

BIOSORPTION OF CHROMIUM (VI) USING IMMOBILIZED *Bacillus subtilis* AND *Pseudomonas aeruginosa*

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Abstract

Heavy metals like chromium do contaminate the environment that comprises of soil, water and air. It affects the growth of flora and fauna which in turn affect human health negatively. Chromium could also bio-accumulate in plants and animals and this becomes dangerous for survival of human if adequate steps are not taken for treatment of industrial and agricultural wastes. Therefore, the batch removal of Cr (VI) from environment water bodies becomes necessary. Its removal from aqueous solution using immobilized *Bacillus subtilis* (IBBS), *Pseudomonas aeruginosa* (IPBS), mixed biomass (IMBS) and Alginate alone (IABS) was carried out. The conditions of influence of initial Cr (VI) concentrations, solution pH, contact time, biomass dosage and temperature were studied. The sorption kinetic models of Cr (VI) onto the biosorbents were examined with pseudo first-order, pseudo second-order, and Elovich kinetics respectively. It was found that the experimental conditions affected the extent of removal of Cr (VI) from aqueous solution. The higher the initial concentration, the larger the amount of Cr (VI) removed while the higher the temperature the lesser the amount removed. The optimum contact time and adsorbent dose for effective removal of Cr (VI) from aqueous solution were found to be 60 mins and 0.01 g respectively. Pseudo second-order kinetic model best correlates the experimental data. Among isotherm models studied, Freundlich adsorption isotherm model gave the best fit.

Key Words: Chromium (VI), Adsorption Kinetics, Adsorption Isotherms, Spectrophotometric Determination, Immobilized Biosorbents

Introduction

Over the years, heavy metals pollution on water bodies has become one of the most serious environmental problems. The presence of some heavy metals even in traces is toxic and detrimental to both plants and animals. Metals may accumulate to toxic level and cause ecological damage (Jefferies and Firestone, 1984). Metals are carried into food web as a result of leaching from waste dumps, polluted soils and water. The toxicity of metal ion is owing to their

ability to bind with protein molecules and prevent replication of DNA and thus subsequent cell division (Kar *et al.*, 2003). Cr (VI) is toxic because it diffuses through the epidermis. Thus the removal is of great importance to human.

Techniques currently in existence for removal of Cr (VI) from contaminated waters include: reverse osmosis, electro dialysis, ultrafiltration, ion-exchange, chemical precipitation and so on. However, these techniques are expensive, not environmentally friendly

while the use of microorganisms with adsorption principle offers a cost-effective and environmental friendly approach (Qazilbash *et al.*, 2006; Adebayo *et al.*, 2010).

Immobilization of the biomass improves the mechanical strength of the biomass, and reduces resistance to fluid flow. This work therefore investigated the influence of experimental conditions, equilibrium and Kinetics on removal of Cr (VI) from aqueous solution using immobilized *B. subtilis* and *P. aeruginosa*.

Materials and Method

Biosorbent Preparation

B. subtilis and *P. aeruginosa* strains were collected from culture centre of the Department of Microbiology, University of Ilorin, Nigeria. They were grown and maintained on both nutrient broth and nutrient agar. They were cultivated at 29 °C in medium containing: 20 gL⁻¹ soluble starch; 10 gL⁻¹ beef extract; 2 gL⁻¹ yeast extract; 5 gL⁻¹ peptone; 5 gL⁻¹ NaCl. (pH adjusted to 7.2). After a 72 h of incubation at 27 °C, biomass were harvested by centrifugation at 10,000 rpm for 10 minutes, washed twice with deionized water and then dried for 24 h at 70 °C in an oven. The dried biomass was then crushed to a fine powder and stored in an air tight pack (Sivaprakash, *et al.*, 2009).

Biomass Immobilization

A 2% (w/v) sodium alginate solution was prepared under constant agitation to form slurry which was allowed to stand for 20 mins to eliminate any trapped air. A range of 0.01 – 0.07 g quantities of biomass cultures were added under agitation conditions to obtain a uniform mixture. The calcium alginate-bacteria biosorbent beads were prepared by injecting in drops 4 ml of 2% slurry into 2% w/v CaCl₂ solution. The beads were allowed to cure for 2 h at 4 °C and

washed repeatedly with deionised water to remove excess calcium ions (Mahamadi and Zambara, 2012).

Biosorbent Characterization

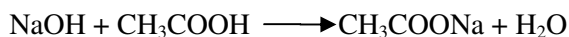
Each of the biosorbents was subjected to triplicate physico-chemical characterization:

(i) A 2.5 g of each biosorbents was weighed separately and dissolved in 50 ml of deionized water. The pH and conductivity of each filtrate as well as bulk density, moisture and ash contents of each adsorbent were determined using standard methods (Okeimen *et al.*, 2004; Gutha *et al.*, 2012).

(ii) Point of Zero Charge (PZC) was determined by mixing 3.0 ml of 0.1 M KNO₃ solution and 6.0 ml of deionized water and placed in each of two 20 ml beakers. The pH of the two solutions was measured. A 1.0 ml of 0.01M KOH solution was added to one beaker (blank) and to another beaker (sample); and their pH was measured. A 0.05 g of each biosorbents was added to the sample container and its pH was also measured. The blank and the sample were titrated with 0.01M HNO₃ solution. The graph of pH versus volume of HNO₃ was plotted on the same graph for both blank and sample and the pH point where the two curves intersected was taken as the point of zero charge (Vakros *et al.*, 2002).

(iii) Cation exchange capacity (CEC) was determined by addition of 50 ml of 0.5 M HCl solution to 0.6 g of each of the samples and shaken rigorously. The mixture was then filtered and the residue washed with deionized water until the filtrate was free from acid and chloride ions. The moistened sample was then transferred into separate 100 ml flask and was shaken at room temperature with 50 ml of 0.25 M ammonium acetate solution. It was then vacuumed filtered and washed with distilled water until filtrate (CH₃COOH) obtained was made up to 200 ml mark. It was titrated with

0.1 M NaOH solution using phenolphthalein as indicator.



Blank determinations of the Cr (VI) were also done (Chapman, 1995) by carrying out similar experiments on distilled water without using the collected sample water. CEC was then calculated from their titre values.

(iv) Iodine numbers of biosorbent types were determined by the modified AWWA procedure (AWWA, 1991). The modification adopted was 5 minutes centrifugation of each biosorbent –iodine mixtures before titration with sodium thiosulphate solution using starch indicator. Methylene blue (MB) adsorption capacities of biosorbent were measured (ASTM, 1989a) while surface area, pore volumes and pore size distribution of the biosorbent types were measured by N₂ adsorption at 77K using the Brunauer –Emmett – Teller (BET) method. A Perkin Elmer 1600 Series FTIR Spectrophotometer Model 1615 was also used in the evaluation of functional groups present on the biosorbent types after crushing with KBr pellet.

Stock solution preparation

A 1000 mg/L of Cr (VI) solution was prepared by dissolving 2.829 g of K₂Cr₂O₇ (potassium dichromate) crystals in 50 ml of deionized water in a 250 ml beaker and made up to 1 L standard flask mark with deionized water. Other concentrations were obtained from the stock by serial dilution.

Biosorption experiments

Factors affecting biosorption

Adsorption experiments were conducted at various pH, contact time, temperature, and initial Cr (VI) concentration in 100 mL conical flasks using 0.01g of the adsorbents and total volume of the each reaction mixture was

30 ml. The pH of solution was maintained at a desired value by adding 0.1 M NaOH or 0.1M HNO₃.

