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Biosorption of Cr(VI) and Ni(II) from aqueous solution onto *Bacillus subtillis* immobilsed in Agarose gel

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ABSTRACT

The objective of this study is to investigate the removal efficiency of Cr(VI) and Ni(II) by Bacillus subtilis immobilized in agarose gel from aqueous solution under different experimental conditions. Batch mode experiments were carried out as a function of solution pH, Cr(VI) and Ni(II) concentration and contact time. It was found that the equilibrium of the process was reached after 60 min. The optimum pH value was found to be 4 for Cr(VI) adsorption and 6 for Ni(II) adsorption. Bacillus subtilis immobilized in agarose gel was found to posses relatively high sorption capacity for Ni(II) compare to Cr(VI). The data obtained from the equilibrium experiment were analyzed using Langmuir and Freundlich isotherm models. The equillibrim data fitted the Langmuir model better than Freundlich with high correlation coefficients for both adsorbate. According to the Langmuir model, the maximum uptake capacities (qe mg g^{-1}) of the biosorbent used for Cr(VI) was 31.77 ± 0.03 mg g^{-1}, 33.64 ± 0.53 mg g^{-1} and 39.78 ± 3.14 mg g^{-1} at 298K, 308K and 318K respectively and for Ni(II) it was 64.94 ± 0.01 mg g^{-1}, 72.61 ± 2.32 mg g^{-1} and 78.13 ± 0.61 mg g^{-1} at 298K, 308K and 318K respectively. This showed that there was increment in adsorption capacity with increasing temperature and this indicated that the adsorption process is endothermic in nature. The pseudo first order and pseudo second order kinetic models were used to describe the dependence of the adsorption on time. The kinetic data fitted with the pseudo second order kinetic model with high correlation coefficient and better than the pseudo-first order kinetic model. Indicating that the adsorption processes were chemisorptions. The results showed that Bacillus subtilis immobilsed in agarose gel can be used to remove Cr(VI) and Ni(II) ions form aqueous system.

Key word: Biosorption, Hexavalent chromium, Nickel and *Bacillus subtilis.*

INTRODUCTION

The discharge of heavy metals into environment has become a matter of concern over the last few decades. The heavy metals like lead, mercury, zinc, aluminum, arsenic, nickel, chromium, cobalt etc. are the common pollutants present in the environment from various natural and industrial sources [1]. Chromium is a toxic metal of widespread industrial use and exists in several oxidation states. The most stable and common forms are the trivalent Cr(III) and the hexavalent Cr(VI) species, which display quite different chemical properties. Cr(VI) is designated and widely recognized to be a human inhalation carcinogen [2]. Cr(III) is less toxic when compared to Cr(VI) and it has low acute and chronic toxicity to humans at high doses. High doses of Cr(VI) compounds are also associated with nephrotoxicity [3]. Acute exposure to high levels of Cr(VI) can produce nervous system damage and liver disorder [4]. Nickel is another metal widely used in many industries. The higher concentration of Ni(II) in ingested water may cause severe damage to lungs, kidneys, gastrointestinal distress, e.g., nausea, vomiting, diarrhea, pulmonary fibrosis, renal edema, and skin dermatitis [5]. Nickel is toxic to plant at concentration as low as 100 ugl^{-1}[6]. It adversely affects reproduction of fresh water crustacean at concentration as low as 0.995 mgl^{-1}[7]. In human (mammals) nickel acts to inhibit insulin release, depress growth and reduces cholesterol [8]. Extensive use of chromium and nickel eg in Ni-Fe battery industry, electroplating, tanning, textile dyeing results in the effluents containing Cr(VI) and Ni(II) at concentrations ranging from tenths to hundreds of milligrams/litre.

Several procedures have been proposed for removal of these metal ions from aqueous systems . Conventional methods for removing these ions from water include: chemical reduction, membrane separation, electrochemical treatment, ion exchange and evaporative recovery [9]. Although the effectiveness of these methods has been proved, they suffer from a major disadvantage, namely lack of cost effectiveness. Other limitations include energy, intensive processing and concentration dependence, low efficiency, not feasible to reduce the Cr(VI) and Ni(II) concentration to levels as low as required by environmental legislation and production of toxic chemical sludge, which needs additional treatment. These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1–100 mg/l [10]. Hence many researchers worked on the biosorption of metal ions using different biosorbents such as *Rhizopus*, dead fungal biomass of *Aspergillus niger*, *Rhizopus oryzae*, *Saccharomyces cerevisiae*, *Ecklonia* biomass, peat moss and modified saw [11,12,13]. Biosorption, an environment friendly technology to clean up the environment based on the utilization of dead biomass and biopolymers can be an efficient and cost effective remedy.

Bacillus subtilis is a Gram-positive, aerobic, rod-shaped bacterium and ubiquitous in soils and waters. Its parietal structure is well known and is composed primarily of peptidoglycan and teichoic acid [14]. Peptidoglycan is a polymer of acetylglucosamine and acetylmuramic acid, which carry mainly carboxylic and hydroxyl functional groups. On the other hand, teichoic acid is a polymer of copyranosyl glycerol phosphate, which carries mostly phosphate and hydroxyl groups. *B. subtilis* is widely used in the commercial production of various enzymes[14]. Agarose is a polysaccharide consisting of linear polymer of D-galactose and 3, 6-anhydro-L-galatose[15]. And it is a biopolymers that is nontoxic, inexpensive and can be effectively use to remove metal ions from aqueous systems[16]. It can be efficiently used to immobilse microorganism in order

to produce adsorbent with good characteristic like ideal size, mechanical strength, rigidity, and porous characteristics that may be lacking if free cell is used. The use of inactive cells in biosorption is most advantageous for removal of metal ions from water, in that, the organisms are not affected by toxic wastes; they do not require a continuous supply of nutrients and they can be regenerated and reused for many cycles [17].

The objectiive of this work was to study the adsorption ability of *Bacillus subtilis* immobilized in agarose for removal of Cr(VI) and Ni(II) and from aqueous solution. The effect of initial metal ion concentration, initial pH, temperature, and contact time were examined. Langmuir and Fruendlich adsorption isotherm were applied to the equilibrium adsorption data. The pseudo first-order and pseudo second-order kinetic models were used to study the dependence of adsorption on contact time.

