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Biosorption of Hexavalent Chromium by *Pseudomonas aeruginosa* Strain ANSC: Equilibria Isothermic, Kinetic and Thermodynamic Studies

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Abstract *Pseudomonas aeruginosa* strain ANSC, a non-genetically modified bacterial strain isolated from soil was used to study and evaluate biosorption potentials for hexavalent chromium (Cr[VI]) from aqueous solution. Living, heat-killed, and permeabilised cells were all used and found to be capable of reducing and sorbing Cr(VI). The influences of initial Cr(VI) ion concentration (50–150 mg/L), contact time (2 h, 10 min intervals), pH (2–8), temperature (30–60°C) and biosorbent mass (1.0–5.0 g/l) were reported. Adsorption of Cr(VI) is highly pH- and temperature-dependent, and the results indicate that the optimum pH and temperature for removal were found to be 2 and 60°C respectively. The hexavalent chromium biosorption equilibrium could be better described by Langmuir isotherm than it could by Freundlich isotherm. A comparison of kinetic models applied to the adsorption of Cr(VI) ions onto the biosorbents was evaluated for the pseudo first-order, pseudo second-order, and intra-particle diffusion kinetic models. Results show that the pseudo second-order kinetic model was evidenced to correlate better the experimental data. The rate of hexavalent chromium adsorption increased following permeabilisation of the outer and/or cytoplasmic membrane by surfactants such as Triton X100, Tween 80, toluene, sodium deoxycholate and sodium dodecyl sulphate. The adsorption process has been found endothermic, and thermodynamic parameters of Gibb's free energy ($\Delta G°$), change in enthalpy ($\Delta H°$) and change in entropy ($\Delta S°$) were calculated. *Pseudomonas aeruginosa* strain ANSC evidenced an effective biosorbent for the removal of hexavalent chromium in aqueous form.

Keywords *Pseudomonas aeruginosa*, Adsorption, Kinetic, Isotherm, Thermodynamic

1. Introduction

Increasing industrialization and technological expansion, rapid urbanization, increased energy utilization and waste generation from domestic and industrial sources have ravaged the environment by discharge of industrial and domestic wastes laden with heavy metals. And contamination of soil and water bodies by organic pollutants and toxic metals has been increased in the past few years due to industrialization, intensive agriculture and anthropogenic activities [1].

Chromium and its compounds are widely used in industries. Hexavalent chromium is present in effluents from electroplating, paint, pigment, cement, mining, dyeing, leather tanning, fertiliser and photography industries. Hexavalent chromium is toxic to humans. Removal of heavy metals from metal-ladened wastewater is often achieved by physico-chemical processes. Physico-chemical processes in use for heavy metal removal from wastewater include precipitation, coagulation, chemical reduction processes, ion exchange, membrane processes such as ultrafiltration, electrodialysis and reverse osmosis, and adsorption [2]. These conventional treatment techniques have reportedly become less effective, more expensive and intensive, requiring highly skilled labour and running energy.

Microbial cells interact with chromium at different levels from the cell wall and periplasm to the cytoplasm and cell organelles [3]. Microorganisms require detecting and regulating intracellular levels of chromium through homeostatic systems that maintain balance between the incorporation, expulsion, and arrest of metal [3].

Therefore, biosorption, the process of attracting various chemical species by biomass (live or dead) through physicochemical mechanisms as adsorption or ion exchange, has presented a more efficient and

environmental-friendly technology to remediate heavy metals. In this current study, live, heat-killed and permeabilised cells of hexavalent chromium tolerant microbe were used to reduce and sorb Cr(VI) from aqueous solution. Also, influences of initial metal concentration of Cr(VI), contact time, pH, temperature and biosorbent dosage were evaluated while correlating the process equilibria adsorption isotherm, kinetics and thermodynamics.

2. Methods

2.1. Screening of Microorganism Showing Resistance to Hexavalent Chromium

Chromate resistant bacterium was isolated from soil with a history of previous contained chemical discharge. The bacterial isolate was screened for tolerance towards hexavalent chromium. The bacterium was grown on Petri dishes containing Luria-Bertani (LB) agar supplemented with 500mg/L of $K_2Cr_2O_7$. The pH of the medium was maintained at 5.3. Plates were incubated at 37°C for 3 days.

2.2. Identification of Isolate by 16S rRNA Gene Sequence

Genomic DNA of the chromate-tolerant bacterial isolate was isolated, and gene fragments specific to the highly variable region of the bacterial 16S rRNA gene amplified by polymerase chain reaction (PCR) (Macrogen Inc., Seoul, Korea). The primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 µL reaction mixture by using an *EF-Taq* (SolGent, Korea) as follows: activation of *Taq* polymerase at 95°C for 2 minutes, 35 cycles of 95°C for 1 minute, 55°C, and 72°C for 1 minute each were performed, finishing with a 10-minute step at 72°C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95°C for 5 minutes, followed by 5 minutes on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). The homology of the 16S rRNA gene sequences was checked with the 16S rRNA gene sequences of other organisms that had already been submitted to GenBank database using the BLASTN (http://www.ncbi.nih.gov/BLAST/) algorithm [4] (Macrogen Inc., Seoul, Korea).

2.3. Reduction of Cr(VI) by Living and Permeabilised Cells of Isolate

Efficiency of Cr(VI) reduction was examined by living and permeabilised cells of isolate. For live cells, isolate was grown overnight in LB broth in the presence of 10mg/L $K_2Cr_2O_7$. The pre-cultures were used for inocula into different cultures containing 0, 100, 200, 300, 400, 500 and 600 mg/L of $K_2Cr_2O_7$. The different Cr(VI)-containing broth cultures were incubated in an orbital shaker at 150 rpm, 37°C. At different times (0, 24, 48, 72, 96, and 120 h), aliquots were harvested in order to measure absorbances at 600nm and 540nm for growth and Cr(VI) reduction respectively. Hexavalent chromium was quantified by employing the *S*-diphenylcarbazide (DPC) method [5].