Time-dependence studies

The kinetics study was carried out with 30 ml volumes of 30 mg/L initial Cr (VI) ion concentration, 0.01g adsorbent dose and pH 2. The mixture were agitated at 140 rpm and 30 °C using various contact time (2-120 min). At pre-determined time, the flasks were withdrawn from the shaker and the reaction mixtures were centrifuged.

Temperature-dependence and isotherm studies

The experiments were performed at 30 to 55 °C while adsorption isotherm study was performed using various concentrations of Cr (VI) solutions (5 – 35 mg/L).

Analysis of Chromium (VI) ion

The filtrates were analyzed for residual metal concentrations using UV-Visible spectrophotometer (Beckman Coulter DU 730). The sorption capacity, q_e for each of the factors was then determined using the equation:

$$q_e = \frac{(C_o - C_e)V}{m} \quad (1)$$

where C_o is initial metal ion concentration, C_e is equilibrium metal ion concentration, V is in L and m is the mass of bio-sorbent.

A 0.25 % w/v solution of 1, 5-diphenylcarbazide was prepared in 50 % acetone. A 15 ml each of the sample solutions containing (5-35 mg/l) concentrations of Cr (VI) was then pipetted out into 25 ml standard flasks. A 2 ml of 3 M H₂SO₄ was added followed by 1 ml of 1, 5-diphenylcarbazide and the total volume was made up to 25 ml using deionised water. Maximum absorbance was recorded at 540 nm using a UV-visible spectrophotometer (Beckman Coulter DU 730). A standard calibration curve was then drawn in the range of 5-35 mg/L by plotting absorbance against

concentration of chromium (VI) (Sivaprakash *et al.*, 2009). By extrapolation, other concentration values were determined.

Results and Discussion

Physicochemical characterization of biosorbents

The pH of all the biosorbents fell within similar range of 6.8 – 7.0 which indicates that they are slightly acidic or neutral while their PZC are less than their pH value. Their conductivity values are low (0.86 – 1.63 mS/cm). The bulk density and CEC of IPBS was highest while that of AIBS was the lowest (Table 1).

However, the prepared biosorbent densities were all found to be higher than 0.25 g/mL minimum bulk density requirement for their application as good adsorbent for treatment of municipal and waste water (AWWA, 1991). Moisture content and ash content of prepared biosorbents were high and are within range of 12.39 – 14.60 % and 9.3 – 12.5 % respectively (Petrov *et al.*, 2000). Moreover, the Iodine numbers values of 370.9, 381.2 and 396.3 mg/g are similar to values {318 – 419 mg/g} reported for active carbons of furfural treated with H₂SO₄, activated in N₂ flow at 600 °C and oxidized in air within range of 200 – 400 °C (Petrov *et al.*, 2000).

Table 1: Physicochemical properties of immobilized bacteria biosorbents

| Parameters | IBBS | IMBS | IPBS | IABS |
|--|----------------|----------------|----------------|----------------|
| pH | 6.8 ± 0.055 | 6.8 ± 0.057 | 7.0 ± 0.064 | 7.1 ± 0.601 |
| PZC | 6.3 ± 0.72 | 6.2 ± 0.54 | 6.3 ± 0.60 | 6.6 ± 1.12 |
| Conductivity (mS/cm) | 1.43 ± 0.027 | 0.86 ± 0.045 | 1.03 ± 0.004 | 1.63 ± 0.031 |
| Bulk density (g/ml) | 0.331 ± 0.002 | 0.376 ± 0.001 | 0.457 ± 0.016 | 0.359 ± 0.032 |
| CEC X 10 ⁻³ (mol/g H ⁺) | 3.27 ± 0.12 | 3.36 ± 0.15 | 3.77 ± 0.12 | 2.26 ± 0.06 |
| Moisture content (%) | 12.40 ± 2.53 | 14.55 ± 2.76 | 14.61 ± 4.14 | 14.52 ± 1.53 |
| Ash content (%) | 10.0 ± 1.32 | 9.3 ± 0.76 | 12.5 ± 0.87 | 10.0 ± 1.32 |
| Iodine number (mg/g) | 370.89 ± 8.094 | 381.23 ± 4.957 | 396.27 ± 5.783 | 269.36 ± 2.650 |
| Methylene blue number (mg/g) | 113.50 ± 2.50 | 241.74 ± 4.93 | 446.28 ± 8.22 | 411.30 ± 7.25 |

(IBBS), (IPBS), (IMBS) and (IABS) represent immobilized *Bacillus subtilis*, *Pseudomonas aeruginosa*, mixed biomass and Alginate alone respectively.

Structural characterization of biosorbents

A BET surface area range of 0.026 – 10.680 m²/g and pore volume range (0.0003 – 0.011 cm³/g) obtained for the biosorbent types are low. These values are not within the minimum range of 500 – 1500 m²/g needed for industrial application and removal of small molecules from aqueous solution (Schwarzenbach *et al.*, 2003). Their pore

size distributions were found to be 5.47, 27.76 and 103.11 nm for IABS, IBBS and IPBS biosorbents respectively; and could be classified as mesoporous for IABS and IBBS while IPBS is macroporous that contribute nothing to adsorption process. However, The presence of broad bands at 3261.6, 3245.0, and 3260.25 cm⁻¹ depicts OH stretching vibrations for IABS, IBBS and IPBS biosorbents respectively (Figures 1

A-C). The peaks at 1085, 1082 and 1081 cm^{-1} correspond to C-O stretching of IABS, IBBS and IPBS respectively. The bands at 1559, 1583 cm^{-1} depicted presence of C=C stretch vibrations while C – O stretch band observed within the range of 1360 – 1050 cm^{-1} indicated simple ethers, alcohols and acid

anhydrides (Petrov *et al.*, 2000; Olivares-Marín *et al.*, 2006). These functional groups might be more responsible for removal of the chromium metal than through pores or available surface area of the biosorbents (Suguna and Kumar, 2013).