MATERIALS AND METHODS

PREPARATION OF ADSORBATE SOLUTION

1000ppm stock Cr(VI) solution was prepared by dissolving 2.828 g of potassium heptaoxodichromate(VI)($K_2Cr_2O_7$) in 200ml of distilled deionised water in a beaker. Then 1.5ml of conc. HNO_3(Sigma-Aldrich) was added. This was then quantitatively transferred into a 1- litre flask and diluted to the mark with distilled deionised water. Similarly,1000ppm stock Ni(II) solution was prepared by dissolving 4.9434g of nickel(II)trioxonitrate(V)hexahydrate ($Ni(NO_3)_2.6H_2O$)(BDH) in 200ml of distilled deionised water in a beaker. Then 1.5ml of conc. HNO_3(Sigma-Aldrich) was added. This was then quantitatively transferred into a 1- litre flask and diluted to the mark with distilled deionised water.Various working solution were prepared from the stock by serial dilution.

GENERATION OF BIOMASS

Pure strains of *Bacillus subtilis* was collected from the Microbiology department of Ahmadu Bello University, Zaria and maintain by monthly subculturing on plate count agar and kept at 4^0C. The biomass grown in a 250 ml corked conical flask containing 100ml Muller Hilton broth medium having the composition (g/l): beef infusion solid (2.0), casein peptone (2.0), starch (1.5), calcium (0.05 mg), magnesium (0.02 mg) at 35^{o}C, 120 rpm in a thermostated water bath for 24 hours. After 24hours, biomass was harvested by centrifugation at 4000 rpm for 15minute at room temperature (25 ± 2) and washed twice with sterile normal saline solution[18]. After washing, the required amount of wet biomass was used for immobilization.

PREPARATION OF THE ADSORBENT

4% agarose solution was prepared by dissolving 10g of agarose in 240 ml of autoclaved deionised distilled water. The solution was heated in a microwave oven until a clear homogenous solution is observed. The heating was such that the mixture was not allowed to boil over. The agarose solution was cooled to about 37^{o}C then 100ml of cell suspension corresponding to 10 McFarland nephelometer standard was thoroughly mixed with the agarose solution. The mixture was poured into petri and kept on ice. After solidification on the petriplate 3 by 3 mm^3 cubes were cut and wash with deionized distilled water. Agarose gel only was prepared to serve as the control.

DETERMINATION OF pH POINT OF ZERO CHARGE OF THE ADSORBENT.
The pH point of zero charge of the adsorbents were determine as described by Onyango *et al.*, 2004 [19].

ADSORPTION EXPERIMENT
Adsorption experiments were conducted at varying pH, contact time, and adsorbate concentration. The experiments were carried out by batch method using 100 mL corked conical flask, 10mg of the adsorbents and the total volume of the reaction mixtures was kept at 50 mL. The pH of solution was maintained at a desired value by adding 0.1 M NaOH or 0.1M HCl. The flasks were shaken for the required time period in a thermostated water bath shaker (Gallenkamp BKS-300010 F model). The isotherm study was performed using various concentrations of Cr(VI) and Ni(II) solutions ranging from 25 to 100 mg/L at 298K, 308K and 318K and pH 6. The kinetics study was carried out with 50ml volumes of 100mg/l initial Cr(VI) and Ni(II) ion concentration, 10mg adsorbent adsorbent dose and pH 4 for Cr(VI) and pH 6 for Ni(II). The mixture was agitated at 120 rpm and 25^{o}C for different contact time ranging from 10-180min. At predetermined time, the flasks were withdrawn from the shaker and the reaction mixtures were filtered through Whatman 1 filter paper. The first 5ml of the filtrate was discarded. All experiments were performed in triplicates. The filtrate samples were analyzed by flame atomic absorption spectrophotometer (Bulk scientific 2000 model). The Cr(VI) and Ni(II) concentration retained on the adsorbent phase was calculated according to the equation below:

$$q_e = \frac{V(C_O - C_e)}{M} \quad (1)$$

The metal percentage removal (%) was calculated using the following equation:

$$\text{Removal}(\%) = \frac{C_e - C_0}{C_o} \times 100 \quad (2)$$

Where q_e is the amount adsorbed in mg/g of the adsorbent at equilibrium, C_o and C_e were the initial and the equilibrium concentrations in mg/l, respectively, V is the volume in liters of the solution used during the experiment and M is the mass of the adsorbent in gram.

RESULTS AND DISCUSSION

pH POINT OF ZERO CHARGE OF THE ADSORBENTS
The pH point of zero charge (pHpzc) of the adsorbents were assessed from the plot of zeta potential (mV) versus initial pH(pHinitial) presented in Figure1. The pHpzc of *Bacillus subtilis* immobilized in agarose gel was 5.21 and that of the Agarose gel only(i.e the control) was 5.53. pHpzc of *Bacillus subtilis* immobilized in agarose was lesser than that of agarose gel only due to more acidic group present in the cell walls of *Bacillus subtilis* [20]. It was reported that the pHpzc of oil palm fibre was 7.1 and on modification with acidic marcapto group it reduced to 6.4 for 0.5 M modification and 6.0 for 1.0 M modification with marcapto aecetic acid [21]. At pH value less than that of pHpzc, the surface charge on the sorbent is a net positive charge, while at pH value greater than that of pHpzc the surface charge on the sorbent is a net negative charge [22]. Therefore, more favorable adsorption will be expected for chromate ion at pH value less

than pHpzc while favorable adsorption of Ni(II) will be expected at pH value greater than pHpzc of the adsorbent.

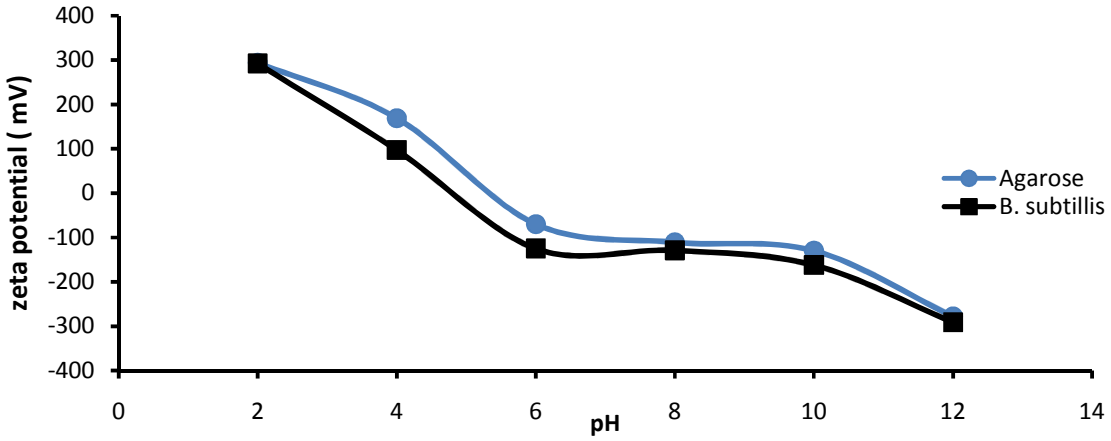

Fig.1 Zeta-potential against initial pH for the determination of pH_{pzc} of the adsorbents.

