaces after As(V) ion biosorption indicates a major morphological difference on mica, the assembly structures changing from rod-like to spindle-like. The FTIR analysis describes the chelating characteristics of metal ion coordination to the functional groups on the B. cereus cell surface, and the functional groups involved may include amide, hydroxyl and amino groups.

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