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ISOTHERM STUDIES FOR DETERMINATION OF REMOVAL CAPACITY OF BI-METAL (Ni AND Cr) IONS BY *ASPERGILLUS NIGER*

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Abstract

Pakistan is among the developing countries where there is a need to establish new industries to meet the demands of a growing population. This has led to industrial setup in various sectors, without proper planning and consideration for treatment of contamination, leading to disposal of untreated wastewater into nearby land and water bodies. This study was planned to investigate an indigenous *Aspergillus niger* for development of biosorbent for the removal of metal ions. The *Aspergillus* isolate's Ni and Cr removal efficiency was determined in batch mode over various pH (4.0-10.0) and temperature (25-40ºC) as single as well as bimetal ions. Using a single metal ion, maximum biosorption potential was obtained at pH 5.0-6.0 and 30-35ºC for both ions. On the other hand, Ni removal was reduced in the presence of Cr, while Ni removal influenced Cr removal with an increase showing maximum removal at an initial adsorbate concentration of 50mg/L, pH 6.0 and 35ºC. Effect of presence of bimetal in a solution on biosorption potential of *Aspergillus niger* was predicted by using equilibrium modelling. Adsorption trends for both nickel (R^2 0.9916) and chromium (R^2 0.8548) followed Langmuir isotherm in single metal removal system, but under bimetal condition chromium adsorption fitted better to Freundlich model and that of nickel followed Temkin isotherm, suggesting considerable change in behaviour and interaction between biosorbent and metal ions. Therefore, we concluded that *Aspergillus niger* a viable strain for development of a biosorbent for removal of a mixture of metal ions.

Introduction

Metal contamination is a global problem. Pakistan is no exception to it, where land and water bodies near industrial zones are reported to have elevated levels of multiple metal ions (Uzair *et al*., 2009). Main reason for this metal contamination is that 80% of the land is irrigated by wastewater generated from industry. The Indus river and its tributaries provide water to over 16 million hectares of land, situated in the mainly arid and semi-arid zones of the country. A rapidly growing population, saline groundwater, a poorly performing irrigation distribution system, and recurrent droughts have led to increased water shortages. Under these conditions, the use of untreated urban wastewater for agriculture has become a common and widespread practice (Ensink *et al*., 2002). Faisalabad city is the third largest city of Pakistan, where more than 2000 hectares are irrigated by untreated wastewater which has led to high heavy metal accumulation (Mn, Cu, Co and Zn) in wastewater irrigated agricultural land (Mahmood & Maqbool, 2006). Although chromium and lead levels were found to be high in almost all ground water sources, however, extremely high concentrations were found in industrial areas of Karachi (Haq *et al*., 2009).

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Chemical precipitation, oxidation/reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange, and membrane technologies are widely applied physico-chemical strategies employed to remove heavy metal from industrial wastewater. But these processes are found to be ineffective or expensive, especially when the heavy metal ions are in solutions containing 1-100 mg dissolved heavy metal ions/L (Rostami & Joodaki, 2002). Removal of heavy metal ions can be also accomplished by using biological material based biosorbent. Bacteria, yeast and algae can take up dissolved metals from their surroundings onto their bodies and can thus serve as biosorbent (Vijayaraghavan & Yun, 2008; Iram *et al.,* 2009; Yahaya *et al.,* 2009). These biosorbent either act as an adsorbent to remove metal ions by a metabolism independent mechanism called biosorption or metabolism dependent metal uptake mechanism termed bioaccumulation. Both processes provide an attractive alternative to physico-chemical methods (Chojnacka, 2007). Thus, research for development of biosorbent involves identification and screening of microbes for newer, efficient and sustainable biosorbent has emerged in the past few years. As wastewaters are usually comprised of a discharge containing multiple metal ions and organic pollutants, this offers difficulties in describing the adsorption of pollutants from such waste streams. When several components are present, interference and competition phenomena for adsorption sites occur. Therefore, multi-metal biosorption studies are conducted in order to find out relationships between the adsorbed quantity of one component and the concentrations of all other components, either in solution or already adsorbed. Several isotherms have been proposed to describe equilibrium for such a system. Equilibrium studies are important for studying these microbes as to determine the capacity of the biosorbent along with relationships between the adsorbent and adsorbate. These studies are based on adsorption isotherms which are computed from ratios between the quantity adsorbed, and that which remains in solution at a fixed temperature at equilibrium (Jalali *et al.*, 2002; Khambhaty *et al.,* 2009).