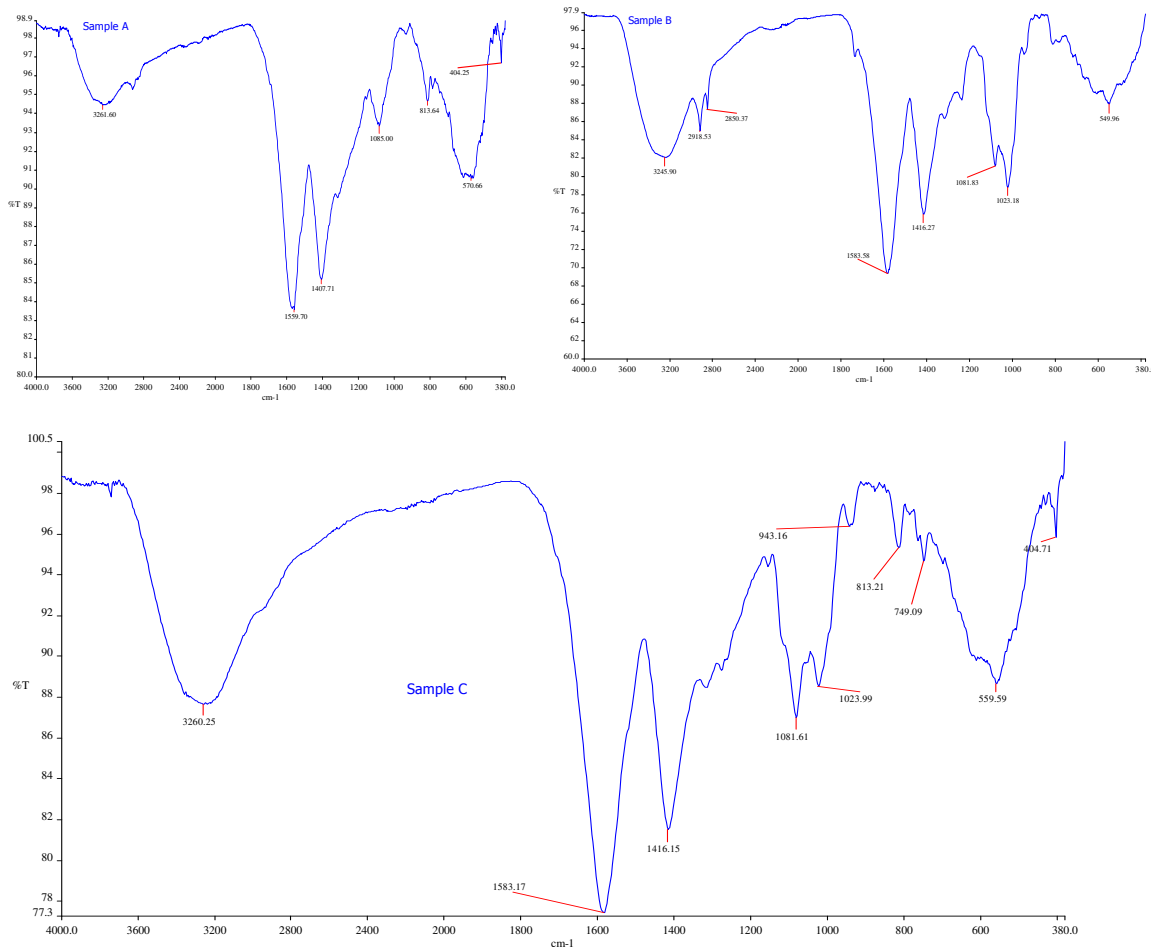


Figure 1 (A – C): FT IR spectra of % transmittance versus wave numbers cm^{-1} of IABS, IBBS and IPBS respectively

Effect of experimental conditions

Effect of initial Cr (VI) ions concentrations

The effect of initial Cr (VI) ions concentrations on uptake capacity Q_e (mg/g) for biosorbents can be seen that increase in uptake capacity with increase

in concentration was obtained. This is due to higher availability of Cr (VI) ions in the solution for biosorption and the affinity it has for the bio-sorbent (Figure 2). A corresponding increase in Cr (VI) concentration from 6.03 to 36.53 mg/L was observed. It is clear that beyond the

equilibrium concentration, there is decrease in uptake capacity which could be attributed to the saturation of the active sites available on the biosorbents. The uptake capacity increased from 6.27 to 31.14 mg/g for IBBS, 5.55 to 30.66 mg/g for IMBS, 6.03 to 25.11 mg/g for IPBS and 3.12 to 19.80 mg/g for IABS while the equilibrium concentration was achieved at 30.49 mg/L.

Effect of contact time on Cr (VI) ions biosorption

The time profile diagram for the IBBS, IMBS, IPBS and IABS are shown in Fig. 3. It can be seen that biosorption of Cr (VI) by IBBS, IMBS and IPBS show a rapid initial adsorption at 2 mins after which a decrease in the adsorption was observed within range of 5 – 10 mins with uptake capacity ranges of 19.56 - 16.47 mg/g, 16.44 - 15.93 mg/g and 17.88 - 17.16 mg/g respectively.

Equilibrium time was attained at 60 mins which corresponded to the uptake

capacity of 41.61, 38.64, 37.89 and 32.10 mg/g for IBBS, IMBS, IPBS and IABS respectively. This could be attributed to the fact that uptake of the Cr (VI) ions was at the surface of the beads which explains the rapid initial adsorptions observed at 2 mins while the second phase represents the diffusion of the Cr (VI) ions into the beads which needs more time because of the high mass transfer resistance (Wu *et al.*, 2004; Soni and Gupta 2011).

Effect of biosorbent dose on Cr (VI) ions biosorption

The effect of biosorbent dose range of (0.01 – 0.07 g) on uptake capacity of Cr (VI) ions using IBBS, IMBS, IPBS and IABS biosorbents showed that uptake capacity increased with decrease in the biosorbent dose (Fig.4). Maximum uptake capacity was achieved at the biosorbent dose of 0.01g for each of the biosorbents.

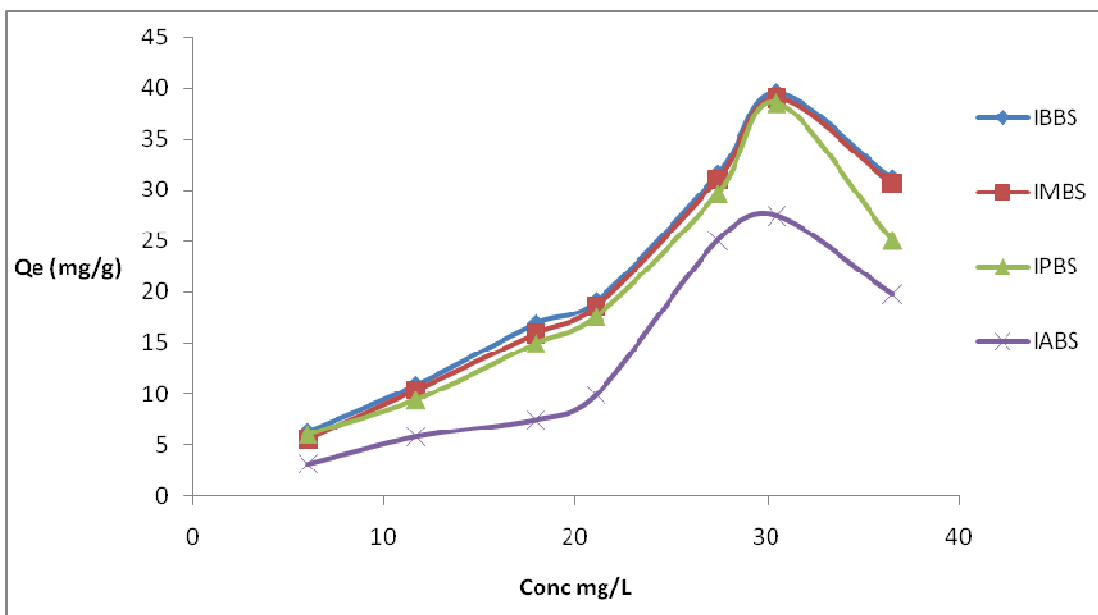


Figure 2: Effect of initial concentration on uptake Cr (VI) ions onto various adsorbents