EFFECT OF SOLUTION pH ON ADSORPTION OF THE METAL IONS

igs. 2 show the variation of sorption percentage of Cr(VI) and Ni(II) ions by the adsorbents with pH of the solution respectively. The highest sorption percent removal by *Bacillus subtilis* occurred at pH of 4 for Cr(VI) ions, while that of Ni(II) occurred at pH of 6. It was observed that Cr(VI) percentage removal increased from 25.6 % to 46.0 % as pH increases from 2.0 to 4.0 and decreased to 24.25 at pH 6. Also, the percentage removal for Ni(II) increased from 54.33 to 89.00% as pH increases from 2.0 to 6.0 and decreased to 64.00% at a pH 8.0.

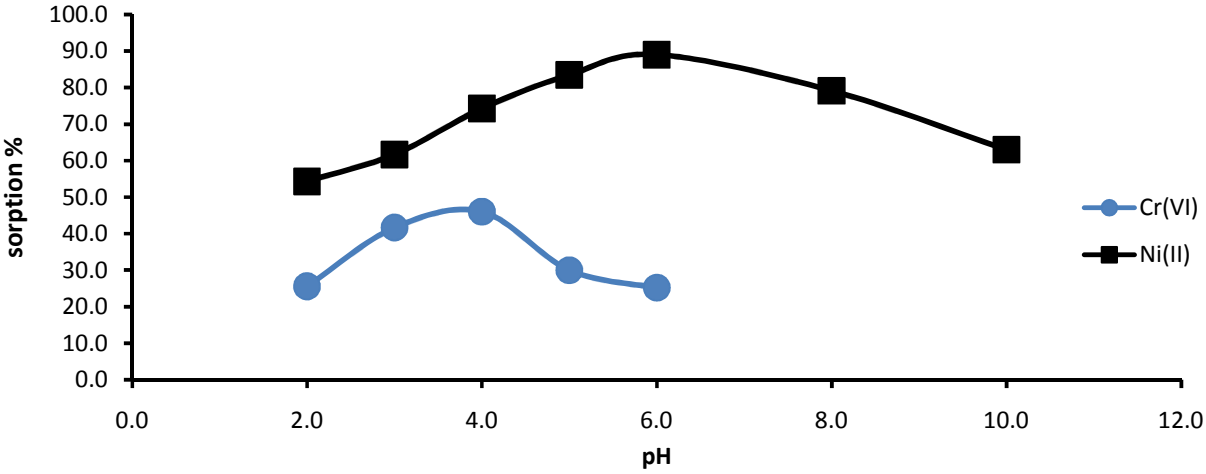

Fig.2. Sorption percentage against pH for the adsorption of Cr(VI) and Ni(II) ion onto *Bacillus subtilis*.

The increase in percentage metal ion removal as pH increases can be explained on the basis of a decrease in competition between proton and the metal ion for the surface sites and by the decrease in positive surface charge, which results in a lower coulombic repulsion of the sorbing

metal ion [23]. Similar observation was made for the adsorption Cd(II) onto activated carbon[24]. Some of the functional groups present in the cell wall of *Bacillus subtilis* , such as amines, are positively charged when protonated and may electro-statically bind with negatively charged metal complexes[25]. Also, it has been reported that at pH value greater than pH_{pzc} the functional groups on the surface of biosorbents has net negative charge and at pH value lower than pH_{pzc} the functional groups on the surface of biosorbents has net positive charge[26]. These accounted for the highest percentage removal observed for Chromium (which exists as $Cr_2O_7^{2-}$ anion in aqueous solution) at pH 4 and that of Nickel (which exists as Ni^{2+} ion) at pH 6.

THE EFFECT OF CONTACT TIME AND TEMPERATURE

Figs.3 and 4 show the variation of adsorption percentage with time (minutes) for the adsorption Cr(VI) and Ni(II) from aqueous solution onto *Bacillus subtilis* immobilized in agarose gel at different temperature.

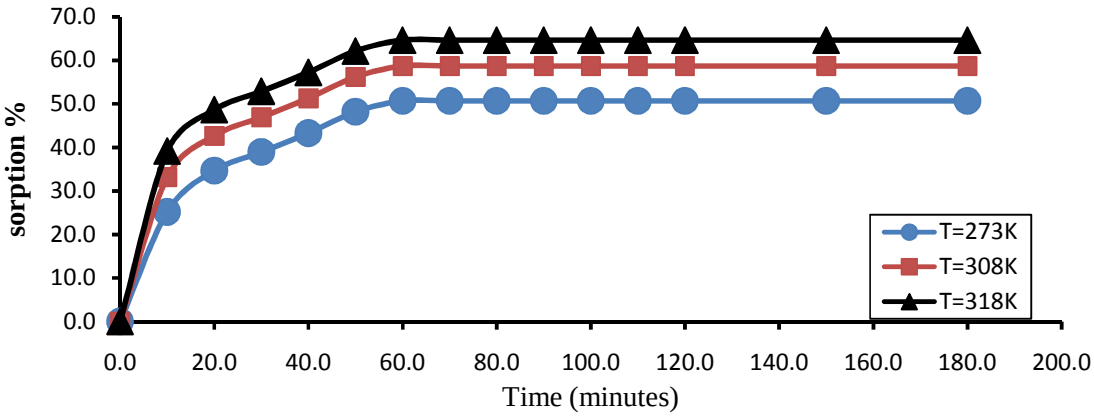

Fig. 2 Variation of Sorption percentage with Time (minutes) for the adsorption of Cr(VI) onto *Bacillus subtilis* at different temperature.