This study was planned to evaluate an indigenous *Aspergillus niger* as a potential biosorbent for removal of nickel and chromium singly, as well as a bimetal, from assimilated wastewater. Equilibrium studies involving Freundlich, Langmuir and Temkin isotherms were used to assess metal ion removal capacity of this strain for bioremediation of contaminated wastewater.

Materials and Methods

Chemicals used: Majority of chemicals and media compounds were obtained from BDH Laboratory Chemical Division (UK), DIFCO Laboratory (USA) and MERCK (Germany).

Preparation of adsorbate solutions: Stock solutions of chromium and nickel were prepared by dissolving $K_2Cr_2O_7$ and $NiCl_2.6H_2O$ in distilled deionized water. Further working solutions were prepared by diluting this stock solution. Initial pH was adjusted using 0.1N HNO_3 and 0.1N NaOH.

Identification: *Aspergillus niger* was initially identified by its phenotypic and cultural characteristics, followed by PCR based identification using primer against internal transcribed spacer (ITS) region of 18S-rDNA sequence, for confirmation. Amplification of DNA extracted from *Aspergillus niger* was done by method proposed by Zhao *et al.,* (2001) with slight modifications, in a Mastercycler Gradient Eppendorf AG 22351

Hamburg, programmed for a first denaturation step of 5 min at 95ºC, followed by second denaturation (30s at 95ºC), annealing (58ºC for 30s) and primer extension (72ºC for 1min., for 40 cycles with final extension for 10 min., at 72ºC.

Batch mode experiments for single metal removal: In order to study biosorption potential of *Aspergillus niger* for chromium and nickel individually different concentration of metal ions (25-100mg/L) were used. In addition, environmental conditions, such as pH (5.0-10.0) and temperature (25-40ºC), were also investigated to find out optimum pH and temperature for further equilibrium studies. *Aspergillus niger* was inoculated in Sabouraud dextrose broth containing different concentrations of Ni and Cr. Each flask was inoculated with $1x10^6$ spores/mL, for six days after adjusting pH to 5.0 at 30ºC, and placed in an orbital shaker (100 rpm) where as controls (without the metal salt and biomass) were also run under same prescribed conditions. Samples and biomass were drawn at the end of specified period of time and then further analyzed for metal content. All the experiments were run in duplicates.

Batch mode experiments for bi-metal removal: Bi-metal ion removal was determined over different concentrations of both metal salts (mixture solution of salts $K_2Cr_2O_7$ and $NiCl_2.6H_2O$). Different salt concentrations were used to make the final concentration equal to 25ppm, 50ppm, 75ppm and 100ppm in distilled deionized water. Bimetal removal and equilibrium studies were performed as described in the previous paragraph.

Analytical procedure: In all sets of experiments to determine residual Cr and Ni, 5mL of samples were drawn from each flask at different time intervals. These samples were digested by HNO_3 for metal analysis, biomass was filtered and dried at 55°C as described earlier (Faryal *et al.,* 2006). Residual Ni and Cr concentrations left in these samples were measured by using air-acetylene flame of Solar Unicam atomic absorption spectrophotometer for Ni and Cr at 232.0.3 and 357.9 nm respectively (Clesceri *et al.,* 1989).

The data obtained in batch mode studies was used to calculate the equilibrium metal uptake capacity (*qe*) and biosorption efficiency (*E*) by using the following equations:

$$q_e = V\left(\frac{C_i - C_f}{m}\right)$$

$$E = \left(\frac{C_i - C_f}{C_i}\right)100$$

where C_i is the initial concentration of the metallic ion (mg/L), C_f is the final concentration of the metallic ion (mg/L), *m* is the mass of the biosorbent in the reaction mixture (g), and *V* is the volume of the reaction mixture (L).