For permeabilised cells, cultures of isolate were grown for five days in an orbital shaker at 37°C, harvested, and washed twice with sterile tri-deionized water, and suspended in 0.1M potassium phosphate buffer (pH 7.0). The suspended biomass was treated with 0.5% v/w triton X-100, 0.5% v/w tween 80, 0.5% v/w toluene, 0.5% w/w sodium deoxycholate and 0.5% w/w sodium dodecyl sulphate, and vortexed for 30 min to achieve cell permeabilisation. Permeabilised cell suspensions (2 wet g) were then added into 20mL of 100µg/mL solutions of $K_2Cr_2O_7$, and incubated for 5 h at 37°C [6]. The samples were filtered. The filtrates containing the residual Cr(VI) solution were evaluated colourimetrically with absorbance read at 540nm using the DPC method.

2.4. Biosorption Studies Using Heat-killed Biomass of Isolate

Adsorption of hexavalent chromium onto heat-killed biomass of isolate was investigated in a batch system. After 5 days of growth in LB broth, the biomass was recovered by centrifugation at 3,000 rpm for 10 min. The recovered biomass was washed as above and heat-killed at 80°C for 24 h. Solutions of chromate containing 50-150 mg/L were prepared using sterile deionized water. The concentrations of chromate studied were prepared from stock solutions. Adsorption experiments were performed by stirring magnetically at 150 rpm, at 30°C for 2 h. The adsorption volume was 20mL, and a 1-5 g biosorbent dosage was used. After centrifugation, the amount of unadsorbed Cr(VI) in the supernatant solutions was measured. The amount of Cr(VI) ion adsorbed onto the biomass at time, q_t, was obtained by using the following expression,

$$qt = \frac{V(Co - Ct)}{W}$$

Metal ion removal percentage was calculated as follows:

$$Removal\ \% = \frac{(Co - Ct)}{Co} \times 100$$

where C_0 and C_t are the initial concentration and concentration at a particular time, t, of metal ion solution

(mg/L) respectively, V is the volume of the chromate solution (L), and W is the weight of the bacterial biomass (g) [7].

The effect of contact time at varying temperature level (i.e., 30, 40, 50 and 60 ℃) on adsorption capacity was evaluated. Also, the effect of pH of the solution on the adsorption capacity of the bacterial biomass was investigated (2.0 – 8.0) at 30℃. Batch experiments were also conducted using different amounts of biosorbent (1.0 and 5.0 g biomass weight) at pH 2.0 and 30℃. To determine the effect of initial concentrations of Cr(VI) on the adsorption rate and capacity of the adsorbent, concentration was varied between 50 and 150 mg/L in the adsorption medium at pH 5.0. For each adsorption experiment, replicate means were reported.

3. Results and Discussion

3.1. Isolation and Identification of Chromate Tolerant Bacterial Isolate

Microorganism was grown on LB agar plates containing 500 mg/L of $K_2Cr_2O_7$. Colonies isolated grew rapidly within three days of incubation at 37℃. Colonies were usually erose, convexed and rough with approximately 4 mm in diameter, and they were fast growing at such elevated concentration of $K_2Cr_2O_7$. In similar works, Camargo and coworkers [8] isolated some chromium resistant bacteria that can tolerate or reduce Cr(VI) at concentrations of 1500–2500 mg/L; and Polti et al. [9] isolated actinomycete strains of *Streptomyces* spp. and *Amycolatopsis* spp. capable of tolerating hexavalent chromium concentration of up to 17mM ($K_2Cr_2O_7$) i.e. ca 5,000 mg/L. The isolate was identified using the 16S rRNA gene sequence analysis (Table 1). Isolate showed 99% sequence identity with *Pseudomonas aeruginosa* strain ANSC.

Table 1. Identification of chromium(VI)-reducing bacterial isolate by 16S rRNA gene sequence analyses

Organism	Accession number	E value	Identity (%)
Pseudomonas aeruginosa ANSC	GU296674.1	0.0	99

3.2. Effect of Chromium(VI) Concentration on Cr(VI) Removal and Growth

The removal of Cr(VI) by *Pseudomonas aeruginosa* ANSC was examined using live cells, and the effect of varying concentrations of Cr(VI) (as $K_2Cr_2O_7$) on bacterial growth was examined as well. Cr(VI) concentrations of 100-600 mg/L were used to examine the Cr(VI) removal and effect on growth. The bacterial cells (ca. 2.06×10^8 cfu/mL), which were cultured in 100 mL LB broth containing 10 mg/L Cr(VI), under orbital shaking at 150rpm at 37℃, were used to seed 25mL LB broth containing 100, 200, 300, 400, 500 and 600 mg/L $K_2Cr_2O_7$. Triplicate assays were examined for both growth and Cr(VI) removal/reduction parameters at a 24-h interval for 120 h. The percentage amount of Cr(VI) removed was determined.

