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BIOREMEDIATION OF ARSENIC (III) AND CHROMIUM (VI) FROM AQUEOUS SOLUTIONS BY LIVING CELLS OF *PSEUDOMONAS PUTIDA* MTCC 3604: EQUILIBRIUM, KINETIC AND THERMODYNAMIC STUDIES

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ABSTRACT

The present research is to investigate the bioremediation of arsenic (III) and chromium (VI) from aqueous solutions by living cells of *Pseudomonas putida* (MTCC 3604) biomass. The various batch parameters affecting the treatment process like biosorbent dose, pH, initial concentration, contact time and temperature are investigated. The maximum removal capacity of *Pseudomonas putida* for arsenic (III) and chromium (VI) was found to be 45.04 mg/g and 52.94 mg/g, respectively, at optimum conditions of pH 5.5, contact time of 1 hr, biomass dosage of 4 g/L, and temperature of 25°C. Biosorption data are fitted to linearly transformed Langmuir isotherm of arsenic (III) and chromium (VI) better than other isotherms with the correlation coefficient (R^2> 0.99). *Pseudomonas putida* cell surface was characterized using SEM-EDX and FTIR. The arsenic (III) and chromium (VI) ions were desorbed from *Pseudomonas putida* using both 1M HCl and 1M HNO_3. The recovery for arsenic (III) and chromium (VI) ions was found to be higher than 88%. The pseudo-second-order equation described is the best kinetic rate-controlling-step than other model with the correlation coefficient (R^2> 0.99). Thermodynamic parameters are also calculated to study the effect of temperature on the removal process. The result reveals the exothermic, spontaneous, and feasible nature of removal process of arsenic (III) and chromium (VI) onto the living biomass of *Pseudomonas putida*.

Keywords: *Pseudomonas putida*; arsenic (III); chromium (VI); Biosorption kinetics; Thermodynamic parameters

1. INTRODUCTION

Biosorption is a potential technology for removal of toxic heavy metals and metalloids from water. The present scenario industrial effluents contain numbers of hazardous anions and cations poses serious problems to the environment and public health. Arsenic is most significant metalloids found in both natural and human activities like magma eruption, aerobic and anaerobic activity, geochemical reactions, smelting of non-ferrous metals and metalloids, fossil fuels combustion and pesticide use etc [1, 2]. Arsenic is present in the environment both inorganic as well as organic forms. Inorganic arsenic exists in the environment in three forms, e.g. metalloid arsenic (As), arsenites (AsO_3^{2-}), and arsenates (AsO_4^{3-}). Long-term exposure to arsenic ions causes melanosis, oedema, keratosis, dark spots on the chest, enlargement of liver and spleen, cancers affect in skin, lung, urinary bladder and kidney [3]. World Health Organization (WHO) has recommended the maximum permissible limit of arsenic in drinking water as 10 µg / Land contaminated water used for human consumption is about 100-300 µg/L [4].

Chromium is one significant metal found natural and anthropogenic sources like effluents discharged from industries e.g. electronics, electroplating, metallurgical, leather industry, wood preservatives, mining processes, cement dust, glassmaking etc [5]. Toxicity of chromium depends on its valence state; trivalent chromium is less toxic, less mobile and mainly found in soil and aquatic environment. Chromium hexavalent highly toxic and mobile in aqueous system exist in the forms of chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) oxyanions. Strong exposure of chromium ions causes genotoxic carcinogens and malignancies to both humans and animals [6]. United States Environmental Protection Agency (USEPA) recommended the maximum permissible limit for chromium (VI) is 0.1 mg L^{-1} for discharge into inland surface waters and 0.05 mg L^{-1} for potable water respectively [7-9]. Biosorption is an efficient technique plays in aqueous solution for elimination of metals and metalloids. The advantages of this technique are low cost effective, minimum operation time, environmental friendly, improved selectivity for specific metals of interest and no production of secondary toxic pollutants.

In this technique utilizes microbial cell wall to removal of metals and metalloids ions from aqueous solutions. Cell metabolism mechanisms based on physic-chemical interactions between metal, metalloid ions and functional groups of the cell wall surface. The cell wall of microorganism mainly consists of polysaccharides, lipids and proteins that serve as binding sites for metals and metalloids. Biosorption is a second part of metal and metalloid ions sequestering process by living microorganisms [10-13]. The objective of the present work is to investigate the biosorption potential of living cells of *Pseudomonas pudida* in the removal of arsenic (III) and chromium (VI) from aqueous solution. Optimum biosorption conditions were determined as a function of pH, biomass dosage, initial metalloid ion concentrations, contact time, temperature and ionic strength. The Langmuir and Dubinin-Radushkevich (D-R) models were used to describe equilibrium isotherms. Biosorption mechanisms of arsenic (III) and chromium (VI) onto *Pseudomonas pudida* biomass were also evaluated in terms of thermodynamics and kinetics. Further *Pseudomonas pudida* biomass is characterized by SEM-EDX and FTIR techniques to know the biosorption of arsenic (III) and chromium (VI).