Equilibrium modelling: *Aspergillus niger* biosorption potential was predicted by using isotherms based equilibrium modelling. Biosorption isotherm data explains distribution of adsorbate molecules (Cr and Ni) between the liquid (metal salt solution) and solid phase (biomass) when the adsorption process reaches equilibrium state (Babarinde *et al.,* 2008; Khambhaty *et al.,* 2009). Three most commonly used adsorption models, namely Freundlich, Langmuir and Temkin were applied to evaluate feasibility of adsorbate-adsorbent (metal ions to biomass) interaction. This adsorption data was analyzed with the help of the following forms of isotherms:

Freundlich Isotherm: $q_e = K_f C_e^{1/n}$

Linearised Freundlich Isotherm: $q_e = logK_f + \left(\frac{1}{n}\right) logC_e$

where, 'K_f' and 'n' are Freundlich constants, which correlated to the maximum adsorption capacity and adsorption intensity, respectively ($1/n = 0$ shows that the partition between two phases does not depend on the concentration, $1/n < 1$ corresponds to a normal L-type Langmuir isotherm while $1/n > 1$ indicates a cooperative sorption involving strong interactions between the molecules of adsorbate).

Langmuir Isotherm: $q_e = \frac{q_{\max} b C_e}{1 + bC_e}$

Linearised Langmuir Isotherm: $\frac{1}{q_e} = \frac{1}{q_{\max}}\left(\frac{1}{bC_e + 1}\right)$

where 'q_{max}' is the maximum metal uptake under the given conditions, 'b' is a constant related to the affinity between the biosorbent and sorbate. This model provides the maximum values where they can not be reached in the experiments. The essential characteristics of the Langmuir isotherms can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L, which is defined as:

$$R_L = \left(\frac{1}{1 + bC_O}\right)$$

The R_L value indicates the shape of isotherm ($R_L > 1$ Unfavourable; $R_L = 1$ Linear; $R_L = 0$ Irreversible; $0 < R_L < 1$ Favourable).

Temkin isotherm is based on the assumption that heat of adsorption would decrease linearly with increase of coverage of adsorbent due to sorbate/sorbent interaction (Yu & Ya-Juan, 2008).

Temkin Isotherm: $q_e = \frac{RT}{b_T} In(a_T C_e)$

Linearised Temkin Isotherm: $q_e = BIna_T + BInC_e$

where a_T (L/g) is the equilibrium binding constant, corresponding to the maximum binding energy, b_T is a constant related to the heat of adsorption, T is the temperature (K), and R is the ideal gas constant (8.315 J mol^{-1} K^{-1}).

Table 1. Influence of initial concentration of adsorbate, pH and temperature on biosorption efficiency of Ni and Cr under single and bimetal conditions.

Variable parameter	Level	Maximum Biosorption Efficiency (%)			
		Ni	Cr	Ni + Cr	
Initial adsorbate Conc. (mg/L)	25	70.96	22.40	70.86	75.35
	50	86.86	32.80	57.32	45.49
	75	39.26	14.90	32.43	34.34
	100	25.76	13.50	24.89	11.15
Temperature (ºC)	25	39.60	25.50	37.00	46.00
	30	86.66	29.50	43.56	56.28
	35	96.92	32.80	56.4	96.64
	40	75.70	23.00	32.56	36.24
pH	3.0	39.70	25.80	54.60	43.60
	4.0	49.50	28.70	74.88	45.56
	5.0	86.60	35.50	83.88	61.70
	6.0	94.47	37.23	94.90	70.32
	7.0	78.30	35.00	78.08	42.00
	8.0	27.60	32.80	57.80	26.20
	9.0	20.15	05.40	44.92	27.44
	10.0	21.67	04.80	32.36	26.96

Results and Discussion

On the basis of morphology, the strain was identified as a member of the genus *Aspergillus*. It was confirmed to be *Aspergillus niger* by PCR based identification using primer against the internal transcribed spacer region (ITS) of 18S-rDNA sequence. The amplified fragment was sequenced using the facility of Macrogen, South Korea and sequence was compared using blast$_n$. This sequence showed 100% homology to *Aspergillus* and was deposited with accession number EU021051 in NCBI database.