Figure 1 shows the percentage Cr(VI) removed by *P. aeruginosa* ANSC under varying concentrations of Cr(VI) against time, with Figure 2 showing the effect of Cr(VI) on *P. aeruginosa* ANSC growth against time. Under 100 and 200 mg/L of chromate, *P. aeruginosa* evidenced a 95.87% and 78.41% Cr(VI) removal respectively by 48 h. However, by 72 h [at 100 and 200 mg/L] the Cr(VI)-DPC assay produced no resultant colour complex with an indication of complete removal of Cr(VI) from the broth. Under 300 mg/L of chromate, *P. aeruginosa* ANSC removed nearly all residual chromate (96.03 %) by 120 h of incubation. Under 400, 500 and 600 mg/L of chromate, however, *P. aeruginosa* ANSC evidenced a steady increase towards removal of Cr(VI), with the bacterium recording the least percentage Cr(VI) removal of 41.53% under 600mg/L of chromate at 120 h. Similar outcomes were reported by Dey and Paul [10] using whole cells of *Arthrobacter* sp. SUK 1205 isolated from metalliferous chromite mine. Batch cultures of *Arthrobacter* sp. under 100 μM of Cr(VI) were used to observe effect and outcome of Cr(VI) reduction and growth inhibition. Complete reduction of 100 μM Cr(VI) was achieved in 48 h of incubation at 35°C under continuous shaking at 120 rpm [10]. Relative trends in chromate removal/reduction were also reported by Pattanapipitpaisal et al. [11] using *Microbacterium liquefaciens*.

For effect on growth, *P. aeruginosa* ANSC proportionately showed increase in cell density with decreasing influence of chromate concentration (Figure 2). Under 100, 200 and 300 mg/L chromate, *P. aeruginosa* ANSC grew exponentially within the initial 48 h. Relatively, all residual Cr(VI) was removed by 48 h of incubation under 100, 200 and 300 mg/L of Cr(VI) concentrations by the isolate. Dey and Paul [10] recorded similar result with respect to time for the removal of 100 μM Cr(VI). Also, initial cell densities were not significantly influenced by the isolate's Cr(VI) removal process with respect to control (without Cr[VI]), except for a slight decrease of initial cell density at 300mg/L Cr(VI) (Figure 2). Similar observations were reported by Pal and Paul [12] using chromate resistant bacteria isolated from serpentine soil. Further, under 600 mg/L Cr(VI), however, initial cell density of *P. aeruginosa* ANSC was greatly retarded by higher Cr(VI) concentration. Therefore, the removal of hexavalent chromium from aqueous solution by *P. aeruginosa* ANSC is growth dependent.

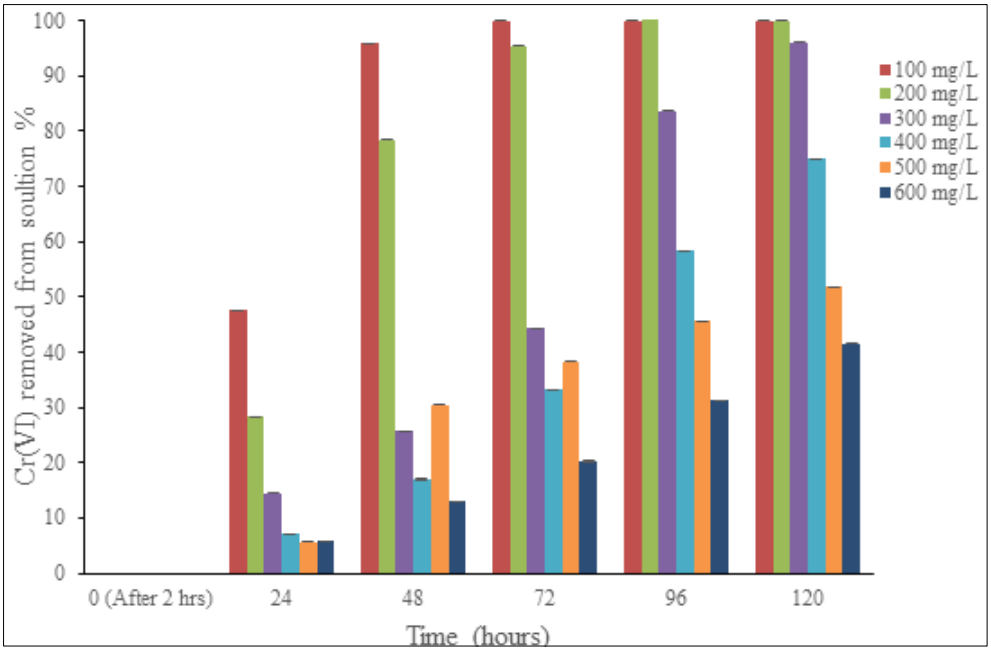

Figure 1. Removal of Cr(VI) as differing $K_2Cr_2O_7$ concentrations in aqueous solution by *P. aeruginosa ANSC.* Error bars denote standard error

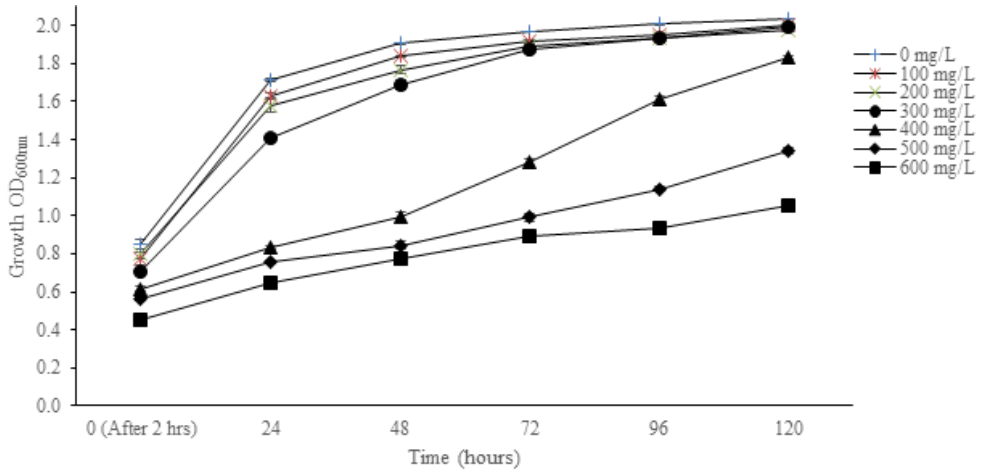

Figure 2. Growth of *P. aeruginosa ANSC* in the presence of different concentrations of Cr(VI). Error bars denote standard errors