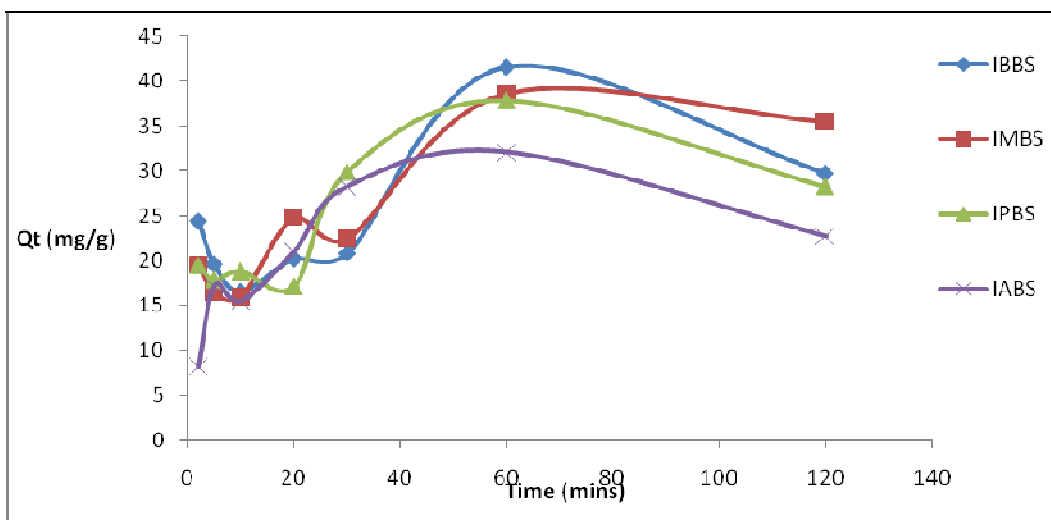


Figure 3: Effect of time on uptake of Cr (VI) ions onto various biosorbents

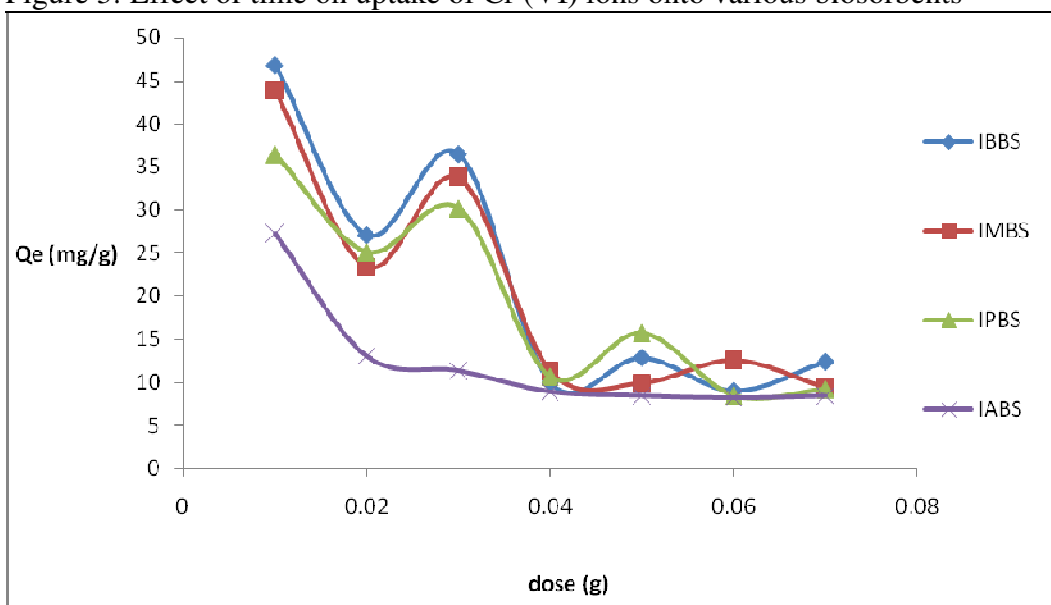


Figure 4: Effect of biosorbent dose on uptake Cr (VI) ions onto various biosorbents

When the biosorbent dose increased from 0.01 to 0.07 g, the removal capacities of the biosorbents decreased from 46.80 to 12.30 mg/g, 43.92 to 9.39 mg/g, 36.45 to 9.15 mg/g and 27.27 to 8.43 mg/g for IBBS, IMBS, IPBS and IABS respectively.

The decrease in Cr (VI) uptake with increasing biosorbents dosages may be as a result of available Cr (VI) ions that are insufficient to cover all the exchangeable sites on the biosorbents surface. It could also be attributed to aggregation of sorbent particles at higher concentrations,

which would lead to decrease in the surface area and an increase in the diffusion path length (Shukla *et al.*, 2002). Similar result was reported when the uptake capacity of Cr (VI) was decreased from 15.57 to 13.48 mg/g when *B. subtilis* biosorbent dose was increased from 1.0 to 3.0 g/L (Sivaprakash *et al.*, 2009).

Effect of pH on biosorption of Cr (VI) ion

The value of pH_{ZPC} for IBBS, IMBS, IPBS and IABS were determined to be 6.3, 6.2, 6.3 and 6.6 respectively (Table 1). It has been reported that sorption of

cations is favoured at $pH > pH_{PZC}$, while the sorption of anions is favoured at $pH < pH_{PZC}$. The specific adsorption of cations shifts pH_{PZC} towards lower values, whereas the specific uptake of anions shifts pH_{PZC} towards higher values (Mall *et al.*, 2006).

It can be observed that the highest uptake capacity for Cr (VI) was obtained at pH 2 which corresponded to uptake capacity of 34.02 mg/g, 33.30 mg/g, 32.82 mg/g and 22.44 mg/g for each of IBBS, IMBS, IPBS and IABS biosorbents respectively. There was a decrease in the amount of Cr

(VI) adsorbed as the pH increased (Fig 5). This can be attributed to the fact that Cr (VI) exists as oxyanions (CrO_4^{2-} , $Cr_2O_7^{2-}$, $HCrO_4^-$) in aqueous solution of which $HCrO_4^-$ and $Cr_2O_7^{2-}$ predominate at pH less than 6 (Soni and Gupta, 2011). At low pH values the surface of the adsorbent is saturated with protons thereby making the surface of the biosorbent to be positively charged, which enhanced the adsorption of the negatively charged Cr oxyanions onto the surface of the bio-sorbent (Gupta and Babu, 2009; Ilesanmi *et al.*, 2013).

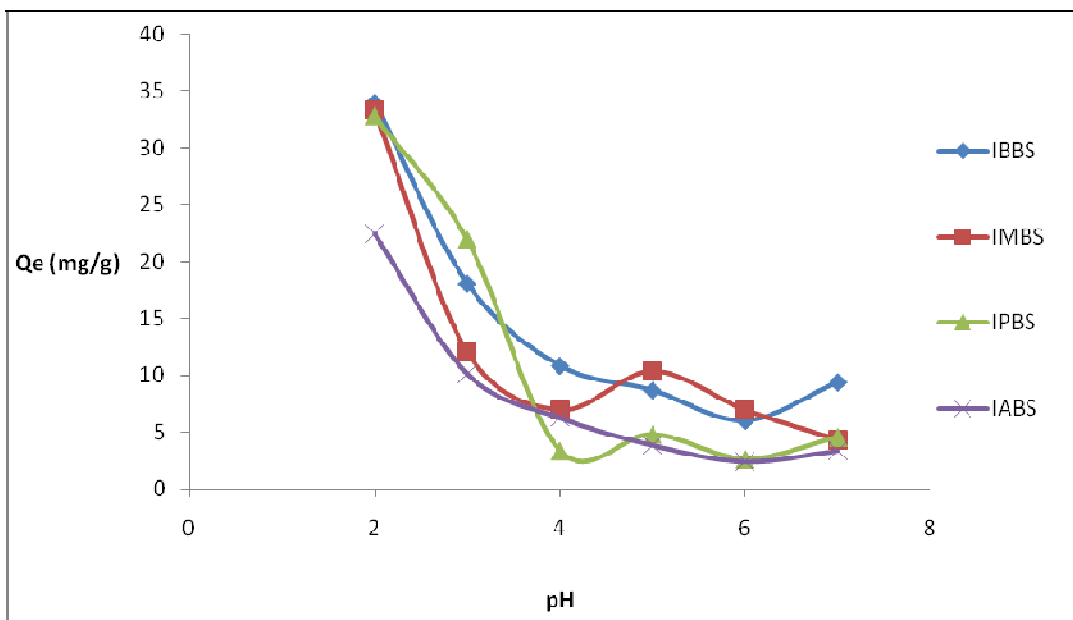


Figure 5: Effect of pH plot on uptake Cr (VI) ions onto various biosorbents

Effect of temperature on Cr (VI) ions biosorption

As temperature increased from 305 to 328 K, it can be observed that the uptake capacity of the various sorption systems decreased from 38.85 to 27.27 mg/g, 44.88 to 29.43 mg/g, 41.76 to 30.18 mg/g and 17.85 to 6.99 mg/g for IBBS, IMBS, IPBS and IABS biosorbents respectively indicating physical adsorption process [Fig. 6(a – d)] (Sag and Kutsal, 1996).