Ni and Cr removal was optimized by growing *Aspergillus niger* under various pH (4.0-10.0) and temperatures (25-40ºC). Results obtained from batch mode study of single as well as bimetal ions performed at various environmental conditions are summarized in Table 1.

In single metal batch system, maximum biosorption of Cr (32.8%) and Ni (86.86%) was seen at 50mg/L, however increasing concentration lead to decrease in removal of both metal ions. This decrease in metal removal was directly reflected in biomass production by the growing fungus (13.5 and 25.76% at 100mg/L for Cr and Ni respectively). This could be attributed to toxicity caused by high concentration of nickel to growing biomass and also due to negative effects on the enzyme machinery (Rajendran *et al.,* 2002). At low metal concentrations (25-50mg/L), the biosorption capacity of the biosorbent was not fully utilized. All this data clearly reveals the existence of a finite heavy metal binding site. Optimum temperature of removal for both nickel (86.66-96.92%) and chromium (29.5-32.8%) was found in range of 30-35ºC. Decreasing temperature below 30ºC decreased fungal growth and enzymatic activity. Furthermore, increase in the temperature to 40ºC, decreased the fungal growth as well as removal efficiency (Sepehr *et al.,* 2005). At higher temperature there was a decrease of biosorption capacity which may be due to the damage of active binding sites in the biomass (Özer & Özer, 2003).

When single ion was used strain showed maximum removal between pH 5.0 and 6.0 *i.e.,* 35.5-37.23% and 86.6-94.47% for chromium and nickel respectively. There was an increase in biomass over pH range of 5.0-7.0 which substantiates that the biosorption increase is in direct correlation with increase in number of functional groups on the mycelia of the *Aspergillus niger* (Murphy *et al.*, 2007). The low level of nickel uptake at lower pH values (3.0-4.0) could be attributed to the increased concentration of hydrogen (H^+) and hydronium (H_3O^+) ions competing for nickel binding sites on the biomass. The increase in nickel biosorption at relatively higher pH (5.0-7.0) may be explained by the ionization of functional groups on the cell surface which serves as the binding sites for the metal ions. On the other hand biosorption efficiency of chromium was relatively less affected by charge in pH (3.0-8.0).

However, in bimetal removal experiments behaviour of both metal ions was different in terms of their biosorption efficiency. In order to assess removal of multiple metal ions at the same time by the selected strain, a mixed salt solution of both nickel and chromium was prepared. With increase in initial concentration of metal ions (25-100mg/L) biosorption efficiency decreased for each metal. Nickel removal was reduced in the presence of chromium from 86.86% to 57.32% at initial concentration 50mg/L. Interestingly chromium removal was increased (32.8% to 45.49% at 50mg/L) showing positive effect of presence of nickel ions in the solution. This probably may be reflective of change in solution chemistry due to presence of second metal salt, as metal salts in the solution dissociate into cations and anions differentially, thus affecting each other causing change in preference of metal adsorption (Romera *et al.,* 2008). Removal of nickel and chromium increased with increase in temperature was observed upto 35ºC, which may be due to energy input requirement in the process of biosorption. The biosorption potential of both metals decreased in bimetal system as compared to single metal system (Hussein *et al.,* 2004; Preetha & Viruthagiri, 2007).

Bimetal removal was enhanced with increase in pH from 3.0-6.0. In case of both metals the removal at all pH levels was higher in comparison to single metal system. This may be due to the fact that the ionic forms of chromium (CrO_4^-) and nickel (Ni^{2+}) tend to balance the initial pH *i.e.* at pH 3.0 the increased number of H_3O^+ and H^+ which inhibit nickel removal in single metal system are balanced by CrO_4^- ions. This assumption could be proved by controlling pH through out the entire contact period until equilibrium is reached (Babalola *et al.,* 2009) but this is a fairly complex task, which necessitates sophisticated instrumentation.