3.3. Adsorption by Permeabilised Cells

Cells of *P. aeruginosa* ANSC were permeabilised using Triton X-100, tween 80, toluene, sodium dodecyl sulphate (SDS) and sodium deoxycholate (SDC). Comparisons of Cr(VI) removal/uptake by permeabilised cells of the isolate were made against resting cells (control) of the isolate under same Cr(VI) concentration and conditions. It was observed that cell permeabilisation increased Cr(VI) removal/uptake, as Triton X-100 permeabilised cells removed 47% of residual chromate, and tween 80 27%, toluene 37%, SDS 29% and SDC 31% (Figure 3) within 5 h. This suggests an efficient intracellular mechanism of chromate uptake by *P. aeruginosa* ANSC.

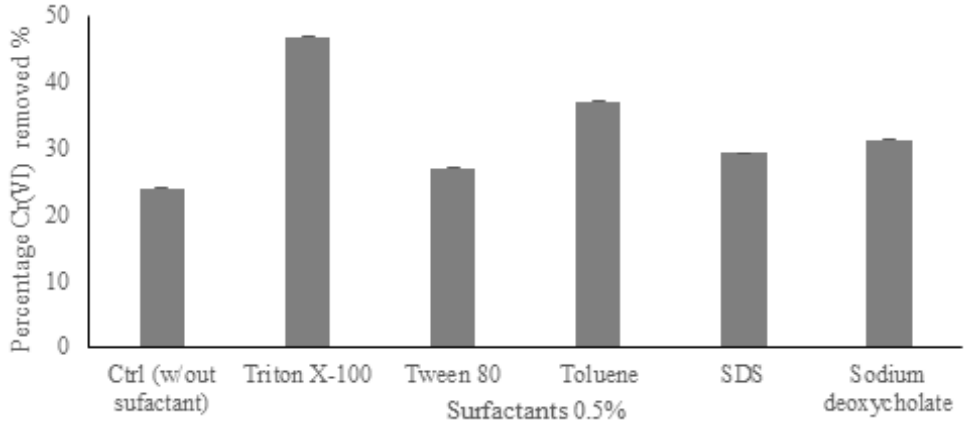

Figure 3. Permeabilized cell assays for Cr (VI) removal by *P. aeruginosa ANSC* at concentrations of 100mg/L of Cr (VI)

3.4. Adsorption Parameters

3.4.1. Effect of Contact Time

The adsorption time of hexavalent chromium on heat-killed biosorbents of *P. aeruginosa* ANSC was investigated in 2 h. As seen in Figure 4, inclined initial gradients for the adsorption curves are observed for temperatures of 60 and 50°C. Also, lesser heightened slopes for temperatures of 40 and 30°C are also observed. These indicate that the first uptake period of Cr(VI) is relatively rapid especially at higher temperatures of 50 and 60°C. This may be due to the fact that at the beginning of the sorption process all the reaction sites are vacant, and hence the extent of removal is high [13]. And, more reaction sites are made easily accessible to Cr(VI) with proportional increase in temperature. Further, after a rapid first uptake, there was a transitionary phase in which the rate of uptake of Cr(VI) was gradual with uptake reaching almost a steady range. Therefore, the adsorption of hexavalent chromium by *P. aeruginosa* ANSC was carried out in two discrete levels, a relatively rapid level followed by a gradual phase.

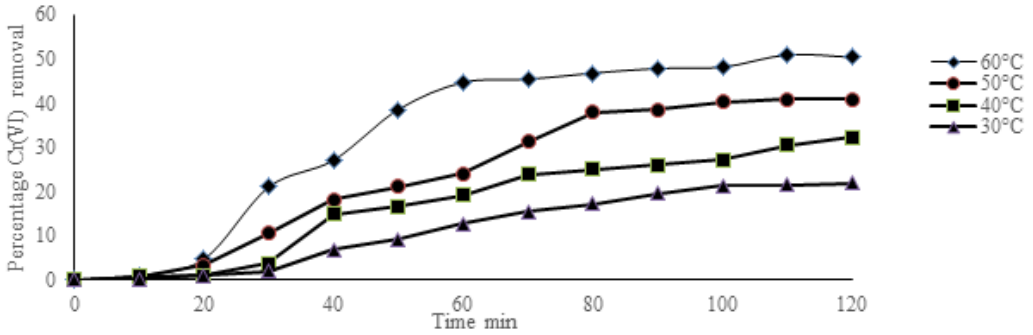

Figure 4. Adsorption time of hexavalent chromium onto dead biomass of *P. aeruginosa* ANSC. Adsorption conditions—initial concentration of Cr(VI): 100 mg/L; medium pH: 5.0; solution volume: 20mL; amount of adsorbent: 1 g/L; temperature: 30-60°C

3.4.2. Effect of pH

Adsorption is primarily influenced by the surface charge of the adsorbent and the degree of ionization of the adsorptive sites [14]. The effect of pH on the adsorption capacity of the heat-killed biomass of *P. aeruginosa* ANSC was studied using chromate under the pH range of 2.0-8.0. As seen in Figure 5, the removal efficiency of Cr(VI) improved with decreasing pH range, with pH 2 and 3 showing the most efficient pH levels for Cr(VI) uptake. The pH value of the solution played a key role in Cr(VI) removal. Expectedly, the ability of sorbing Cr(VI) is related to the acidic surface functional groups of the adsorbents. At higher pH values, the Cr(VI) removal ability of the adsorbents is restricted by increasing acidic surface functional, as a result of the weak interactions between the adsorbents' rich carboxyl group and the anionic dichromate ions. At lower pH values, however, the acidic surface functional groups almost have no effect on the Cr(VI) removal by the adsorbents from aqueous solution.