Adsorption Isotherms study

The analysis of the isotherm data by fitting them into different adsorption

isotherm models is an important step in finding a suitable model that can be used for design purpose.

Freundlich isotherm

The Freundlich isotherm equation is given as:

$$\log q_e = \log k_f + \frac{1}{n} \log C_e \quad (2)$$

where q_e is the amount adsorbed at equilibrium in (mg/g), C_e is the equilibrium residual Cr (VI) concentration. A plot of $\log q_e$ against $\log C_e$ gave a straight line which indicated the conformation of the experimental data

to the Freundlich adsorption isotherm [Figs. 7(a – d)] (Schwarzenbach *et al.*, 2003). The biosorption processes for all the biosorbents are favourable since values of $1/n$ (sorption intensity) obtained fell below 1 and their correlation coefficient, R^2 is within range of 0.96 – 0.99. Their Freundlich biosorption

capacity, K_f fell within range of 1.461 – 2.033 mg/g (Table 2). The Cr (VI) ions sorption isotherm data fitted into the Freundlich model better than the other two models. This is reflected in the values of their correlation coefficients R^2 , obtained for each isotherm (Table 2).

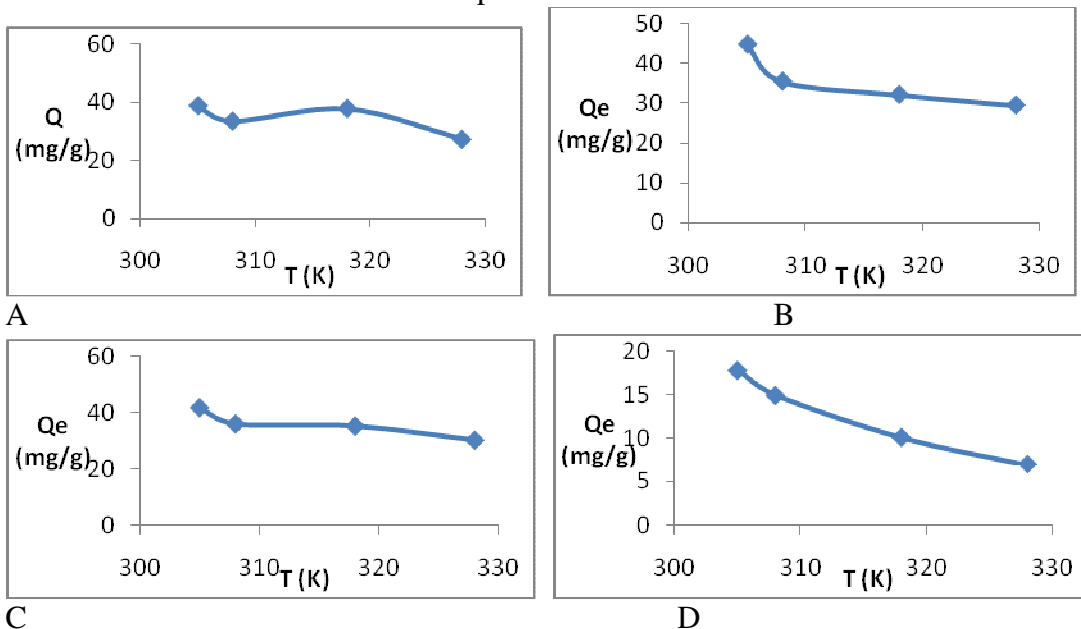


Fig. 6 (a-d): Effect of temperature on adsorption capacity of IBBS, IMBS, IPBS and IABS adsorbents

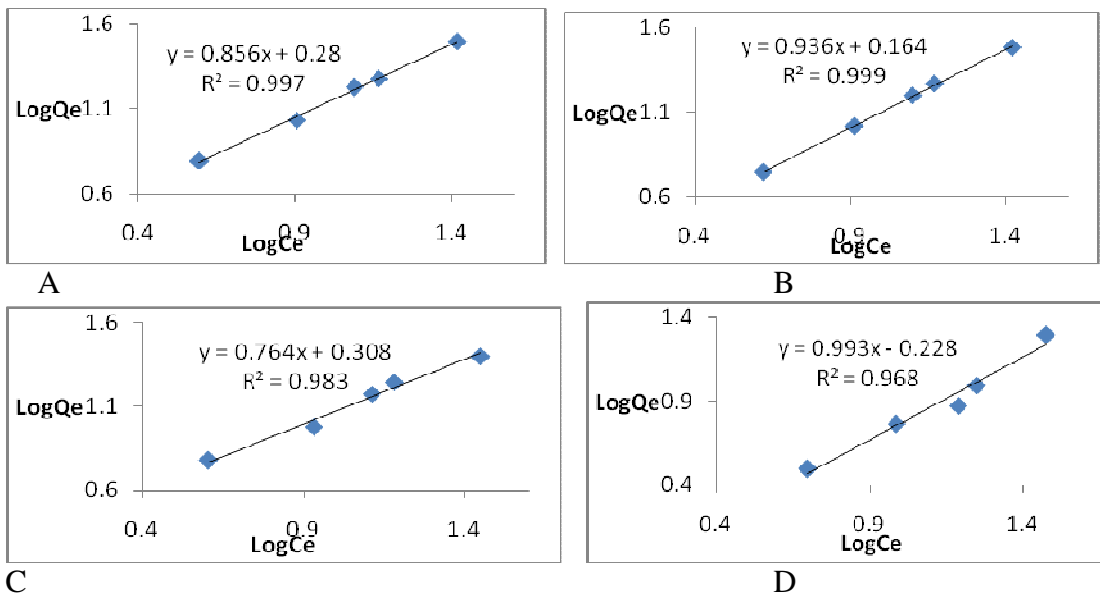


Figure 7(a-d): Freundlich plot for adsorption of Cr (VI) ion onto IBBS, IMBS, IPBS and IABS adsorbents

Langmuir adsorption isotherm

The Langmuir model assumes that the uptake of metal ions occurs on a homogeneous surface by monolayer adsorption without any interaction between the adsorbed ions. Its linear form is as follows:

$$\frac{C_e}{q_e} = \frac{1}{bq_o} + \frac{C_e}{q_o} \quad (3)$$

where, q_o (mg/g) is the maximum adsorption capacity, b (L/mg) is a constant related to the affinity of binding sites or bonding energy. The plot of C_e/q_e versus C_e gave a straight line [Fig. 8(a-d)] with lower R^2 values of 0.85, 0.84 and 0.95 for IBBS, IPBS and IMBS respectively (Schwarzenbach *et al.*, 2003).