2. EXPERIMENTAL PROCEDURE

2.1. Bacterial biomass preparation

Pseudomonas putida (MTCC NO: 3604) of microbial type culture and collection which is obtained from the Institute of Microbial Technology, Chandigarh, India to undertake the study. The identified species of *Pseudomonas putida* is a gram-positive, rod shaped bacterium. The biomass of *Pseudomonas putida* cells is grown aerobically at 30°C in agitated liquid medium consisted of: yeast extract 2g/L, beef extract 1.5 g/L, peptone 5 g/L, and sodium chloride 5 g/L, for 72 h with growth condition (pH 7.2-7.5, stirring speed 160 rpm). The medium is sterilized by autoclaving at a pressure (1.5 bar) and temperature of 121°C. Then bacterial is harvested by centrifugation at7000 rpm for 30 min rinsed four to six times with distilled deionied (DDI) water. The biomass is freeze-dried at -50°C at reduced pressure over 24h with a freeze dryer (ALPHA 1-2 LD, Germany, CHRIST).

2.2. Preparation of standards and reagents

All the chemicals used in the studies are of analytical grade which are used without further purification. In all experiments, double distilled water (Milli-Q Millipore 18.2 MΩ cm^{-1} conductivity) is used for preparation, dilution and analytical purposes of solutions. A stock arsenic (III) solution of 1000 mg/L was prepared bydissolving 4.164 g of sodium arsenite ($NaNO_3$) in a 1000 mL of deionized water. The stock solutions are preserved with 1% trace metal grade nitric acid. The 500-mL$NaBH_4$ solution is prepared by dissolving 2.5 g NaOH and 2.0 g $NaBH_4$, in double-distilled water and diluting up to mark. The $NaBH_4$ reagent is always prepared immediately before use. A stock Cr(VI) solution of 1000 mg/L was prepared by dissolving 2.828 g of anhydrous potassium dichromate ($K_2Cr_2O_7$) and add 1.5 mL 1M HNO_3 in a 1000 mL of deionized water [14]. Sodium phosphate buffer (0.1mol/L) was prepared by adding an appropriate amount of phosphoric acid to sodium dihydrogen phosphate solution to result in a solution of pH 2. Ammonium acetate buffers (0.1mol/L) were prepared by adding an appropriate amount of acetic acid to ammonium acetate solutions to result in solutions of pH 4-6. Ammonium chloride buffer solutions (0.1mol/L) were prepared by adding an appropriate amount of ammonia to ammonium chloride solutions to result in solutions of pH 9.

2.3. Equipment

The pH measurements of metal ions in aqueous solution were made using a calibrated pH meter (Orion two stars, USA). The As (III) and Cr (VI) concentrations were measured using flame atomic absorption spectrophotometer (Perkin-Elmer P 200, USA). FT-IR spectra of the samples were obtained by using PerkinElmer FT-IR Spectrometer Spectrum RX-I. The surface micro-morphology of materials was investigated using a scanning electron microscope (SEM) and qualitative element composition was analyzed using energy dispersive X-ray (EDX) by JOEL model JSM-6480LV (Japan). The sample was coated with platinum for 90 seconds at a current of 50 MA before the SEM-EDX micrograph is obtained. The micrographs are taken at a magnification of 15,000.

2.4. Batch biosorption experiment

Biosorption experiments were optimized out at the desired pH value, contact time and biomass dosage level using the necessary biomass in a 250mL stoppered conical flask containing 50mL of test solution. Necessary amount of the biomass was then added and 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 25, 35, 45, and 55 °C. The time required for reaching the equilibrium condition was estimated by drawing samples at regular

intervals of time till equilibrium was reached. The contents of the flask were filtered through filter paper and the filtrate was analyzed for metal concentration by using flame AAS. Biosorption experiments for the effect of pH were conducted by using a solution having 10 mg/L of As (III) and Cr (VI) concentration with a biomass dosage of 4 g/L. Throughout the study, the contact time was varied from 5 to 90 min, the pH from 2 to 9, the initial metal concentration from 10 to 400 mg/L, and the biosorbent dosage from 0.5 to 15 g/L. The results of the biosorption are reported as concentration of As (III) and Cr (VI) in mg/g. The amount of As (III) and Cr (VI) sorbed (removal) is calculated as equation:

$$\text{Biosorption (\%)} = (C_i - C_f)/C_i \times 100 \qquad (1)$$

Where $C_i$and C_f are the initial and final concentrations of the chromium (VI) and arsenic (III) ions in the aqueous solution (mg L^{-1}), respectively. *V* is the volume (L); and *M* is the mass of biosorbents in (g) used.

2.5. Desorption procedure

The sample volume of 50mL, containing 10 mg/L of As (III) and Cr (VI), was transferred separately into a beaker; 10mL of buffer solution was added. After a fast shaking, 4 g/L of onto *Pseudomonas putida* was added and the mixture was shaken again for 90 min at 150 rpm. The system wasfiltered with blue band filter paper. Then the filter and constituents were washed with distilled water. In order to elute the sorbed analytes by onto *Pseudomonas putida*, 10mL of 1M HCl and 10mL of 1M HNO_3 was used separately. Analyte contents of the final solution were determined by AAS. The same procedure was applied to the blank solution. In order to use the biomass for the next experiment, the biomass was washed with excess of 1M acid solution and distilled water, sequentially.

3. RESULTS AND DISCUSSION

3.1. Effect of pH

pH is one of the important factors affecting the biosorption of metal/metalloid ions in the solution. Generally, metal biosorption involves complex mechanisms of ion entrapment in inter and intrafibrillar capillaries and spaces of the cell structural network of a biosorbent. The effect of pH on the biosorption of As (III) and Cr (VI) ions onto *Pseudomonas putida* biomass was studied at pH 2-9 for initial metal/metalloid concentration of 10 mg/L As (III) and Cr (V) solution and the results were presented in Fig. 1.