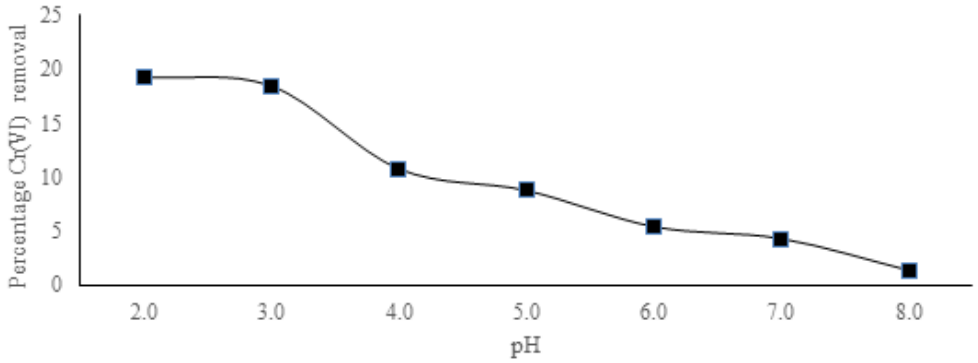

Figure 5. Effect of pH on the adsorption capacity of *P. aeruginosa* ANSC dried biomass for hexavalent chromium. Adsorption conditions—initial concentration: 100 µg/ml; temperature: 30 °C; solution volume: 20 mL; amount of biomass: 1.0 g/L; adsorption time:

3.4.3. Effect of Adsorbent Dosage

The reliance of hexavalent chromium adsorption on adsorbent dosage was investigated by varying the amount of adsorbents in the medium from 1.0 to 5.0 g while keeping other parameters such as initial concentration of Cr(VI) (100 mg/L), pH (2.0), temperature (30°C), stirring rate (150 rpm) and contact time (2 h) constant. The removal efficiency of the adsorbent was enhanced with increasing adsorbent dosage (as seen in Figure 6). Therefore, the higher the dosage of adsorbents in the adsorption medium, the plentiful the availability of sorption sites for the Cr(VI) ions. Overall, the percentage Cr(VI) removed by dosage increased from 21 to 42% with an increase in adsorbent weight of 1.0 to 5.0 g. As the adsorbent dose increased, surface area and available sites for Cr(VI) ions also increased. Therefore, better adsorption was recorded.

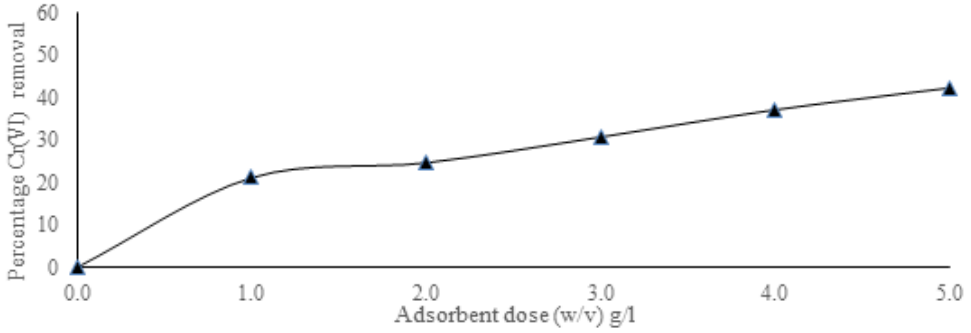

Figure 6. Effect of *P. aeruginosa* ANSC biomass dosage on hexavalent chromium adsorption. Adsorption conditions—initial concentration of Cr(VI): 100 mg/L; medium pH: 2.0; solution volume: 20 mL; amount of adsorbent: 1.0-5.0 g/L; temperature: 30 °C.

3.4.4. Effect of Initial Concentrations of Cr(VI) on the Adsorption Efficiency

The initial concentration of adsorbate provides a key influence to overcoming resistances of all mass transfer of the adsorbate molecules between aqueous and solid phases [13]. The concentrations selected for the study were 50, 75, 100, 125, and 150 mg/L. As seen in Figure 7, removal increases with decreasing initial concentration of Cr(VI). The data showed that as the initial concentration of residual chromate increased from 50 to 150 mg/L, the percentage adsorption decreased from 43 to 8%. This is indicative of the dependence of Cr(VI) removal on adsorbate concentration.

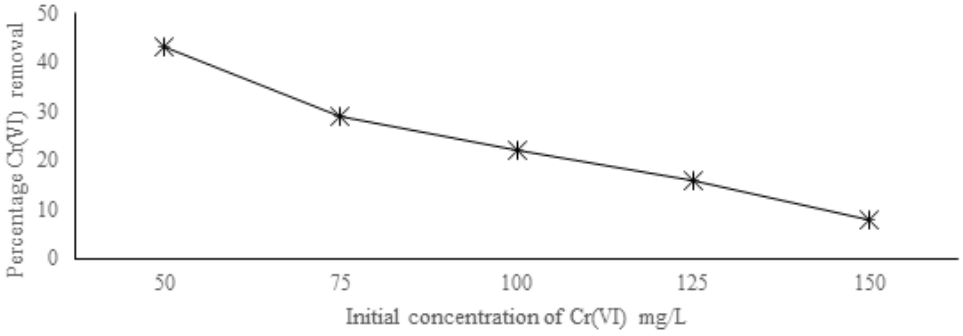

Figure 7. Effect of initial concentration of Cr(VI) on the adsorption capacity of *P. aeruginosa* ANSC dried biomass Adsorption conditions—initial concentration: 100 mg/L; temperature: 30 °C; pH 2.0; solution volume: 20 mL; amount of biomass: 1.0 g/L