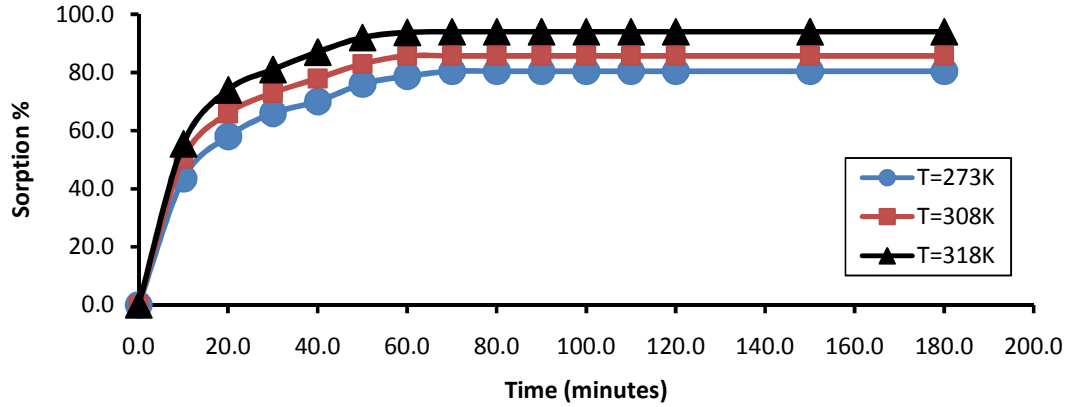

Fig. 4 Variation of Sorption percentage with Time (minutes) for the adsorption of Ni(II) onto *Bacillus subtilis* at different temperature.

Obviously, the rate of adsorption was rapid in the first 30minutes. Almost 60 percent removal of the metal ions by the adsorbents occurred at this time. There was an increase in the concentration of metal ion adsorbed, but at slower rate, until 60 minutes after which, there was no significant change in the adsorption percentage with further increase in contact time. This indicated that 60

minutes was the time required to achieve equilibrium and the uptake and unadsorded metal ion concentration at the end of 60 minutes were given values q_e (mg/g) and C_e (mg/l) respectively. Such a fast adsorption rate before 30 minutes could be attributed to available functional group on the surface of the adsorbent [27]. The slower rate of adsorption in the latter stages may be attributed to great decrease of the binding site on the surface of the adsorbent [28]. A short contact time necessary to reach equilibrium in adsorption studies indicates that the predominant mechanism of reaction is chemical adsorption [29].

Considering the effect of temperature on the trend of adsorption, it is evident that the amount of metal ion adsorbed increases with increase in temperature. This indicate that the adsorption of the metal ions support the mechanism of chemical adsorption. For a chemical adsorption mechanism, the extent of adsorption is expected to increase with increase in temperature as observed in this study [30]. With increase in temperature, the attractive forces between adsorbents' surfaces and metal ions became stronger and these usually resulted in increase in extent of adsorption [31]. This behavior is typical for the adsorption of most metal ions from their solution onto natural materials [32]. It was reported that adsoption of Ni(II) on *Syzygium aromaticus* attained equilibrium within 40 minutes[28]. Similar rapid metal uptake has been reported for the biosorption of Pb(II) using *Ecklonia radiate* wherein the system reached over 50-60% of the equilibrium uptake capacity in 10 min [33]

EFFECT OF INITIAL METAL ION CONCENTRATION

Fig. 5 show the effect of initial metal ion concentration on the adsorption of Cr(VI) and Ni(II) by *Bacillus subtilis* immobilized in agarose gel at 298 K. The increase in initial metal ion concentration decreased the percentage removal and increased the amount of metal ion uptake per unit mass of the adsorbent (mg/g). The figures revealed that for Cr(VI) ions percentage metal ion removal decreased from 50.66 % (6.33 mg/g) to 32 % (20.00 mg/g) and for Ni(II) it decreased from 75 % (9.38 mg/g) to 61 % (38.13 mg/g) by increasing the concentrations of the adsorbates from 25 to 125 mg/L. The increase in the amount of metal ion uptake per unit mass of the adsorbent (mg/g) observed as initial concentration is increased was as a result of increase in concentration gradient, the driving force for adsorption. Though an increase in metal uptake (mg/g) was observed, the decrease in percentage adsorption may be attributed to lack of sufficient surface area to accommodate more metal ion available in the solution as the concentration increases.

At lower concentrations, all metal ions present in solution could interact with the functional group and binding sites on the surface of the adsorbent and thus the percentage adsorption was higher than those at higher metal ion concentrations. At higher concentrations, lower adsorption yield is due to the saturation of adsorption sites. Thus, purification yield can be increased by diluting the wastewaters containing high metal ion concentrations.

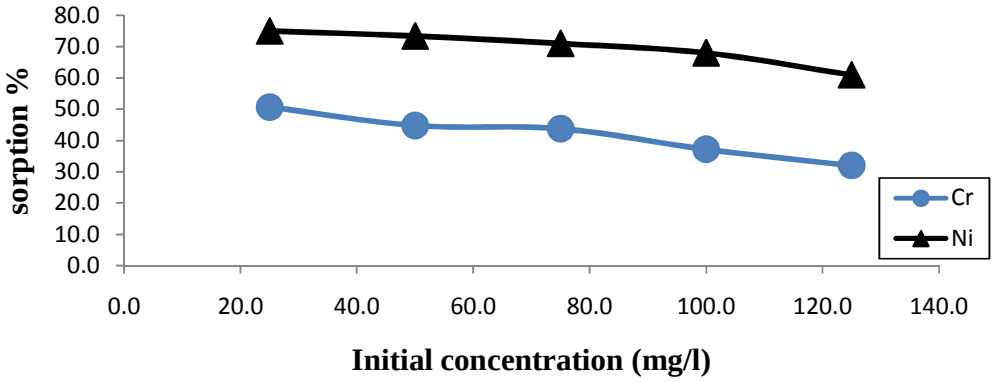

Fig.5 Variation of Sorption percentage with Initial concentration for the adsorption of Cr(VI) and Ni(II) onto *Bacillus subtilis* immobilized in agarose.

ADSORPTION ISOTHERMS

Adsorption isotherms were used to characterize the interaction of each Cr(VI) and Ni(II) species with the adsorbent. This provides a relationship between the concentration of Cr(VI) and Ni(II) in the adsorption medium and the amount of Cr(VI) and Ni(II) adsorbed on the solid phase when the two phases are at equilibrium. Langmuir and Freundlich adsorption isotherms, the two widely used isotherms were used to study the adsorption processes.