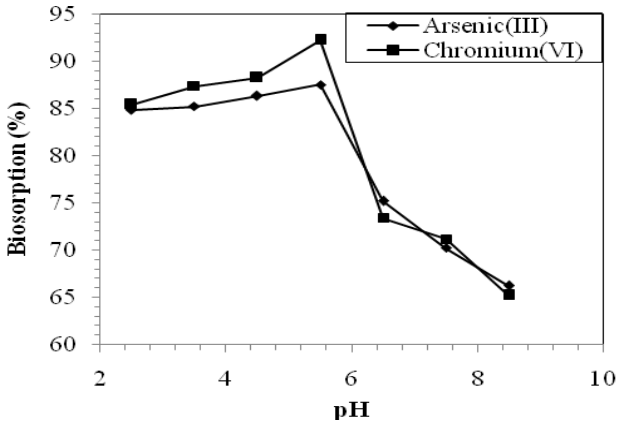

Fig.1: Effect of pH on the biosorption of arsenic (III) and chromium (VI) onto *Pseudomonas putida* biomass (Initial concentration: 10 mg/L; temp.: 25 °C).

The sorption efficiency increases, as the pH of the solution increases from 2.0 to 5.5 and then after decreases with further increase in pH up to 9.0. The maximum biosorption was found to be 85.34% for As (III) and 88.25% for Cr (VI) ions at pH 5.5. At pH 2.0 - 5.5, H_3AsO_4 and $H_2AsO_4^-$ exists, however the predominating is $H_2AsO_4^-$. At low pH (2.0-5.0) the surface of biosorbent is highly protonated and as a result, a strong attraction exists between oxyanion and positively charged surface of the biosorbent [15-16]. The further decrease in metal uptake with increase in pH (5.5 - 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 sorbent, or both. A repulsive force may develop between the negatively charged surface and the anions [17]. Cr (VI) exists as $HCrO_4^-$, $Cr_2O_7^{2-}$ in solution at optimum sorption pH has a tendency to bind the protonated active sites of the biosorbent. The dominant form of hexavalent chromium at acidic pH is $HCrO_4^-$ which arises from the hydrolysis reaction of the dichromate ion ($Cr_2O_7^{2-}$). But as pH of the solution increases, bacterial cell wall becomes more and more negatively charged due to functional groups, which repulse the negatively charged chromate ions thereby affecting Cr (VI) adsorption on the bacterial surface, resulting in reduced sorption efficiency [18].

3.2. Effect of biomass dosage

The biomass dosage is an important parameter because this determines the capacity of a biosorbent for a given initial concentration. The biosorption efficiency for As (III) and Cr (VI) ions as a function of biomass dosage was investigated (Fig. 2). The percentage of the metal

biosorption increases with the biomass loading up to 4 g/L. This result can be explained by the fact that the biosorption sites remain unsaturated 1 during the biosorption reaction whereas the number of sites available for biosorption site increases by increasing the biosorbent dose. The maximum biosorption, 86 % for As (III) and 89 % for Cr (VI), of the metal ions was attained at biomass dosage, 4 g/L. It is observed that after dosage of 4 g/L, there is no significant change in percentage of removal of arsenate. It may be due to the higher dosage could produce a 'screen effect' on the cell wall, protecting the binding sites, thus in lower arsenate uptake [19]. So, 4 g/L is considered the optimum dose and is used for further study.

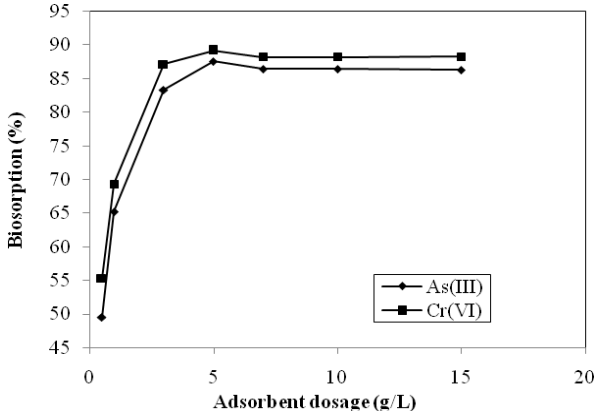

Fig. 2: Effect of biosorbent dosage on the biosorption of arsenic (III) and chromium (VI) on to *Pseudomonas putida* biomass (Initial concentration: 10 mg/L; pH 5.5; temp.: 25 °C).

3.3. 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 desirable parameters. Fig 3 shows the effect of contact time on the biosorption of As (III) and Cr (VI) ions onto *Pseudomonas putida*. It is clear from the figure the biosorption increased considerably with increasing contact time up to 30 min and after then, it was nearly constant. Therefore, the optimum contact time was selected as 30 min for further experiments. Fig 3 shows the biosorption of As (V) and Cr (VI) ions as a function of the temperature. The biosorption percentage decreased from 86% to 56% for As (III) and from 89% to 61% for Cr (VI) as temperature was increased from 25 to 55°C for the equilibrium time, 30 min. These results indicated the exothermic nature of As(III) and Cr (VI) biosorption onto *Pseudomonas putida* biomass. These results indicated the exothermic nature of As(III) and Cr (VI) biosorption onto *Pseudomonas putida* biomass. A decrease in the biosorption of As(III) and Cr (VI) ions with the rise in temperature may be due to increasing tendency to desorbs metal ions from the interface to the solution [20-21]. Optimum temperature was selected as 25°C for further biosorption experiments.