Table 2: Freundlich, Langmuir and Temkin adsorption isotherms for uptake of Cr (VI) onto various biosorbents

| Sorbents | Adsorption isotherm types | | | | | | | | | |
|----------|---------------------------|---------------|------------|-------|-------|-----------------|--------|-----------------|-------|-------|
| | Langmuir | | Freundlich | | | | Temkin | | | |
| | q_o (mg/g) | b (L/mg) | R_L | R^2 | $1/n$ | K_f (mg/g) | R^2 | K_T (mg/g) | B_T | R^2 |
| IBBS | 115.05 | 0.013 | 0.678 | 0.85 | 0.86 | 1.906 | 0.99 | 1.168 | 0.072 | 0.93 |
| IMBS | 227.27 | 0.006 | 0.820 | 0.95 | 0.94 | 1.461 | 0.99 | -15.97 | 30.79 | 0.93 |
| IPBS | 61.35 | 0.025 | 0.523 | 0.84 | 0.76 | 2.033 | 0.98 | -9.65 | 9.99 | 0.95 |
| IABS | - | - | - | - | 0.99 | 1.691 | 0.97 | -12.81 | 8.54 | 0.81 |

q_o , K_f , and K_T are adsorption constants of Langmuir, Freundlich and Temkin isotherms, b and B_T are energy of adsorption due to Langmuir and Temkin isotherms while R_L is the dimensionless constant Langmuir isotherm. R^2 is the correlation coefficient.

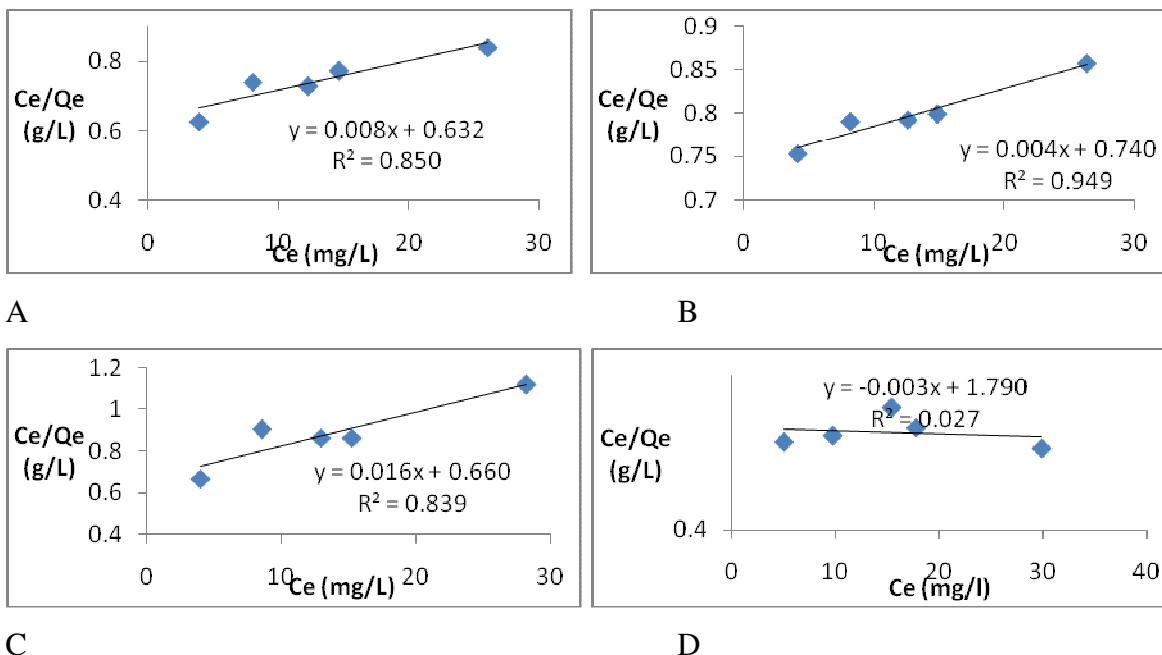


Figure 8(a-d): Langmuir plot for adsorption of Cr (VI) ion onto IBBS, IMBS, IPBS and IABS adsorbents

The essential characteristics of a Langmuir isotherm can be expressed in

terms of a dimensionless separation factor, R_L , which is defined

by the following equation (Zheng *et al.*, 2009):

$$R_L = \frac{1}{1 + bC_o} \quad (4)$$

where b is the Langmuir constant, C_o is the initial equilibrium concentration (mg/L), and R_L indicates the shape of the isotherm as follows: $R_L > 1$ Unfavourable, $R_L = 1$ Linear, $0 < R_L < 1$ Favourable, $R_L = 0$ Irreversible. So, the biosorption processes were also favourable to Langmuir adsorption isotherm since all R_L values fell between 0 and 1 (Table 2).

Temkin adsorption isotherm

The Temkin isotherm has been used in the following linearized form:

$$q_e = B_T \log k_T + B_T \log C_e \quad (5)$$

Where $B_T = \frac{RT}{b^1}$, q_e is the amount adsorbed at equilibrium and C_e is the residual equilibrium concentration, T is the absolute temperature (K) and R is the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$).

The linear plots of q_e versus $\log C_e$ enabled the determination of the constant k_T (L/g) and b^1 from the slope and intercept respectively [Fig.9 (a-d)] (Srivastava *et al.*, 2006). The binding energy, B_T was highest for IMBS (30.785 L/g) and lowest for IBBS (0.072 L/g) while their Temkin biosorption capacity, K_T for IBBS, IMBS, IPBS and IABS are

found to be 1.168, -15.970, -9.654 and -12.807 mg/g respectively.

Adsorption kinetics

The effects of contact time at pH of 2, 30 mg/l initial concentration and different temperatures on chromium removal were analyzed from the kinetic point of view using the following kinetic models: pseudo-first order, pseudo-second order, Elovich and the intra-particle kinetic models.

Pseudo-first order equation

The linearized pseudo-first order is given by:

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \quad (6)$$

Where, q_e and q_t are the adsorption capacity at equilibrium and after time t , respectively (mg/g); k_1 is the rate constant for pseudo first-order (L/min). When the values of $\log (q_e - q_t)$ are linearly correlated with t , a straight line which indicates a good fit for the pseudo-first order kinetic model is obtained (Fig. 10). k_1 and q_e can be determined from the slope and intercept of the plot, respectively (Table 3).

Pseudo-second order equation

The linearized pseudo-second order kinetic is expressed as (Ho and Mackay, 2000):

$$\frac{t}{q_t} = \frac{1}{q_e} t + \frac{1}{k_2 q_e^2} \quad (7)$$

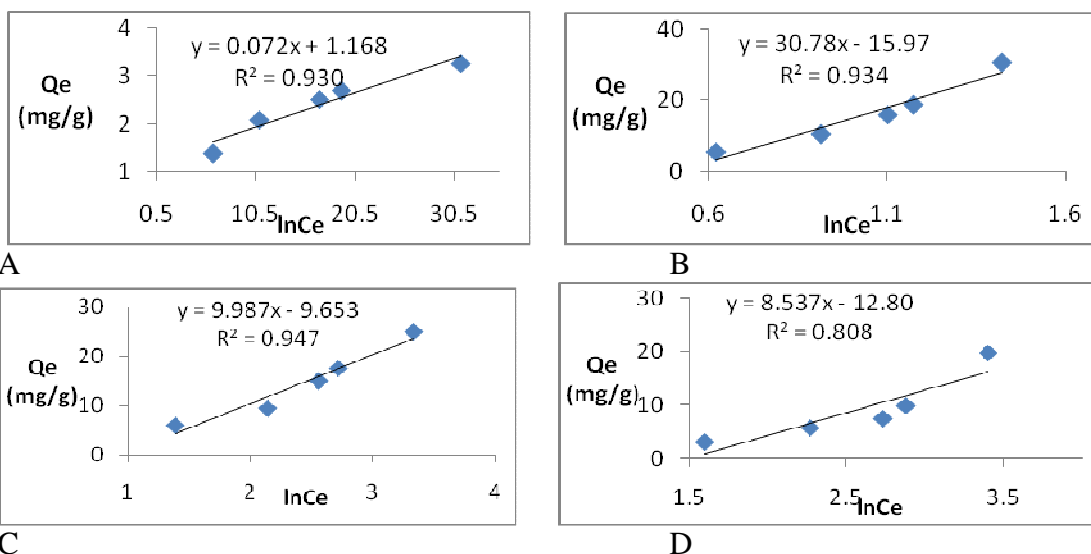


Figure 9(a-d): Temkin plot for adsorption of Cr (VI) ion onto IBBS, IMBS, IPBS and IABS adsorbents