3.5. Kinetic Parameters

The kinetics of adsorption are critical from the view point that they control process efficiency [13]. Therefore, kinetic models were used to test the experimental data in order to evaluate the controlling mechanism of the biosorption process. The kinetics of Cr(VI) adsorption onto dried biomass of *Pseudomonas aeruginosa* ANSC were determined with three different kinetic models i.e. Pseudo first-, second-order and intra-particle diffusion models. The first-order rate equation of Lagergren is one of the most widely used equations for the sorption of solute from a liquid solution [15, 16].

$$\log\left(\frac{qeq}{qeq - qt}\right) = k1.\frac{t}{2.303}$$

where k_1 is the rate constant of pseudo-first-order adsorption (min^{-1}), and q_{eq} and q_t denote the amount of adsorption at equilibrium and at time t (mg/g), respectively. The slopes and intercepts of plots of $\log(q_{eq}-q_t)$ versus t were used to determine the pseudo first-order rate constant k_1 and q_e.

Additionally, pseudo-second-order equation based on sorption equilibrium capacity may be expressed in this form [16]:

$$\frac{1}{qt} = \frac{1}{k2qeqt} + \frac{1}{qeq}$$

where k_2 (g/(mg.min)) is the rate constant of pseudo-second-order adsorption. The rate constant (k_2) and adsorption at equilibrium (q_{eq}) can be obtained from the intercept and slope, respectively. The pseudo-second order model is based on the assumption that the rate-determining step may be a chemical sorption involving valence forces through sharing or exchange of electrons between adsorbent and sorbate [16].

According to experimental and theoretical kinetic data in Table 2, adsorption of hexavalent chromium onto dried biomass of *P. aeruginosa* ANSC at optimum conditions of pH, contact time and dose of adsorbent were found to comply with the Pseudo-second order kinetic. Theoretical q_{eq} values parameterised from the first-order kinetic model gave significant different values compared to the experimental values. The results showed that the first-order kinetic model did not fit the sorption system. However, in the case of the second-order kinetic, the theoretical q_{eq} values for the dried biomass of *P. aeruginosa* ANSC were in close proximity to the experimental q_{eq} values. The correlation coefficients for the linear plots of $1/q_t$ against $1/t$ for the second-order equation are greater than 0.4. The pseudo-second-order equation at different temperature levels relatively fitted well with the experimental data for hexavalent chromium (Table 2).

To assess the extent of the diffusion process for adsorption of hexavalent chromium onto dried biomass of strain *P. aeruginosa* ANSC, evaluations were made to ascertain the diffusion coefficients. The intra-particle diffusion model was proposed by Weber and Morris [17]. By linearisation of the curve $q = f(t^{0.5})$, the initial rate of intra-particle diffusion is calculated as:

$$q = k_{id}.t^{0.5}$$

where q (mg/g) is the amount of adsorbed Cr(VI) on the dried biomass of *P. aeruginosa* ANSC at time t (min), and k_{id} is the diffusion coefficient in the adsorbent ($mg/g.min^{0.5}$). k_{id} has been determined by a plot $q = f(t^{0.5})$.

It is assumed that Cr(VI) ions are transported into the cell of the adsorbent by intra-particle transport mechanism when the Cr(VI) sample solution is stirred. The intra-particle transport through this mechanism is meant to be the rate-controlling step. However, it can be said that the amount of adsorbed Cr(VI) species varies proportionately with the retention time of the adsorbent. The results indicated that the representation in Figure 8 was not linear over the whole-time range for all plots. Although the representations showed similar general contours (i.e. an initial linear portion followed by a climax). The initial linear portion can be attributed to the intra-particle diffusion. However, such a digression of the straight line from the origin could likely be due to the difference in the rate of boundary layer diffusion in the initial stage of adsorption [13]. The intercept of the plot of q_t *vs*. $t^{0.5}$ generally provides an insight about thickness of boundary layer, as the larger the value of the intercept, the greater the boundary layer diffusion effect is, and vice versa [13]. The values of intra-particle diffusion rate constant, k_{id}, are presented in Table 2. These results reveal that the Cr(VI) ions diffused steadily and slowly among the adsorbents at the onset of the adsorption process, and then intra-particle diffusion decreased steadily and stabilised eventually. If the regression of q *vs*. $t^{0.5}$ is linear and crosses through the point of origin, then intra-particle diffusion is the singular rate-limiting step. However, the digression of the straight lines from the point of origin indicates that intra-particle transport is not the rate-limiting step.

Table 2. Pseudo-first- and second-order kinetics and intra-particle diffusion models for adsorption of Cr(VI) onto *P. aeruginosa* ANSC

Metal	Temp (K)	q_{eq} exp (mg/g)	Pseudo-first order		Pseudo-second order			Intra-particle diffusion	
			K_1 (min^{-1})	q_{eq} (mg/g)	R^2	K_2 (g/(mg·min)	q_{eq} (mg/g)	R^2	K_{id} mg/(g.$min^{0.5}$)
Cr(VI)	333	0.0294	-37.671	-1.278	0.2764	2.524	0.0219	0.916	0.00350
	323	0.0246	-37.989	-1.355	0.333	-8.085	0.0198	0.939	0.00289
	313	0.0201	-89.770	-1.605	0.2764	4.271	0.0123	0.934	0.00217
	303	0.0138	-77.088	-1.754	0.4284	-12.866	0.0122	0.938	0.00161