The Langmuir model is based on the assumption of surface homogeneity such as equally available adsorption sites, monolayer surface coverage, and no interaction between adsorbed species. This model assumes :(i) no change in the properties of the adsorbed molecules (ii) no lateral interaction between adsorbed molecules (iii) one adsorption site per molecule and (vi) that all adsorption sites have the same affinity for the sorbate [34]. The following represents the linearised Langmuir isotherm equation,

$$\frac{C_e}{q_e} = \frac{1}{K_L Q^o} + \frac{C_e}{Q^0} \quad \text{----------(3)}$$

Where K_L (L/g) is a constant related to the adsorption/ desorption energy and Q^0 (mg/g) is the maximum sorption upon complete saturation of the adsorbent (biosorbent) surface[35]. The experimental data were fitted into the equation (3) by plotting of $\frac{C_e}{q_e}$ againstC_e (Figures 6 and 7). K_L (L/g) and Q^0 (mg/g) were calculated from the slope and the intercept of the plots respectively.

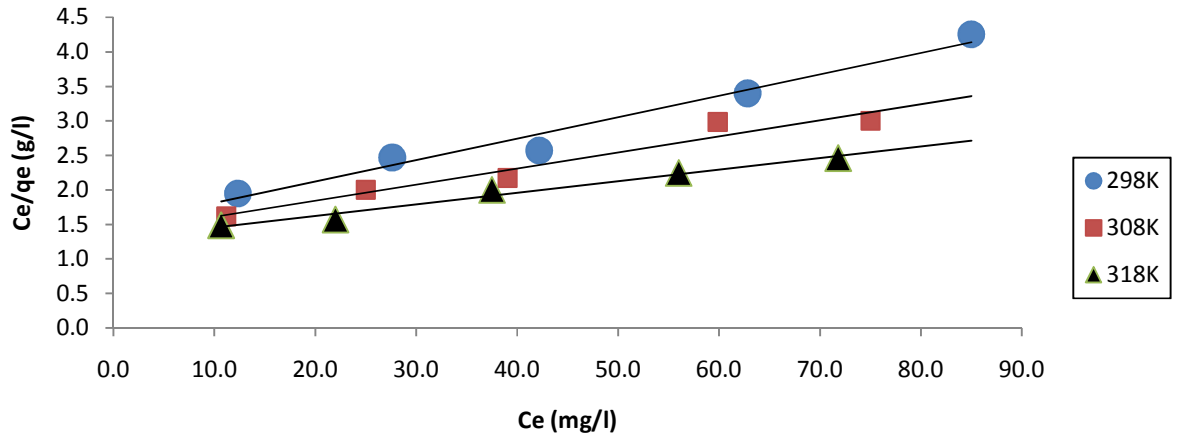

Fig.6 Langmuir isotherm at different temperature for Cr(VI) ion adsorption on *Bacillus subtilis* immobilized in agarose.

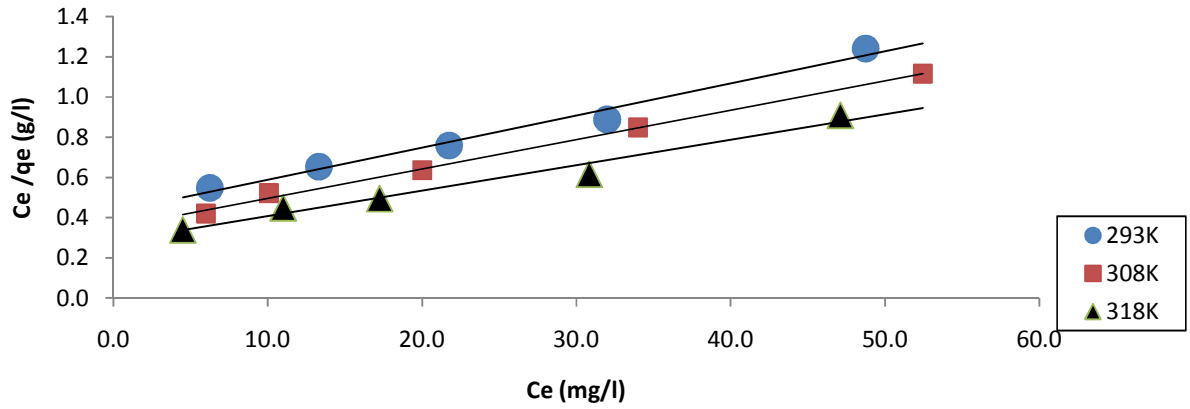

Fig.7 Langmuir isotherm at different temperature for Ni(II) ion adsorption on *Bacillus subtilis* immobilized in agarose.

The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of a metal ion binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. One limitation of the Freundlich model is that the amount of adsorbed solute increases indefinitely with the concentration of solute in the solution. The linearised empirical equation takes the form

$$Inq_e = InK_l + \left(\frac{1}{n}\right) InC_e \text{ ----------(4)}$$

Where q_e (mg/g) is the adsorption density, C_e is the concentration of metal ion in solution at equilibrium (mg/l), K_f and n are the Freundlich constants which determines the curvature and steepness of the isotherm [36]. The experimental data were fitted into the equation (4) by plotting

InC_e against Inq_e (Figure 10 and 11).The value of $\frac{1}{n}$ and InK_l were determined from the slope and intercept of the plots respectively.

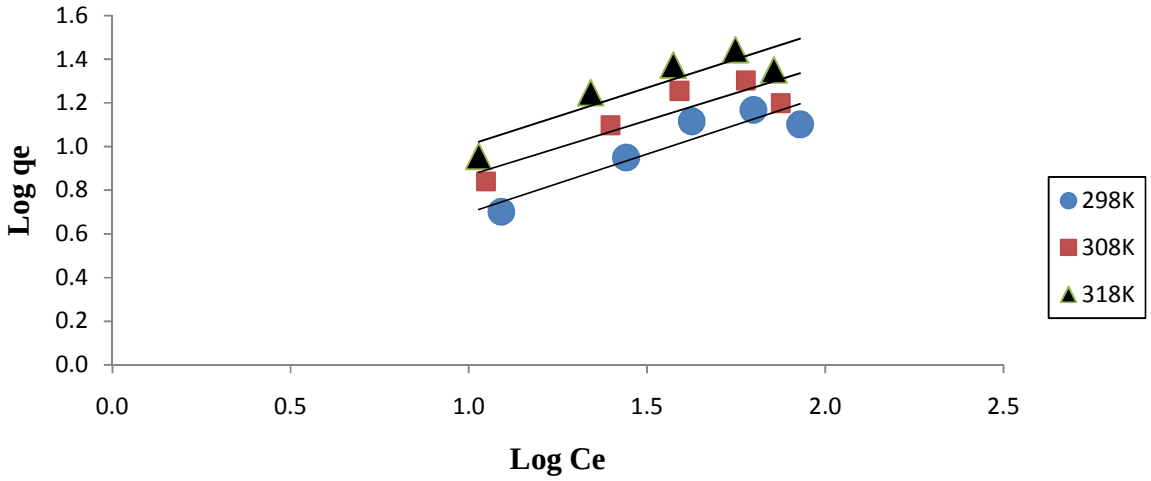

Fig.8 Freundlich isotherm at different temperature for Cr(VI) ion adsorption on *Bacillus subtilis* immobilized in agarose.