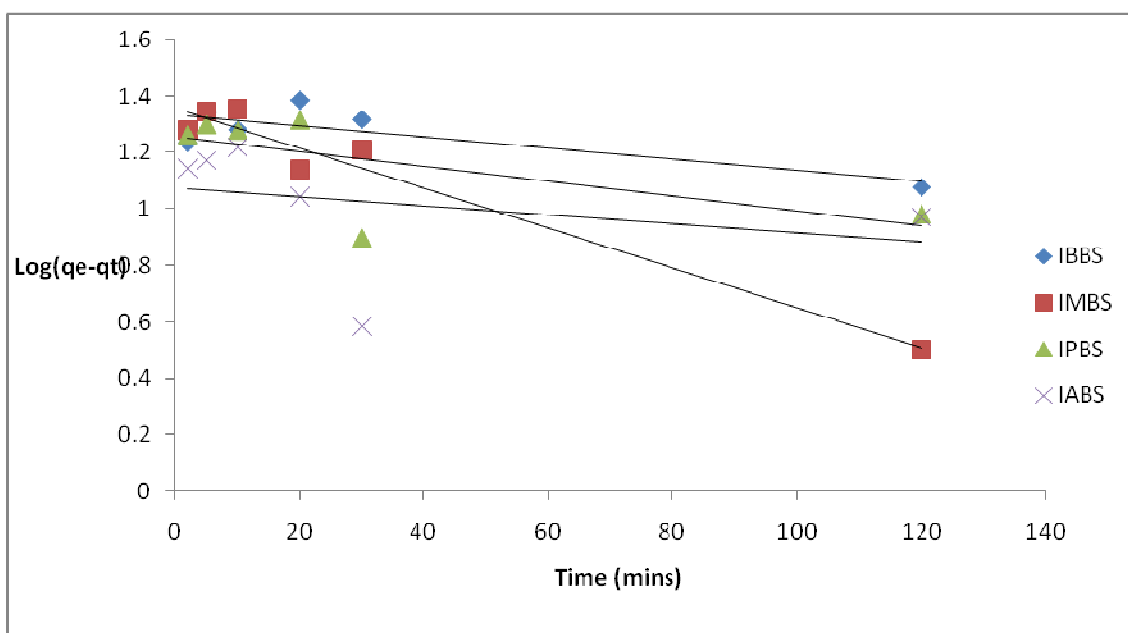


Figure 10: Pseudo-first order plot for Cr (VI) ions at 305K.

Table 3: Pseudo-first order kinetics model parameters for Cr (VI) ions uptake

| Sorption system | T (K) | k_1 (min^{-1}) | Q_e cal (mg/g) | Q_e exp (mg/g) | R^2 |
|-----------------|-------|-----------------------------|------------------|------------------|--------|
| IBBS//Cr (VI) | 305 | 4.61×10^{-3} | 21.66 | 41.61 | 0.6468 |
| IMBS//Cr (VI) | 305 | 1.64×10^{-3} | 22.91 | 38.64 | 0.9632 |
| IPBS//Cr (VI) | 305 | 5.99×10^{-3} | 18.06 | 37.89 | 0.4159 |

k_1 is pseudo-first order rate constant, Q_e cal and Q_e exp are the calculated and experimental equilibrium uptake capacities, T is the absolute temperature and R^2 is correlation coefficient.

The plot of (t/q_t) against t using equation (7) gave a linear relationship from which q_e and k_2 were determined from the slope

and intercept of the plot, respectively (Fig. 11, Table 4).
Elovich kinetics model

The Elovich model describes the chemical nature of the adsorption and is generally expressed as (Günay *et al.*, 2007):

$$\frac{dq_t}{dt} = \alpha \exp(-\beta q_t) \quad (8)$$

where: α is the initial adsorption rate ($\text{mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$) and β is the Elovich constant related to the surface coverage ($\text{g}\cdot\text{mg}^{-1}$).

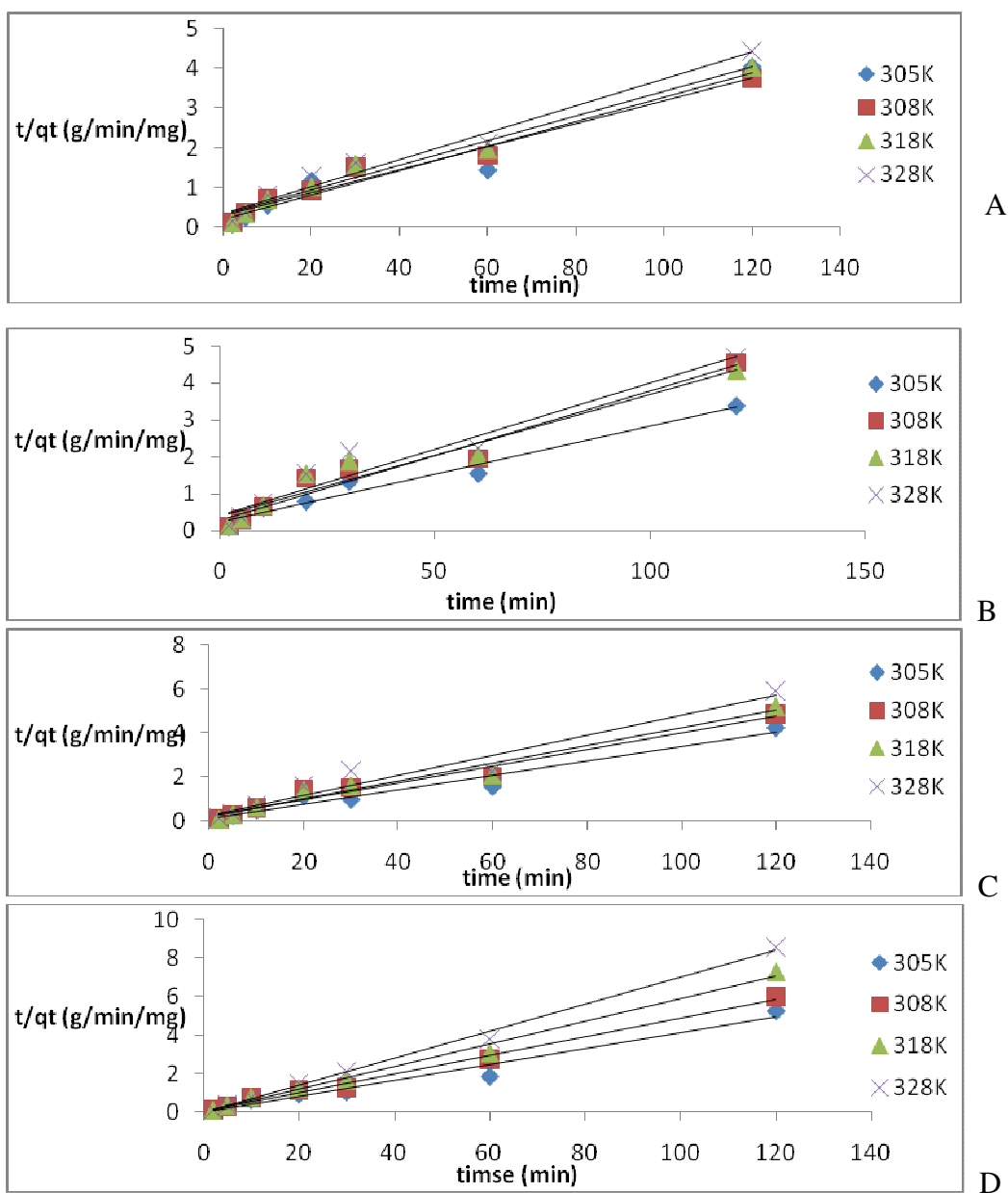


Figure 11(a- d): Pseudo-second order plot for Cr (VI) using IBBS, IMBS, IPBS and IABS