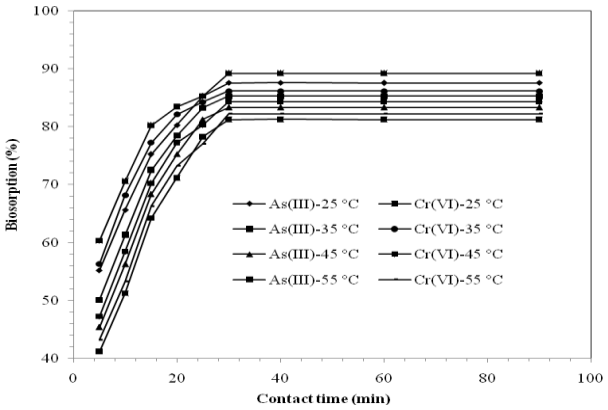

Fig. 3: Effect of contact time and temperature on the biosorption of arsenic (III) and chromium (VI) onto *Pseudomonas putida* biomass (Initial concentration: 10 mg/L; pH 5.5; biomass dosage: 4 g/L).

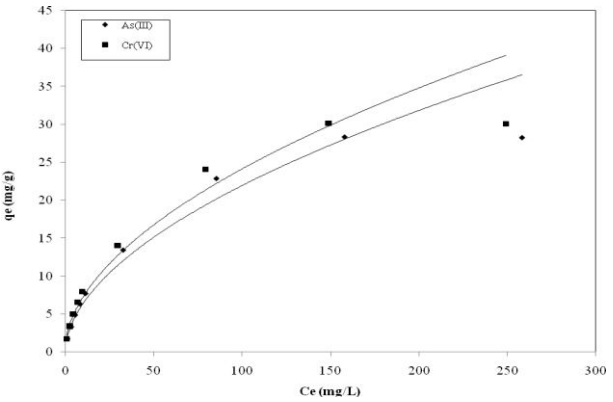

Fig. 4: Langmuir plots (non linear and linear) for the biosorption of arsenic (III) and chromium (VI) onto *Pseudomonas putida* biomass (biomass dosage: 4 g/L; contact time: 60 min; pH: 5.5; temp.: 25 °C).

3.4. Biosorption isotherm models

The capacity of a biomass can be described by 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 the Langmuir and Dubinin–Radushkevich isotherm models were analyzed. Langmuir model are the sorption equilibrium between the concentrations of sorbed metal ions and solid biosorbent. A basic assumption of the Langmuir theory is that sorption takes place at specific homogeneous sites within the sorbent. This model can be written in non-linear form [22].

$$q_e = q_0 K_c C_e / 1 + K_c/C_e \quad (2)$$

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 *K*C is the Langmuir biosorption constant (L/mg) related with the free energy of biosorption. Fig. 4 indicates the non-linear relationship between the amount (mg) of As (III) and Cr (VI) ions sorbed per unit mass (g) of *Pseudomonas putida* biomass against the concentration of As (III) and Cr (VI) ions remaining in solution (mg/L). The coefficients of determination (*R*) were found to be 0.994 and 0.997 for As (III) and Cr (VI) biosorption, respectively. The maximum biosorption capacity (*q*0) of *Pseudomonas putida* biomass was found to be 45.04 mg/g for As (III) and 52.94 mg/g for Cr (VI). The *K*C value was found as 0.018 L/mg for As (III) ion and 0.0094 L/mg for Cr (VI) ion. From this data it can be concluded that the maximum sorption corresponds to a saturated monolayer of sorbate molecules on biosorbent surface. The comparison of biosorption capacity (q_0: mg/g) of *Pseudomonas putida* biomass for As(III) and Cr (VI) ions onto *Pseudomonas putida* biomass for As (III) and Cr (VI) ions with that of various biomasses reported in literature [23-25] and presented in **Table 1**.

Table 1: Comparison of biosorption capacity of *Pseudomonas putida* biomass for arsenic (III) and chromium (VI) with that of different biosorbents

Biosorbent	pH	As(III) mg/g	pH	Cr(VI) mg/g	References
L. nigrescens	2.5	45.5	-	-	[29]
A. niger biomass	3.5	0.20	-	-	[33]
Tea fungal biomass	7.20	4.95	-	-	[21]
Methylated biomass	6.5	3.75	-	-	[22]
I. hispidus	2.0	59.6	-	-	[34]
Chitosan-coated biosorbent	4.0	96.46	-	-	[16]
M. oleifera seed powder	2.5	2.16	-	-	[28]
Bacillus circulans	-	-	2.5	34.5	[23]
Bacillus megaterium	-	-	2.5	32.0	[23]
Rhizopus nigricans	-	-	4.0	200	[24]
Aspergillus flavus	-	-	3.0	0.335	[25]
Rhizopus arrhizus	-	-	-	5.1	[37]
Pseudomonas putida biomass	5.5	45.04	5.5	52.94	Present study