To determine the biosorption behaviour of Ni and Cr in single and bimetal system, equilibrium data were evaluated using three widely used biosorption isotherms, viz., Langmuir, Freundlich and Temkin isotherms. The individual adsorption constants for each component obtained by evaluating the isotherms are listed in Table 2 along with the linear regression coefficients.

The Langmuir model was slightly better fit to the adsorption data of Ni (R^2 0.9916) than Freundlich model (R^2 0.9886) in the studied concentration range (25-100mg/L) in a single metal removal system (Fig. 1a, 2a, 3a). Cr adsorption data exhibited a better fit for Langmuir model (R^2 0.8548) in comparison with Temkin (R^2 0.5987). Although R^2 value of Cr was high (0.9479) but data did not follow Freundlich isotherm as the value of '*n*' was less than one (0.27). The strain showed better biosorption potential for nickel as compared to chromium as indicated by high maximum biosorption capacity 'q_{max}' (28.1mg/g and 6.97mg/g) and higher binding capacity 'K_f' (0.15 and 3.04 x 10^{-6} L /g), for Ni and Cr respectively. High values of '*b'* are reflected in the steep initial slope of a sorption isotherm, indicating desirable high affinity (Table 2). As a higher '*b'* value was obtained for chromium than nickel, it indicates higher adsorption energy and relatively faster increase in adsorption at low concentrations of adsorbate (Fig. 1a, 2a) as cited by Davis *et al.* (2003). R_L value for nickel and chromium biosorption lied between 0.68-0.89 and 0.5-0.91 respectively indicating favourable adsorption for both metals.

Table 2. Langmuir, Freundlich and Temkin isotherm constants under single and bimetal conditions.

Isotherms	Parameters	Nickel	Chromium	Ni + Cr	
				Nickel	Chromium
Langmuir	q_{max} (mg/g)	28.10	6.97	1.49	1.06
	b (L /mg)	0.00462	0.01	-1.36	-0.773
	R	0.995	0.924	0.802	0.485
	R^2	0.9916	0.8548	0.6437	0.2350
Freundlich	K_f (L /g)	0.15	3.06×10^{-6}	2.39	2.295
	n	1.09	0.27	7.57	4.48
	R	0.994	0.973	0.629	0.750
	R^2	0.9886	0.9479	0.3960	0.5626
Temkin	a_T (L /g)	0.0063	8.338×10^{-10}	2.06	1.68
	b_T (kJ/mol)	0.862	0.310	-163.2	-178.68
	R	0.940	0.773	0.809	0.514
	R^2	0.8842	0.5987	0.6560	0.2624

Adsorption trend changed considerable in bimetal system, as indicated by the fact that equilibrium data of Cr removal better fitted to Freundlich (R^2 0.5626) where as that of Ni fitted to Temkin model with R^2 value of 0.6560 (Fig 3a, 3b, 3c). Analysis of Freundlich constant for chromium removal under single and bimetal system showed considerable increase in both adsorption capacity (K_f 2.295 L /g) and heterogenosity (n 4.485) of cell surface. Low K_f (3.06×10^{-6} L /g) and n (0.27) for Cr in case of single metal system may suggest the involvement of very few and similar functional groups, whereas with addition of Ni in the solution the different functional groups were exposed as a result of interaction of Ni^{+2}(cations) and CrO_4^- (anions).