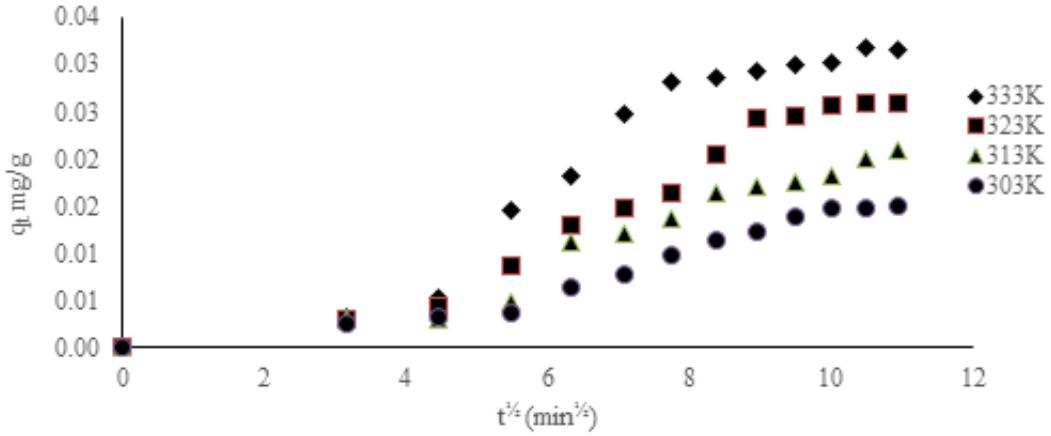

Figure 8. Intra-particle diffusion plot for the adsorption of Cr(VI) at 333, 323, 313, and 303K

3.6. Adsorption Isotherms

Adsorption isotherms are equilibria models used to evaluate the affinity of studied adsorbent (dried biomass of *P. aeruginosa* ANSC) for the removal of Cr(VI) from aqueous solution. In this present study, two (2) isotherm models (*viz*. Langmuir and Freundlich models) were used. The Langmuir isotherm model assumes that a monolayer of adsorbate is adsorbed over a uniform adsorbent surface. Hence,

$$q = \frac{qm.Kl.Ce}{1 + Kl.Ce}$$

The constant K_L is related to the energy of adsorption; C_e is the equilibrium concentration of the Cr(VI) in the solution; q is the amount of adsorbed Cr(VI) on the adsorbent surface; and the constant q_m represents the maximum binding at the complete saturation of adsorbent binding sites. The essential features of a Langmuir isotherm can be expressed in terms of a dimensionless constant, separation factor or equilibrium parameter, R_L, which is used to predict if an adsorption system is "favourable" or "unfavourable" [7].

$$Rl = \frac{1}{1 + Kl.Co}$$

The value of R_L indicates the shape of the isotherm to be either unfavourable ($R_L > 1$) or linear ($R_L = 1$) or favourable ($0 < R_L < 1$) or irreversible ($R_L = 0$) [where C_o (mg/L) is the initial Cr(VI) concentration].

Freundlich isotherm model, an empirical equation used to describe the adsorption of organic and inorganic compounds on a wide variety of adsorbents, takes into account the fitting of experimental adsorption data from heterogeneous sorbent systems; hence,

$$\log qe = \log Kf + \frac{1}{n}\log Ce$$

where K_F (L/mg) is an indicator of the multilayer adsorption capacity and $\frac{1}{n}$ is the adsorption intensity, and both indicate the relative distribution of energy and the heterogeneity of the adsorbent sites, respectively [18].

The results showed that the Langmuir model was most suitable for describing the adsorption of hexavalent chromium onto the dried biomass of *P. aeruginosa* ANSC at elevated temperatures of 323 and 333 K (Table 3). Figures 9 and 10 show the respective fit of both Langmuir and Freundlich models for the adsorption of Cr(VI) onto *P. aeruginosa* ANSC at 30 to 60 °C. The relative high values of R^2 in the range of 0.883 to 0.968 indicate minimal deviation from the fitted equation. Also, there exist close proximity of values of modeled q_m with experimental q_m. Further, the fact that all R_L values for the adsorption of hexavalent chromium onto dried biomass of *P. aeruginosa* ANSC are approximately unity at 303 to 333 K for 100 µg/mL initial Cr(VI) concentration is a confirmation that the bacterial biosorbent is favourable for adsorption of hexavalent chromium under the studied conditions. Freundlich isotherm model, on the other hand, produced adsorption intensity of greater than unity across studied temperature.

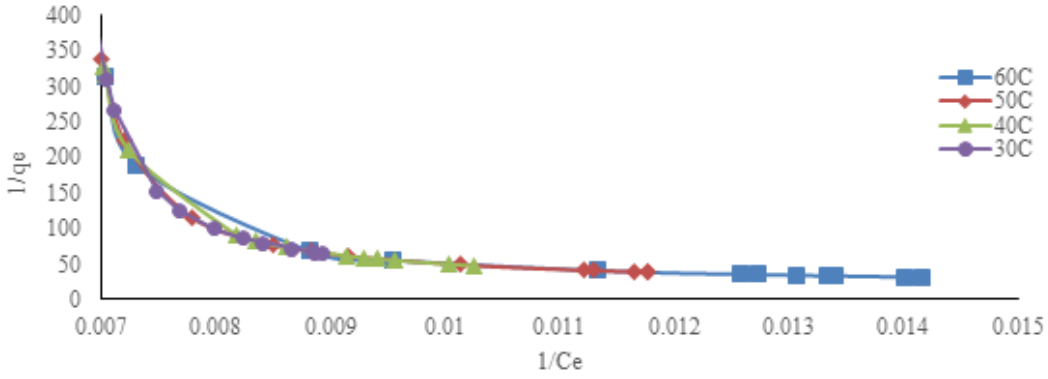

Figure 9. Langmuir adsorption isotherm for chromium (VI)–*P. aeruginosa* ANSC dried biomass system: temperature = 30-60°C; pH 2; biomass = 1 g; time = 2 h