It is known that the Langmuir and Freundlich biosorption isotherm constant do not give any idea about the biosorption mechanism. The equilibrium data were also subjected to the D-Risotherm model to determine the nature of biosorption processes as physical or chemical. The D-R sorption isotherm is more general than Langmuir isotherm, as its derivation is not based on ideal assumptions such as equipotent of the sorption sites, absence of steric hindrance between sorbed and incoming particles and surface homogeneity on microscopic level [26-27]. The linear presentation of the D-R isotherm equation [28] is expressed by

$$\ln q_e = \ln q_m - \beta\varepsilon^2 \quad (3)$$

where *q*e is the amount of metal ions adsorbed on per unit weight of biomass (mol/L), *q*m is the maximum biosorption capacity (mol/g), β is the activity coefficient related to biosorption mean free energy (mol^2/kJ^2) and ε is Polanyi potential ($RT \ln(1+1/Ce)$). The D-R isotherm model well fitted the equilibrium data since the R^2 value was found to be 0.997 and 0.996 for As (III) and Cr (VI), respectively (**Fig. 5**). D. R. isotherm constants β and *q*m are calculated from the slope and intercept of the plots to be 3.18 x 10-3 mol^2 kJ^{-2} and 0.0039 mol/g for As (III) biosorption and 3.21 x 10^{-3} mol2 kJ^{-2} and 0.0037 mol/g for Cr (VI) biosorption, respectively. The biosorption mean free energy (*E*; kJ/mol) is as follow:

$$E = (-2K)^{-1/2} \quad (4)$$

The *E* (kJ/mol) value gives information about adsorption mechanism, physical or chemical. If it lies between 8 and 16 kJ/mol, the adsorption process takes place chemically and while $E < 8$ kJ/mol, the adsorption process proceeds

physically. The mean biosorption energy was calculated as 12.65 and 12.48 kJ/mol for the biosorption of As(III) and Cr (VI) ions. These results suggest that the biosorption processes of both metal ions onto *Pseudomonas putida* biomass could be taken place by chemical ion-exchange mechanism because the sorption energies lie within 8-16 kJ/mol.

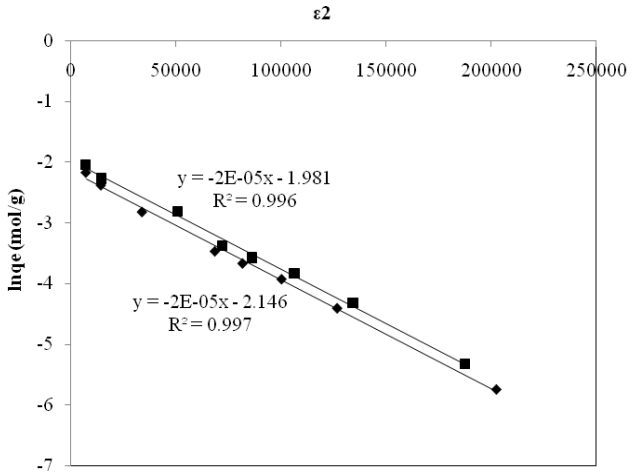

Fig. 5: D-R isotherm plots for the biosorption of arsenic (III) and chromium (VI) onto *Pseudomonas putida* (biomass dosage: 4 g/L; contact time: 60 min; pH: 5.5; temp.: 25 °C).

3.5. Desorption efficiency

Desorption of adsorbed analyte ions onto *Pseudomonas putida* were also studied by using HCl and HNO3 at various concentrations in Table 2.

Table 2: Influence of various eluents on desorption of arsenic (III) and chromium (VI) ions from *Pseudomonas putida*.

Eluent		Recovery (%)	
		As(III)	Cr(VI)
0.5 mol L^{-1}	HCl	72.23±3	77.11±3
1mol L^{-1}	HCl	81.33±3	87.21±3
0.5 mol L^{-1}	HNO_3	78.34±3	84.55±2
1mol L^{-1}	HNO_3	90.55±3	90.23±2

For these studies, 10mL of each eluent was used. Analyte ions were desorbed from *Pseudomonas putida* with both 1M HCl and 1M HNO_3. The highest recovery for both metal ions was found to be 90% using 1M HNO_3 and 80% using 1M HCl. The effects of volume of 1M HNO_3 as eluent were also investigated in the range of 5.0-10.0 mL. The highest recovery values (90%) were obtained for both metal ions after 8.0mL of 1M HNO_3. Subsequent elution with 10mL 1M HNO_3 readily strips the sorbed metal ions from *Pseudomonas putida*. In addition, as it can be seen from **Fig. 6,** the high stability of *Pseudomonas putida* permitted ten times of adsorption-elution process along the studies without a decrease about 10% in recovery of both metal ions.

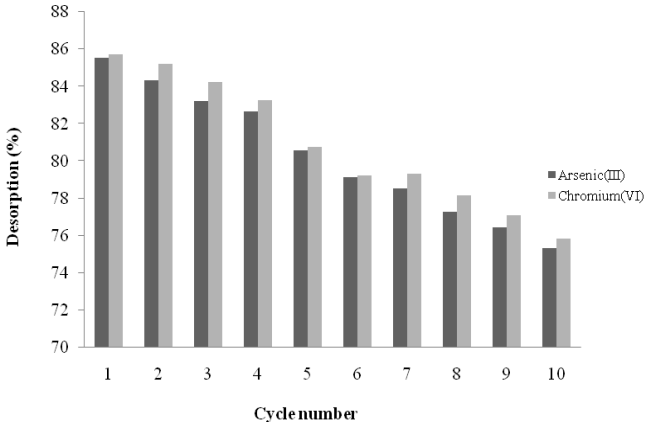

Fig. 6: Desorption efficiency of *Pseudomonas putida* biomass with cycle number.