Nickel biosorption in bimetal system followed Temkin isotherm, where as in single metal system it fitted better to Langmuir isotherm indicating change in interaction between adsorbate and biosorbent (Fig. 1a, 3c). The constant b_T reflects bonding energy which in turn dictates the type of interaction. Typical bonding energy range for ion-exchange mechanism is reported to be 8–16 kJ/mol (Helfferich, 1962). It is observed that upto −20 kJ/mol is surface indicative of physisorption process due to electrostatic interaction between charged molecules whereas more negative than −40 kJ/mol involves chemisorption (Zafar *et al.,* 2007). In our study of bimetal ion removal system, high value of b_T (-163.2 kJ/mol) indicate chemisorption, whereas low values in the case of single metal study (0.862 kJ/mol) indicate that interactions between the sorbate and sorbent are neither purely through ion-exchange nor purely through physisorption (Kiran & Kaushik, 2008).

Although the data in case of bimetal ions did not fit well to Langmuir model but it helped in prediction of maximum biosorption capacity, which indicated that biosorption capacity in our study for nickel was slightly more severely effected by the presence of chromium (dropping from 28.1 to 1.49mg/g) than that of chromium by nickel (dropping from 6.97 to 1.06 mg/g).

The present study also revealed that the chemical interaction of multiple ions is complex and thus requires further analysis and research for development of a viable bioreactor for metal removal from wastewater containing multiple metal ions.

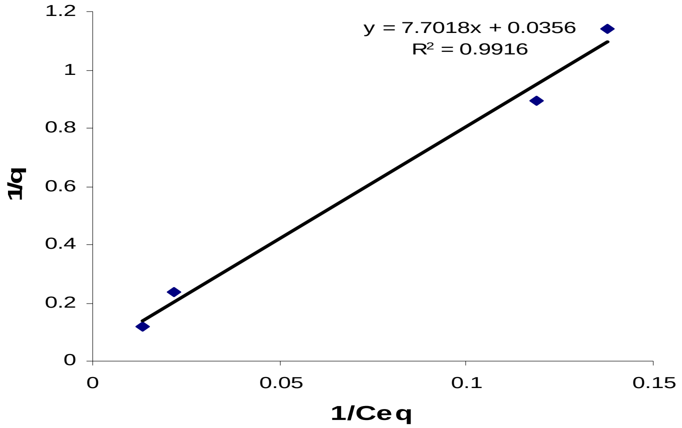

Fig. 1a. Langmuir adsorption isotherm for Ni biosorption by *Aspergillus niger* under single metal system.

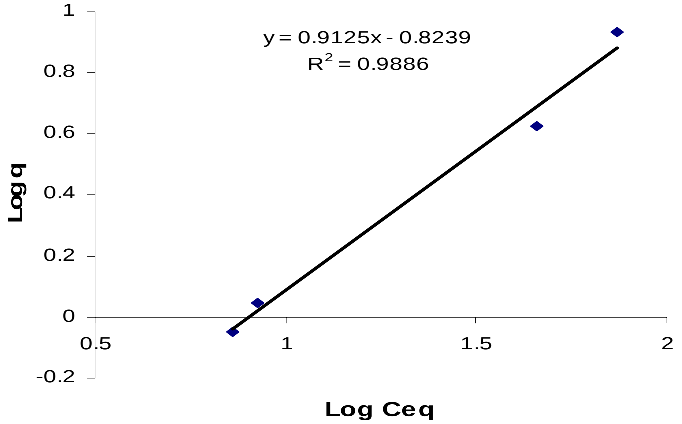

Fig. 1b. Freundlich adsorption isotherm for Ni biosorption by *Aspergillus niger* under single metal system.

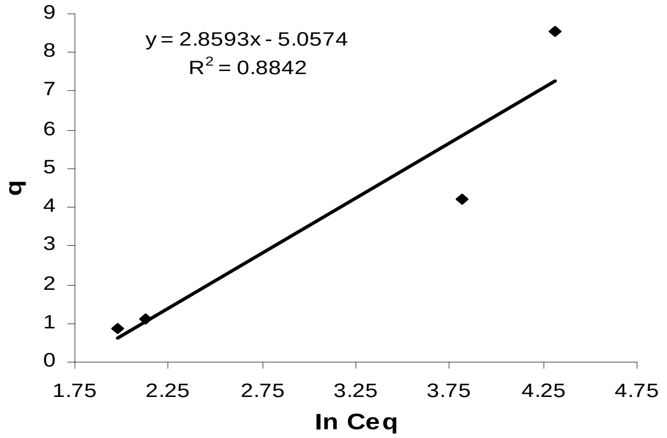

Fig. 1c. Temkin adsorption isotherm for Ni biosorption by *Aspergillus niger* under single metal system.