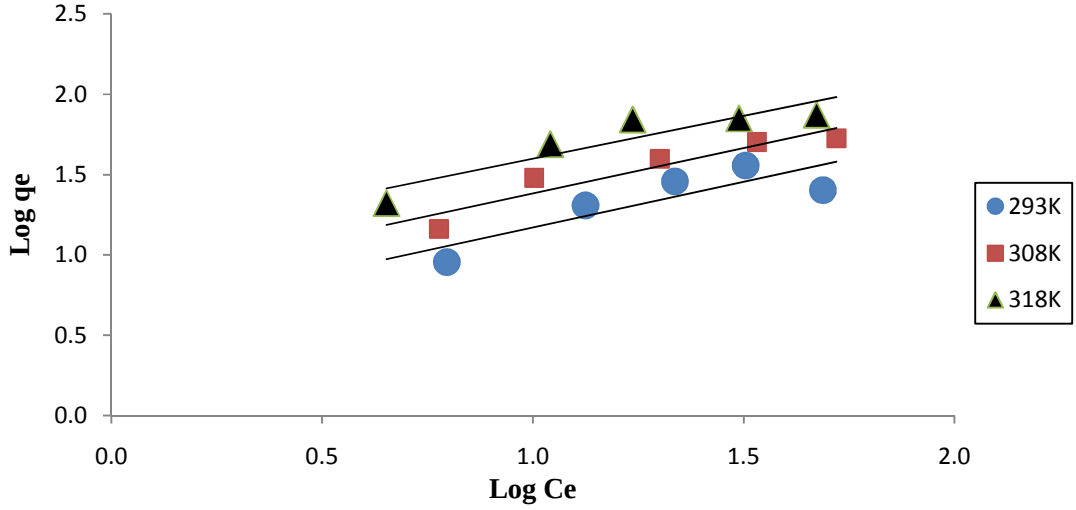

Fig.9 Freundlich isotherm at different temperature for Ni (II) ion adsorption on *Bacillus subtilis* immobilized in agarose.

The adsorption constants and correlation coefficients obtained from the Langmuir and Freundlich isotherms are provided in Table 1. From Table 1 it can be concluded that the data generally fitted Langmiur adsorption isotherm with hiigher correlation coefficients greater than 0.9 and also better than the freundlich adsorption isotherm. This implied that a monolayer adsorption occurred over a surface containing a finite number of adsorption sites and the adsorbed ions cannot migrate across the surface or interact with neighboring molecules [37].

Table 1 Langmuir and Freundlich isotherms constants and correlation coefficients for Cr(VI) and Ni(II) adsorption onto *Bacillus subtilis* and *Escherichia coli* immobilized in agarose.

	LANGMUIR ISOTHERM					
	Bacillus subtilis immobilized in agarose			Agarose gel only		
TEMPERATURE	298K	308K	318K	298K	308K	318K
	CHROMIUM					
q_m(mg/g)	31.77 ± 0.03	33.64 ± 0.53	39.78 ± 3.14	17.73 ± 0.89	19.84 ± 0.27	24.14 ± 1.97
K_L (L/mg)	0.014 ± 0.00	0.018 ± 0.00	0.021 ± 0.00	0.009 ± 0.00	0.010 ± 0.01	0.013 ± 0.00
R^2	0.973 ± 0.00	0.957 ± 0.00	0.974 ± 0.00	0.973 ± 0.00	0.957 ± 0.01	0.974 ± 0.00
	NICKEL					
q_m(mg/g)	64.94 ± 0.01	72.61 ± 2.32	78.13 ± 0.61	24.18 ± 1.86	27.71 ± 0.54	32.42 ± 2.92
K_L (L/mg)	0.032 ± 0.00	0.036 ± 0.01	0.040 ± 0.01	0.017 ± 0.01	0.024 ± 0.01	0.027 ± 0.01
R^2	0.986 ± 0.00	0.960 ± 0.04	0.984 ± 0.01	0.963 ± 0.01	0.978 ± 0.01	0.989 ± 0.01
	FREUNDLICH ISOTHERM					
	CHROMIUM					
K_F (mg/g)	1.180 ± 0.39	1.313 ± 0.18	1.587 ± 0.06	0.220 ± 0.01	0.317 ± 0.06	0.677 ± 0.19
1/n	0.598 ± 0.06	0.591 ± 0.07	0.559 ± 0.06	0.860 ± 0.00	0.799 ± 0.00	0.725 ± 0.05
$*R^2$	0.879 ± 0.03	0.899 ± 0.02	0.876 ± 0.06	0.901 ±0.01	0.853 ± 0.01	0.836 ± 0.00
	NICKEL					
K_F (mg/g)	3.457 ± 0.60	6.023 ± 0.55	9.970 ± 1.82	0.387 ± 0.02	0.583 ± 0.02	1.146 ± 0.08
1/n	0.635 ± 0.06	0.577 ± 0.01	0.536 ± 0.00	0.819 ± 0.01	0.773 ± 0.01	0.759 ± 0.01
$*R^2$	0.815 ± 0.08	0.879 ± 0.00	0.872 ± 0.03	0.895 ±0.02	0.903 ± 0.01	0.913 ± 0.00

Note: $R^2$and $*R^2$ are the correlation coefficient of Langmiur and Freundlich isotherms respectively

The Langmuir constants K_L and q_m which are related to the affinity between the adsorbent and adsorbate and adsorption capacity were found to increase with increasing temperature for all the adsordate on the adsorbents used. This indicated that the adsorption processes involved more heat of adsorption and increased adsorption capacity for the adsorbent with increasing temperature [38].Thus, the process is endothermic [39].