3.6. Biosorption kinetics

The prediction of sorption rate gives important information for designing batch biosorption systems. The experimental data are applied to pseudo-first-order and pseudo second-order models to clarify the sorption kinetics of As (III) and Cr (VI) onto *Pseudomonas putida* biomass. Sorption of metal ions is rapid for the first 30 minutes and its rate slowed down as it approaches towards equilibrium. The linear form of the pseudo-first-order rate equation by Lagergren is given as [29-30].

$$\log (q_e - q_t) = \log q_e - (K_1)t / 2.303 \quad (5)$$

where $q_t$and q_e (mg/g) are the amounts of the metal 1 ions biosorbed at equilibrium (mg/g) and t (min), respectively, and $k1$ is the rate constant of the equation (min^{-1}). The biosorption rate constants ($k1$) can be determined experimentally by plotting of log (qe - qt) vs t. The plots of log (q_e - q_t) vs t for the pseudo-first-order model were not shown as figure because the coefficients of determination for this model at studied temperatures is low. It can be concluded from the R^2 values in Table 3 that the biosorption mechanisms of As(III) and Cr(VI) ions onto *Pseudomonas putida* biomass does not follow the pseudo-first-order kinetic model. Experimental data were also tested by the pseudo-second-order kinetic model which is given in the following form [31-32].

$$t/q_t = 1/K_2 q_e^2 + t/q_e \quad (6)$$

Where, $k2$ (g/mg min) is the rate constant of the second-order equation, qt (mg/g) is the amount of biosorption time t (min) and qe 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 [33-34].

Table 3: Kinetic parameters obtained from pseudo-first-order and pseudo-second-order for arsenic (III) and chromium (VI) bisorption onto *Pseudomonas putida* biomass at different temperatures

Temperature (˚C)	Pseudo-first-order		Pseudo-second-order	
	K_1(min^{-1}) x 10^2	R^2	K_2 (g/mg min) x 10^2	R^2
		As(III)		
25	0.3927	0.974	0.0138	0.998
35	0.3654	0.971	0.0114	0.997
45	0.3558	0.962	0.0092	0.996
55	0.3432	0.923	0.0076	0.993
		Cr(VI)		
25	0.4537	0.984	0.0175	0.999
35	0.4353	0.981	0.0149	0.999
45	0.3555	0.967	0.0102	0.996
55	0.3346	0.939	0.0084	0.994

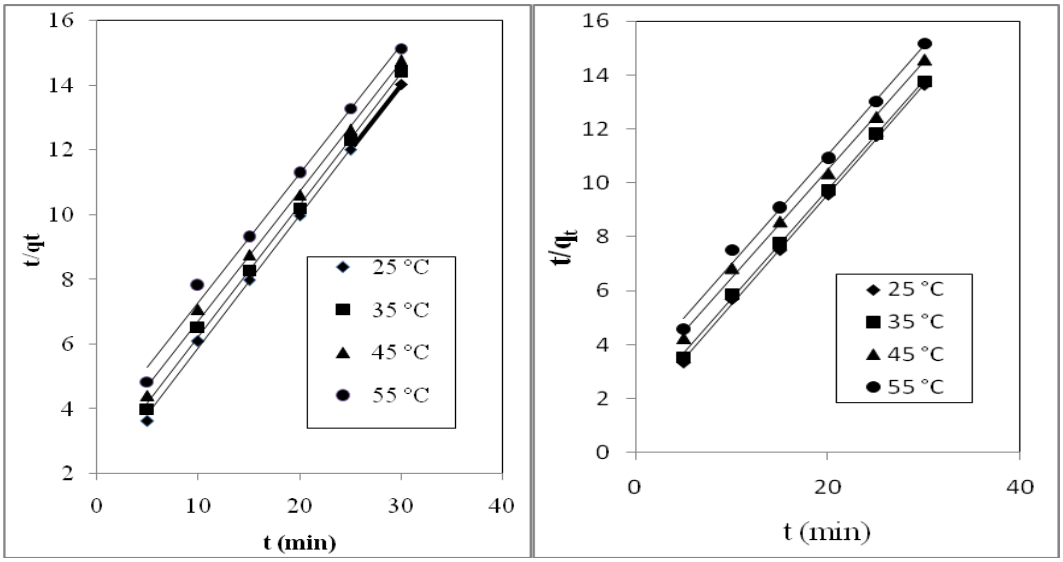

Fig. 7: Pseudo-second-order kinetic plots at different temperatures for; (a) arsenic (III) and (b) chromium (VI) biosorption (initial concentration: 10 mg/L; pH: 5.5; biomass dosage: 4 g/L).

The linear plots of t/qt vs t for the pseudo-second-order model for the biosorption of As(III) and Cr(VI) ions onto *Pseudomonas putida* at 25-55˚C were shown in **Fig. 7(a)** and **(b),** respectively. The rate constants ($k2$), the $R2$ and qe values are given in Table 3. It is clear from these results that the R^2 values are very high (in range of 0.993 - 0.998 for the As (III) biosorption and 0.994 - 0.999 for the Cr (VI) 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(III) and Cr (VI) ions onto *Pseudomonas putida* in contrast to the pseudo-first-order model.