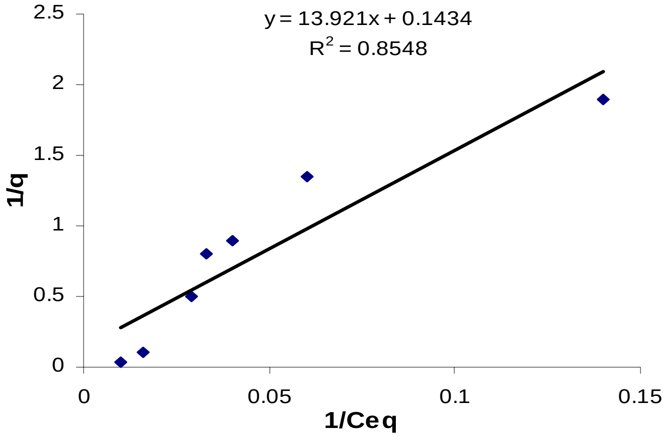

Fig. 2a. Langmuir adsorption isotherm for Cr biosorption by *Aspergillus niger* under single metal system.

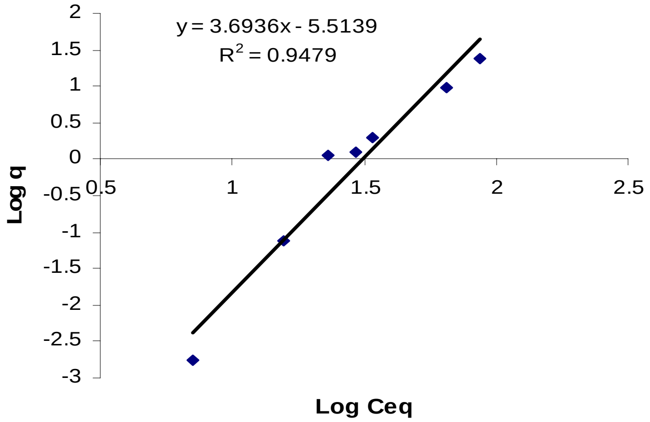

Fig. 2b. Freundlich adsorption isotherm for Cr biosorption by *Aspergillus niger* under single metal system.

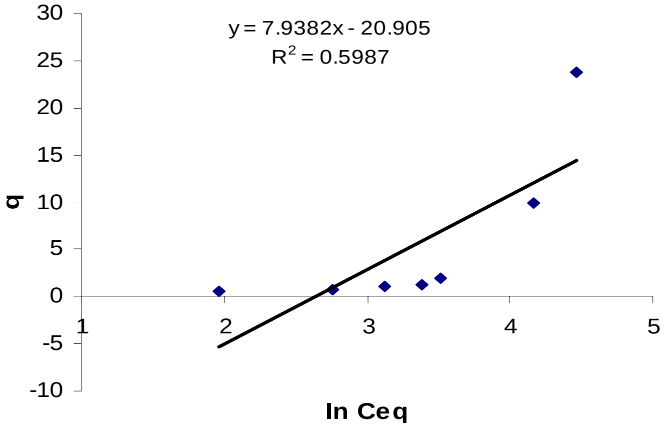

Fig. 2c. Temkin adsorption isotherm for Cr biosorption by *Aspergillus niger* under single metal system.