It was observed from the table that *Baccillus subtilis* immobilized on agarose gel has greater affinity for the positively charged Nickel (II) than the negatively charged Chromate ion at all the temperature of study. Hence greater qe(mg g^{-1}) is obtained for the Ni(II) ion compare to the Cr(VI) ion. This may be as a result of the fact that the functional groups of the peptidoglycan layer of bacterial cell wall generally assume net negative charge in aqueous system. It was reported that the functional groups on bacterial surfaces are similar to those found on mineral surfaces in that they are proton active. However, unlike functional groups on mineral surfaces that become doubly protonated and positively charged at low pH, bacterial surface functional groups behave like monoprotic organic acids. Most bacterial surface groups are either protonated and neutrally charged or deprotonated and negatively charged, although amine groups are positively charged when protonated [40]

The Langmuir based adsorption capacity (q_m) of several adsorbents was reported in mg/g, for Novel saw dust (24.44 mg/g)[41], *Tamarindus indica* (25.34 mg/g)[42], resting cells of *Aspergillus sp* (34.8 mg/g)[43],Activated sludge (30 mg/g)[37] Algae, Nile water (37.43 mg/g), *Fucus spiralis* (64 mg/g), marine-algae dead biomass (80 mg/g) [44]. These values were in

agreement with the data reported in Table 1. This implies that *Bacillus subtilis* immobilized in agarose gel is an effective potential adsorbents for the removal of Cr(VI) and Ni(II), from aqueous solution.

ADSORPTION KINETICS

Kinetics of adsorption in terms of solute uptake rate, which governs the residence time, is one of the important characteristics defining the efficiency of adsorption processes. Kinetic parameters as a function of temperature were determined to predict the adsorption behaviour of Cr(VI) and Ni(II) onto *Bacillus subtillis* immobilized in agarose gel. Pseudo-second order and pseudo-first-order (Lagergren) kinetic models were used to fit the experimental data obtained at different temperature.

The integrated form of pseudo-first order kinetic equation can be written as:

$$Log\ (q_e - q_t) = Log\ q_e - \frac{k_1}{2.303} t \quad \text{---------- (8)}$$

q_e(mg g^{-1}) and qt(mg l^{-1}) are the adsorption capacities at equilibrium and at time t, k_1(l min^{-1}) is the rate constant of pseudo-first-order adsorption. The kinetic data were fitted into the equation and k_1(l min^{-1}) and q_e(mg g^{-1}) were determined from the slope and the intercept of the log (q_e - q_t) against "t" (Figs. 10 and 11).

The integrated form of pseudo-second order kinetic equation is express as [45]

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad \text{---------- (9)}$$

Where q_e(mg g^{-1}) and qt(mg l^{-1}) are the adsorption capacities at equilibrium and at time "t" respectively, k_2 (g mg^{-1} min^{-1}) is the rate constant of pseudo-second-order equation. The kinetic data were fitted into the equation andq_e and k_2 were determined from the slope and intercept of the plot of t/q_t against "t" (Figs. 12 and 13)

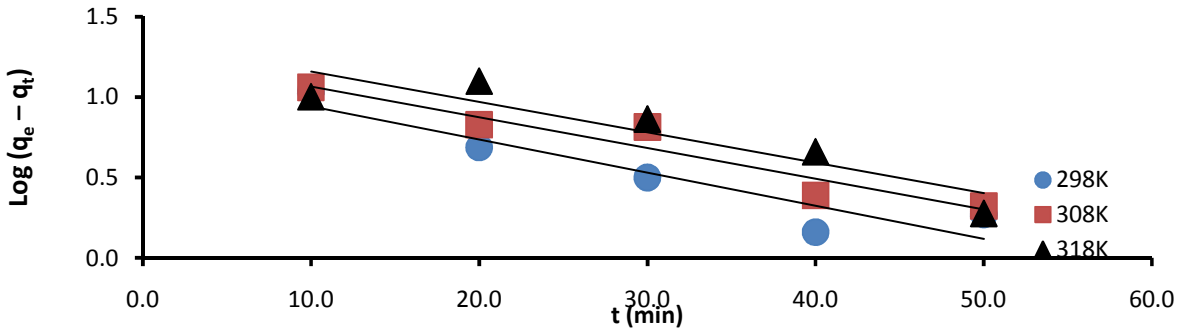

Fig. 10 Pseudo-first order kinetic plot at different temperature for the adsorption of Cr (VI) onto *Bacillus subtilis* immobilized in agarose.

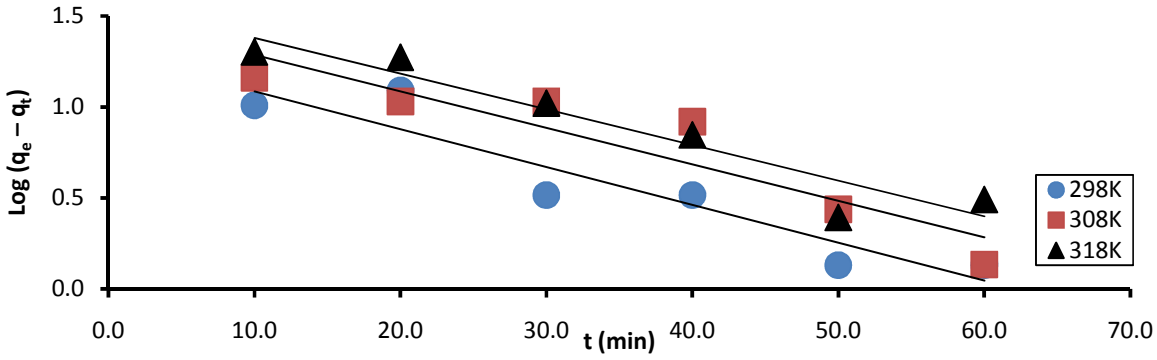

Fig.11 Pseudo-first order kinetic plot at different temperature for the adsorption of Ni(VI) onto *Bacillus subtilis* immobilized in agarose.