3.7. Biosorption thermodynamics

In order to describe thermodynamic behavior of the biosorption of As(III) and Cr (VI) ions onto *Pseudomonas putida* biomass, thermodynamic parameters including the change in free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) were calculated from following equations [35-36].

$$\lg Kc = \Delta S / 2.303R + \Delta H / 2.303RT \quad (7)$$

$$\Delta G = \Delta H - T\Delta S \quad (8)$$

Where, R is the universal gas constant (8.314 J/mol K), T is temperature (K) and KD (qe/Ce) is the distribution coefficient. The enthalpy (ΔH) and entropy (ΔS) parameters were estimated from the following equation.

According to Eq. (8), the ΔH and ΔS parameters can be calculated from the slope and intercept of the plot of ln KD vs $1/T$ yields, respectively (**Fig. 8**). Gibbs free energy change (ΔG) was calculated to be -49.37, -43.71, -38.42, and -33.70 kJ/mol for As (III) biosorption and -56.06,- 48.8, -42.60, and -35.22 kJ/mol for the biosorption of Cr(VI) at 25, 35, 45, and 55°C, respectively. The negative ΔG^{o} values indicated thermodynamically feasible and spontaneous nature of the biosorption. The decrease in ΔG values with increase in temperature shows a decrease in feasibility of biosorption at higher temperatures. The ΔH parameter was found to be -68.01 and -48.85 kJ/mol for As (III) and Cr (VI) biosorption, respectively. The negative ΔH^{o} indicates the exothermic nature of the biosorption processes at 25-55°C. The ΔS parameter was found to be 418.24 J/mol K for As (III) biosorption and 396.53J/mol K for Cr (VI) biosorption. The positive values of ΔS suggest increased randomness at the solids/solution interface during the biosorption of metal ions onto biosorbent.

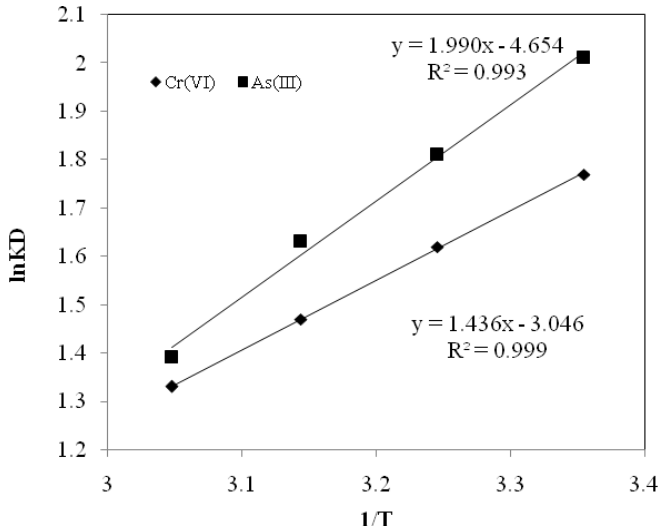

Fig. 8: Plot of ln K_D vs 1/T for the estimation of thermodynamic parameters for biosorption of arsenic (III) and chromium (VI) onto *Pseudomonas putida*.

3.8. Characterization of the biosorbent

The surface morphology of *Pseudomonas putida* cells without and with sorption of As (III) and Cr (VI) during biosorption process is observed with the help of SEM-EDX as shown in Fig. 9.

Pseudomonas putida bacteria without As (III) and Cr (VI) ions exposure in the control blank are rod-like in shape with a smooth surface (the dimension of these cells 1 is about 3.476μm long and 1.136 μm wide, on average, as shown in Fig. 9(a)). After the As (III) and Cr (VI) ions 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 (III) and Cr (VI) sorption, as shown in **Fig.9 (b)** and **Fig. 9(c),** respectively. The morphological changes of the sample can be attributed to the interactions between heavy metal and the surface of *Pseudomonas putida* cells. These agree with the results of FT-IR spectra analysis.