Table 4: Pseudo-second order kinetics model parameters for Cr (VI) ions uptake

| Adsorbent types | Temperature (K) | K_2 (g/mg/min) | Q_e calculated, (mg/g) | Q_e expected, (mg/g) | R^2 |
|-----------------|-----------------|------------------------|--------------------------|------------------------|--------|
| IBBS | 305 | 4.84×10^{-3} | 32.68 | 41.61 | 0.9386 |
| | 308 | 3.09×10^{-3} | 32.72 | 33.06 | 0.9722 |
| | 318 | 3.14×10^{-3} | 32.15 | 30.42 | 0.9761 |
| | 328 | 3.43×10^{-3} | 29.41 | 28.50 | 0.9756 |
| IMBS | 305 | 2.73×10^{-3} | 38.61 | 38.64 | 0.9703 |
| | 308 | 4.37×10^{-3} | 28.49 | 30.63 | 0.9594 |
| | 318 | 2.84×10^{-3} | 30.12 | 29.46 | 0.9385 |
| | 328 | 2.98×10^{-3} | 27.90 | 26.79 | 0.9400 |
| IPBS | 305 | 8.98×10^{-3} | 30.58 | 37.89 | 0.9609 |
| | 308 | 5.93×10^{-3} | 26.53 | 29.19 | 0.9676 |
| | 318 | 7.46×10^{-3} | 24.94 | 28.50 | 0.9698 |
| | 328 | 7.25×10^{-3} | 22.03 | 26.79 | 0.9469 |
| IABS | 305 | -2.97×10^{-3} | 24.10 | 32.10 | 0.9692 |
| | 308 | 3.78×10^{-3} | 20.66 | 23.64 | 0.9931 |
| | 318 | 1.02×10^{-3} | 17.04 | 19.32 | 0.9917 |
| | 328 | 2.09×10^{-3} | 14.33 | 15.69 | 0.9962 |

k_2 is pseudo-second order rate constant, Q_e cal and Q_e exp are the calculated and experimental equilibrium uptake capacities, T is the temperature.

By applying the boundary conditions $qt = 0$ at $t = 0$ and $qt = qt$ at $t = t$, the integrated form of Equation. (8) is:

$$q_t = \frac{1}{\beta} \ln(\alpha\beta) + \frac{1}{\beta} \ln(t) \tag{9}$$

The plot of qt vs $\ln(t)$ yielded a linear relationship with a slope of $(1/\beta)$ and an intercept of $(1/\beta)\ln(\alpha\beta)$, (Fig. 12) and its values are summarized in Table 5.

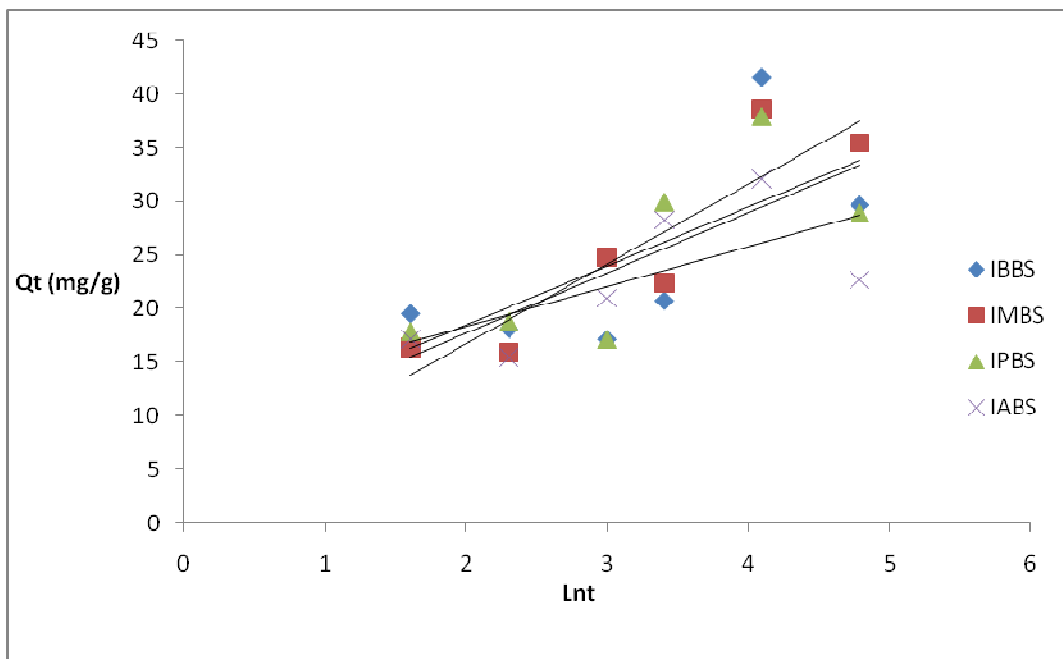


Figure 12: Elovich kinetics model plot for Cr (VI) at 305K

Table: 5 Elovich kinetics model parameters for Cr (VI) ions

| Sorption system | T (K) | α (mg/g/min) | β (g/mg) | R^2 |
|-----------------|-------|---------------------|----------------|--------|
| IBBS//Cr (VI) | 305 | 17.881 | 0.178 | 0.4703 |
| IMBS//Cr (VI) | 305 | 5.134 | 0.178 | 0.8684 |
| IPBS//Cr (VI) | 305 | 21.367 | 0.181 | 0.5756 |
| IABS//Cr (VI) | 305 | 67.472 | 0.267 | 0.4589 |
| IABS//Cd (II) | 305 | 4.089 | 0.218 | 0.8320 |

β is Elovich constant related to the surface coverage, α is initial adsorption rate

Conclusion

The *B. subtilis* and *P. aeruginosa* immobilized with sodium alginate may be inexpensive and effective biosorbent for the removal of Cr (VI) ions from water bodies. The physicochemical properties (bulk density, CEC) of the biosorbents compared favourably with those of the commercial biosorbent resins. The biosorption of Cr (VI) was found to be dependent on initial metal concentration, biosorbent dose, contact time, pH and temperature. Equilibrium conditions were established after contact time of 1 hour, pH 2; and biosorbent dose of 0.01g. The sorption capacity of Cr (VI) is highest in IBBS followed by IMBS which may be attributed to a thicker peptidoglycan layer in the gram positive organism. The kinetic data were

best explained by Pseudo-second order kinetics and Freundlich model explained adsorption Equilibrium data best. The IBBS and IMBS biosorbents could be used for removal of Cr(VI) ions even at low concentration.

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