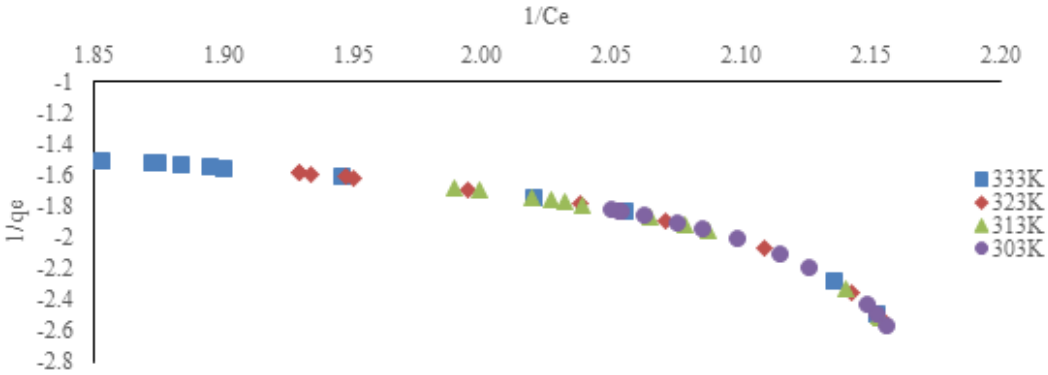

Figure 10. Freundlich adsorption isotherm for chromium (VI)–*P. aeruginosa* ANSC dried biomass system: temperature = 30-60°C; pH 2; biomass = 1 g; time = 2 h

Table 3. Isotherm model's constants, correlation coefficients and thermodynamics for adsorption of Cr(VI)

Metal	Temp (K)	q_{exp} (mg/g)	Freundlich			Langmuir			Thermodynamics		
			1/n (L/g)	K_F *10^4 (mg/g)	R^2	K_L *10^3 (L/mg)	q_m *10^3 (mg/g)	R^2	ΔG° (kJ/mol)	ΔH° (kj/mol)	ΔS° (j/k/mol)
Cr(VI)	333	0.0294	-2.86	0.75	0.988	0.0367	2.59	0.883	-28.27	5.43	-6.12
	323	0.0246	-3.52	18.97	0.989	0.0248	2.06	0.896	-28.47		
	313	0.0201	-5.10	37790	0.930	0.0123	1.21	0.956	-29.41		
	303	0.0138	-6.59	55118215	0.947	0.0077	0.84	0.968	-29.65		

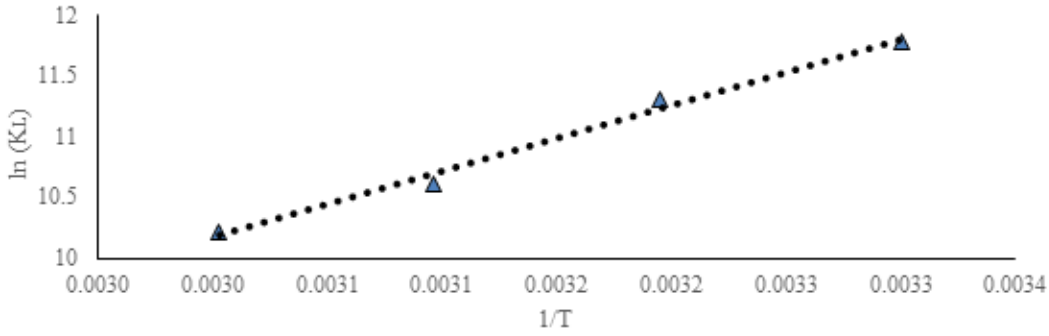

Figure 11. Thermodynamic study of adsorption of Cr(VI) onto *P. aeruginosa* ANSC biomass

3.5. Thermodynamic Parameters

It has been evidenced that the adsorption capacity of the bacterial biosorbent increases with increasing temperature levels; thereby, indicating an endothermic adsorption process. Therefore, in order to assess the feasibility and efficiency of effect of temperature for hexavalent chromium adsorption onto the bacterial biosorbent, thermodynamic parameters of standard Gibbs free energy (Δ*G*°), enthalpy (Δ*H*°), and entropy (Δ*S*°) changes needed

to be derived. The Gibbs free energy change of the adsorption process was calculated using the following equation:

$$\Delta G^{\circ} = -RT \ln Kl$$

where K_L is the dependency of the equilibrium association constant from Langmuir isotherm models; T, solution's absolute temperature, and R, universal gas constant (8.314 kJ/mol.K). Applying the above equation resulted in negative values of ΔG°; thereby indicating the spontaneity of adsorption process under the conditions studied (Table 3). Further, the changes with temperature of the free energy change and the equilibrium constant were represented as follows;

$$\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ}$$

where values of ΔH° and ΔS° can be calculated from the slope and intercept of the plot between ln K_L versus $\frac{1}{T}$ [7] (Figure 11). The positive value of ΔH° reveals the adsorption is endothermic and physical in nature (Table 3). However, the negative ΔS° value of Cr(VI) adsorption process onto *P. aeruginosa* ANSC indicates a rather regular increase of randomness at the biosorbent-solution interface during adsorption.

Therefore, in this study, *Pseudomonas aeruginosa* ANSC has been evidenced to reduce hexavalent chromium using its free cells, and Cr(VI) reduction by the strain is growth-associated. Also, efficiency for adsorption of hexavalent chromium in aqueous solution by the strain has been enhanced by cell permeabilisation. The adsorption process for hexavalent chromium has been better described by the pseudo-second order kinetic and Langmuir isotherm model. The sorption system was also found to be endothermic and spontaneous.

4. Conflicts of Interest

The authors hereby declare no conflict of interest as it regards the publication of this study.

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