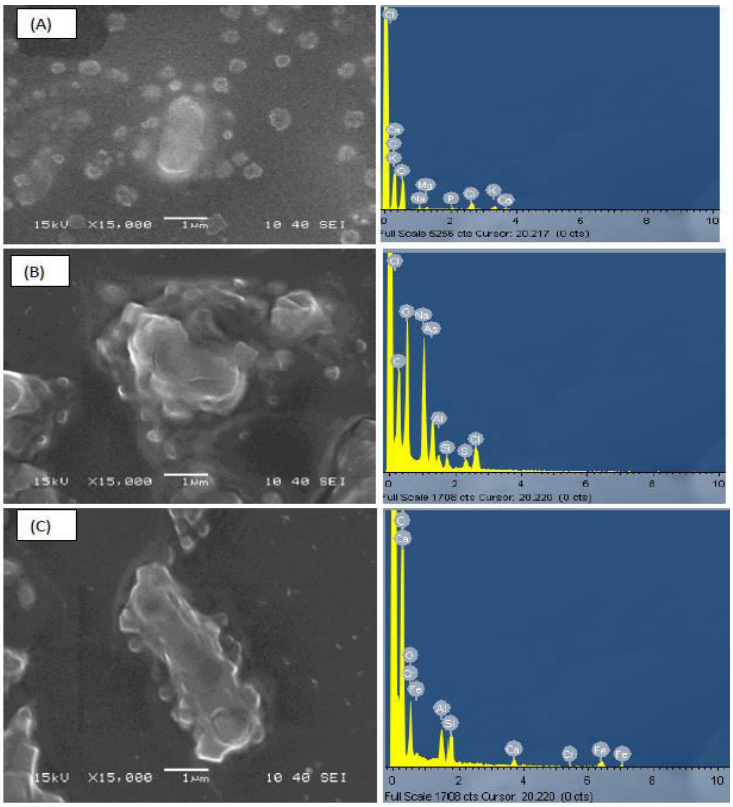

Fig. 9: SEM-EDX image of *Pseudomonas putida* cells (ion strength 0.01 mol/L; pH 5.5) (a) control blank of *Pseudomonas putida*; (b) 10 mg/L arsenic(III) ion-exposed cells; (c) 10 mg/L chromium(VI) ion-exposed cell

Infrared spectra of the *Pseudomonas putida* biomass with and without As (III) and Cr (VI) loaded were obtained to determine which functional groups may have contributed to the As (III) and Cr (VI) absorption on the *Pseudomonas putida* biomass as shown in **Fig. 10**. The spectra revealed

biosorbent heterogeneity, evidenced by different characteristic peaks with the possible presence of amino, amide, carboxyl and hydroxyl groups. The IR absorption bands and corresponding possible groups able to interact with protons or metal ions are presented in **Table 4**.

Table 4: IR absorption bands and corresponding possible groups observed on raw and treated (arsenic (III) and chromium (VI)) spore forming bacterium *Pseudomonas putida*.

Living bacteria			Assignments	Probable site for functional group
without treated of As (III) and Cr(VI) wave number (cm^{-1})	with As (III) treated wave number (cm^{-1})	with Cr(VI) treated wave number (cm^{-1})		
3466.32	3450.21	3460.29	N–H and O–H stretching vibrations from polysaccharides and proteins	Cell wall –direct interaction of OH group with chromium and arsenic ions [35, 38]
2311.29	2294.34	2282.91	-CH stretching	-CH stretching vibration of alkyl chains
1652.44	1648.97	1652.62	Amide I (protein C=O stretching)	Peptides- amino acids /amides [39, 35]
1467.78	1412.83	1450.38	COO^- symmetric stretching from amino acid side chains and fatty acids	Amino acid - cell wall of the bacterial cells [34, 39]
1113.61	1118.07	1116.26	C-N<	C-N stretching vibrations of amino groups [40-41]
848.12	849.69	879.51	Glycogen units, polysaccharides	Cell wall [42]

It was observed from the table after sorption of As (III) and Cr (VI) ions there was a significant shift of few absorption peaks indicating the coordination of metal ions on the surface of the biomass. This clearly manifests the binding of As (III) and Cr (VI) ions on the surface of the biomass.

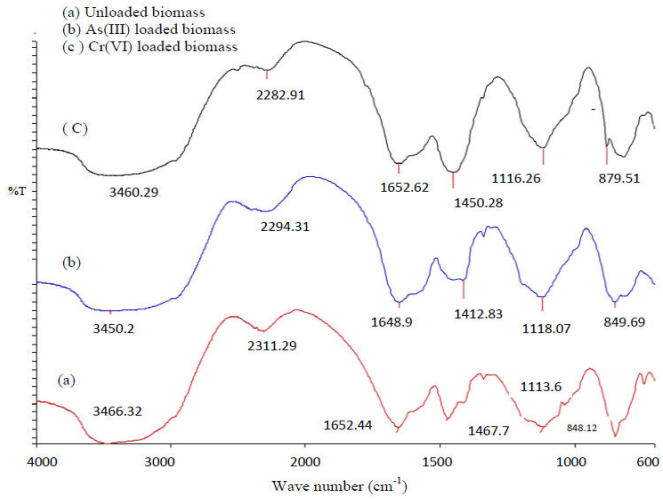

Fig.10: FT-IR spectra of the *Pseudomonas putida* biomass without (control) and with (10 mg/L) arsenic (III) and chromium (VI) ions biosorption.

4. CONCLUSIONS

The operating parameters, pH of solution, biomass dosage, contact time, and temperature, were effective on the biosorption efficiency of As (III) and Cr (VI). The biosorption capacity of *Pseudomonas putida* biomass was found to be 45.04 mg/g for As (III) and 52.94 mg/g for Cr (VI), respectively, at optimum conditions of pH 5.0, contact time of 30 min and temperature of 25°C. The mean free energy values evaluated from the D-R model indicated that the biosorption of As (III) and Cr (VI) onto *Pseudomonas putida* biomass was taken place by chemical ion-exchange. The kinetic data signified that the biosorption of As (III) and Cr (VI) ions onto *Pseudomonas putida* followed well the pseudo-second-order kinetic model. The thermodynamic calculations showed the feasibility, exothermic and spontaneous nature of the biosorption of As (III) and Cr (VI) ions onto *Pseudomonas putida* biomass at 25-55°C. The SEM-EDX imaging of *Pseudomonas putida* biomass surfaces after As (III) and Cr (VI) ions 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 ions coordination to the functional groups on the *Pseudomonas putida* cell

surface, and the functional groups involved may include amide, carboxyl, hydroxyl and amino groups. *Pseudomonas putida* is low-cost biomass with considerable high biosorption capacity.

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