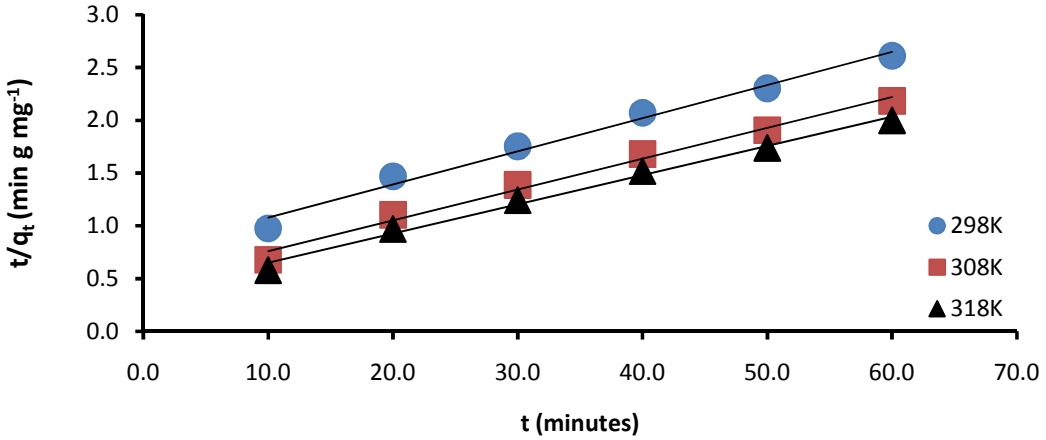

Fig. 12 Pseudo-second order kinetic plot at different temperature for the adsorption of Cr (VI) onto *Bacillus subtilis* immobilized in agarose.

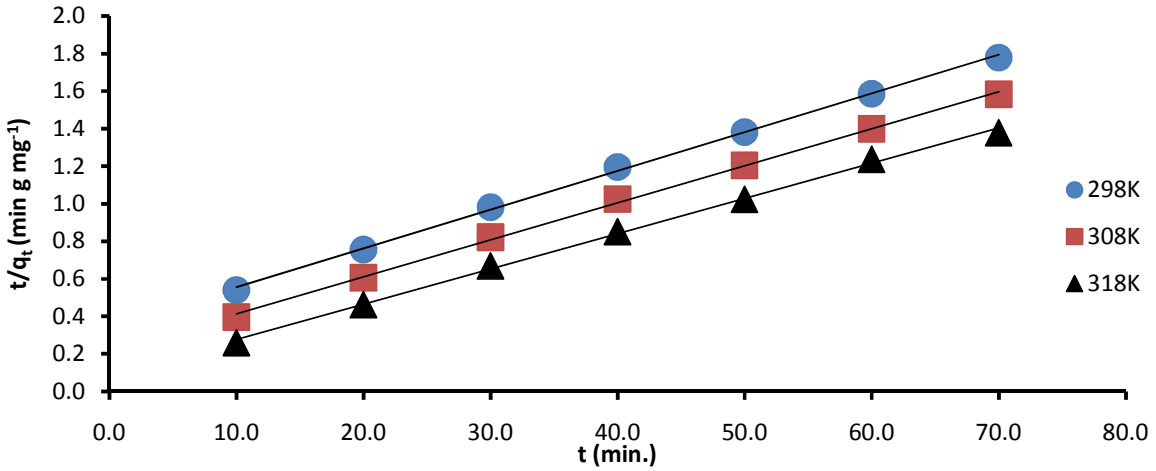

Fig. 13 Pseudo-second order kinetic plot at different temperature for the adsorption of Ni(II) onto *Bacillus subtilis* immobilized in agarose.

Kinetic parameters obtained from the plots and their correlation coefficients (R^2) are presented in Table 2. From Table 2, it can be concluded that pseudo-second order kinetic model fitted the adsorption data for both Cr(VI) and Ni(II) better than pseudo-first order kinetic model as presented in their respective correlation coefficient (R^2). This indicated that the adsorption process can be described as chemisorptions [31]. At the equilibrium time, for each of the adsorbent used, the amount of metal ion adsorbed per unit gram of the adsorbent (q_e mg g^{-1}) increased with increase in temperature, indicating the chemisorptive nature of the adsorption processes [30].

Table 2 Pseudo first order and pseudo-second order kinetic parameters for the adsorption of Cr(VI) and Ni(II) onto *Bacillus subtilis* immobilsed in agarose.

	PSEUDO FIRST ORDER KINETICS					
	CHROMIUM			NICKEL		
Temperature	q_e(mg g-1)	$k_1 \times 10^{-2}$(min^{-1})	R^2	q_e(mg g-1)	$k_1 \times 10^{-2}$(min^{-1})	R^2
298K	15.19 ± 1.10	42.50 ± 0.10	0.8883 ± 0.03	31.78 ± 0.58	59.38 ± 0.01	0.8976 ± 0.04
308K	19.20 ± 1.07	43.31 ± 0.08	0.9051 ± 0.02	39.60 ± 2.77	61.33 ± 0.01	0.8744 ± 0.03
318K	24.59 ± 2.24	45.40 ± 0.10	0.8850 ± 0.04	45.15 ± 1.42	63.33 ± 0.01	0.8743 ± 0.06
	PSEUDO FIRST ORDER KINETICS					
	q_e (mgg^{-1})	$k_2 \times 10^{-3}$ ($g\ min^{-1}\ mg^{-1}$)	R^2	q_e (mgg^{-1})	$k_2 \times 10^{-3}$ ($g\ min^{-1}\ mg^{-1}$)	R^2
298K	30.71 ± 1.20	1.220 ± 0.06	0.944 ± 0.01	44.88 ± 1.42	1.261 ± 0.05	0.997 ± 0.00
308K	33.59 ± 1.16	1.752 ± 0.06	0.989 ± 0.00	51.16 ± 2.15	1.845 ± 0.05	0.984 ± 0.01
318K	36.40 ± 1.33	1.995 ± 0.06	0.982 ± 0.01	56.63 ± 1.16	3.963 ± 0.02	0.973 ± 0.02

CONCLUSION

The results of this study revealed that *Escherichia coli* immobilized in agarose gel could be used as an effective adsorbents material for the removal of Cr(VI) and Ni(II) from water solution. The adsorption of the metal ions onto adsorbents used was found to be time, concentration, pH and temperature dependent. Maximum percentage removal occur at pH 4 for Cr(VI) and pH 6 for Ni(II) ions. The milligrams of metal ion adsorbed per gram of the adsorbent (q_e) were found to increase with increasing temperature, indicating endothermic and chemisorptive nature of the adsorption processes. The adsorption equilibrium data fitted Langmuir model better than Freundlich models. Time dependent data indicate the applicability of pseudo-second order kinetics model rather than pseudo-first order, which shows that the adsorption process was chemisorptions.

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