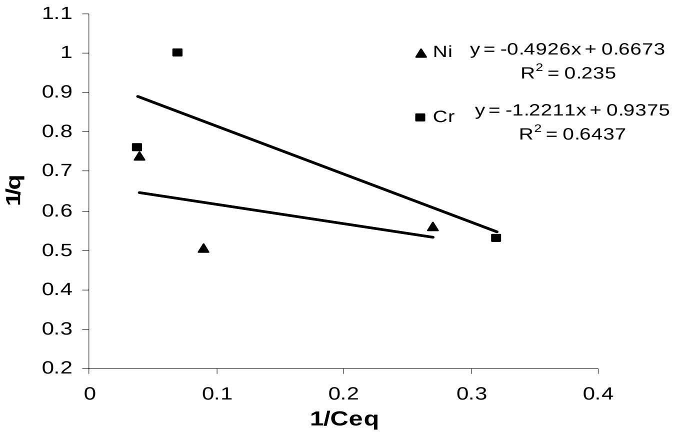

Fig. 3a. Langmuir adsorption isotherm for Ni and Cr biosorption by *Aspergillus niger* under bimetal system.

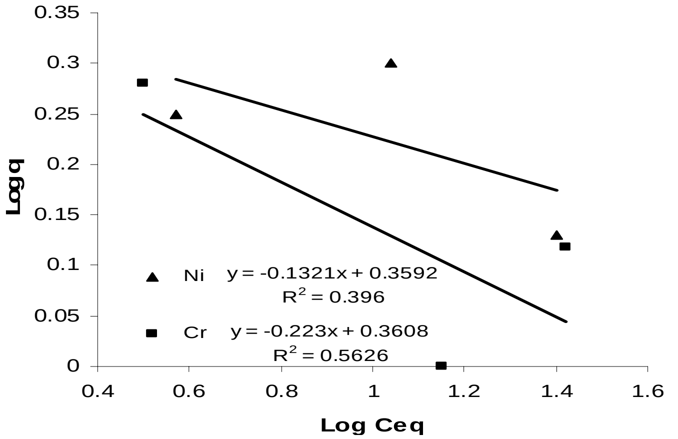

Fig. 3b. Freundlich adsorption isotherm for Ni and Cr biosorption by *Aspergillus niger* under bimetal system.

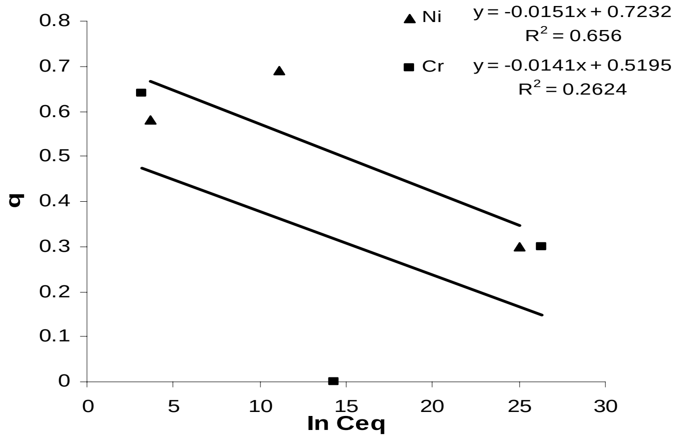

Fig. 3c. Temkin adsorption isotherm for Ni and Cr biosorption by *Aspergillus niger* under bimetal system.

Conclusion

This study showed that *Aspergillus niger* can efficiently remove both chromium and nickel ions from aqueous solution. It also showed that the biosorption process was pH, contact time, temperature and concentration dependent. The competitive effect was exerted by the both heavy metal ions on each other, leading to greater Cr removal Ni. Although, it seems that metals are not necessarily bound by exactly similar mechanisms but in a mixture interfered with each other's uptake. Biosorption equilibrium data for both chromium and nickel fitted well to Langmuir model in single metal removal system. On the other hand, Ni biosorption in bimetal system followed Temkin isotherm better than other two models, showing chemisorption and indicating change in behaviour and interaction between biosorbent and metal ions. There are only a couple of studies on metal ion removal from Pakistan, and this study reports *Aspergillus niger* a candidate for bimetal ion removal over wider range of pH and temperature, without toxicity to fungus.

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