



Equilibrium Modelling of Simultaneous Biosorption of Chromium (VI) and Nickel (II) onto Dried Fungal Biomass

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 3-9-2010
Accepted: 29-9-2010

Key Words:

Chromium (VI), Nickel (II)
Biosorption
Binary system
Adsorption models
Isotherms

ABSTRACT

The biosorption of Cr (VI) and Ni (II) ions, both singly and in a binary system, by dried biomasses of *Rhizopus arrhizus* and *Mucor hiemalis* was investigated in a batch system as a function of initial pH. The working initial pH values for Cr (VI) and Ni (II) in a single biosorption system were determined as 2.0 and 8.0 respectively. In a binary system, the optimum pH for sorption of Cr (VI) remained the same (2.0) while for Ni (II) it changed to 5.0. Although both the biomasses had a higher adsorption capacity for Cr (VI) and Ni (II) at single-component situation due to the initial pH of the solution, the equilibrium uptakes of Cr (VI) and Ni (II) in the binary mixture were found to be decreasing because of the antagonistic interaction between the components. It was observed that the co-ion effect on the equilibrium uptake became more pronounced as pH level increased for Cr (VI) and decreased for Ni (II). Adsorption isotherms were developed for the dual-metal ion system at these two pH values and expressed by the mono-component Langmuir and Freundlich adsorption models. The model parameters were estimated by the non-linear regression.

INTRODUCTION

Heavy metal releases into the environment have been increasing continuously as a result of rapid industrial and technological development, posing a significant threat to the environment and public health because of their toxicity, biomagnification in the food chain and persistence in nature (Ceribasi & Yetis 2001). Chromium and nickel are released into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc. (Congeevaram et al. 2007, Preetha & Viruthagiri 2007). These two metals are of major concern because of their large usages in developing countries and their non-degradable nature. Chromium exists in several oxidation states out of which Cr (III) and Cr (VI) are the most stable forms. Hexavalent chromium is highly soluble in water and is of special concern because of its high toxicity and potential carcinogenicity (Tewari et al. 2005). Ni (II) is more toxic and carcinogenic metal when compared with Ni (IV). Due to their adverse effects on the living systems, stringent limits have been stipulated for the discharge of chromium and nickel into the environment. According to ISI: Bureau of Indian Standard (BIS) the industrial effluent permissible discharge level of Cr (VI) and Ni (II) into inland water is 0.1 and 3.0 mg/L respectively (Congeevaram et al. 2007).

With increased environmental awareness, development of new methods for metal removal of heavy metal ions and recovery from dilute solutions (1 to 100 mg/L) has become imperative (Ceribasi & Yetis 2001). The conventional

processes for the removal of heavy metals from water such as flocculation, precipitation, electrolysis, ion exchange, crystallization, etc. are expensive and non-ecofriendly. These methods also suffer from severe constraints, such as incomplete metal removal, high reagent or energy requirements, generation of toxic sludge or other waste products that require safe disposal (Tewari et al. 2005). Thus, there is a need for the development of new cost effective methods that are environment friendly.

In this endeavour, microbial biomass has emerged as a potent bioremediator, presenting an option for developing economic and ecofriendly wastewater treatment processes (Preetha & Viruthagiri 2007). Nonliving and dead microbial biomass may passively sequester metal(s) by the process of biosorption from dilute solutions. Generally, biosorptive processes can reduce capital costs by 20%, operational costs by 36% and total treatment costs by 28% when compared with conventional systems (Loukidou et al. 2003). The process can be made economical by procuring natural bulk biomass or spent biomass from various fermentation industries. The performance of a biosorbent can further be improved by various physical and chemical treatments. The economics of these sorbents merit their commercialization over chemical ion exchangers (Sag et al. 2001).

Much of the work on the biosorption of heavy metal ions by various kinds of microorganisms has been focused on the uptake of single metal. Bioremoval of single species of metal ions is affected by several factors viz., the specific surface properties of the microorganisms and the physico-chemical

parameters of the solution such as temperature, pH, initial metal ion concentration and biomass concentration. Nevertheless, in practice, wastewaters are usually comprised of a discharge containing multiple metal ions and organic pollutants. When several components are present, interference and competition phenomena for adsorption sites occur. The combined effects of two or more metal ions on microorganisms depend on other factors as well, such as the number of metal ions competing for binding sites, metal ion combination, levels of metal ion concentration and order of metal ion addition (Aksu et al. 2002). This offers difficulties in describing the adsorption of pollutants from such waste streams. Hence, to design a cost-effective sorption system, the biosorption capacity of a metal in the presence of other metals has to be quantified. 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 at a fixed temperature at equilibrium (Chong & Volesky 1995, Khambhaty et al. 2009, Munir et al. 2010). Equilibrium studies determine the capacity of the biosorbent along with relationships between the adsorbent and adsorbate.

The objective of the present study was to investigate the use of the dead biomasses of *Rhizopus arrhizus* and *Mucor hiemalis* as potential biosorbents for the removal of Cr (VI) and Ni (II) simultaneously in a binary system and compare it with single metal [Cr (VI) or Ni (II)] situation in a batch system. The choice of metals has been made with regard to their industrial use and potential pollution impact. Equilibrium studies involving Langmuir and Freundlich isotherms were used to assess metal ion removal capacity of these fungal biomasses.

MATERIALS AND METHODS

All chemicals (AR grade) were procured from Hi Media Laboratories, Mumbai.

Preparation of adsorbate solutions: 1000 ppm stock solutions of chromium and nickel were prepared by individually dissolving 0.280g of $K_2Cr_2O_7$ and 0.480 g of $NiSO_4 \cdot 7H_2O$ in 1000 mL deionized distilled water. Further working solutions of 50 ppm were prepared by diluting the stock solutions appropriately. For single metal condition, 100mL of 50 ppm Cr (VI) and Ni (II) were added separately into 250mL Erlenmeyer flasks, whereas in case of binary metals, 100mL of binary metal solution containing 50 ppm each of both the metals were added in the same flask. Initial pH was adjusted using 0.01N HCl and 0.01N NaOH.

Preparation of the biosorbents: Pure strains of *Rhizopus arrhizus* NCIM 997 and *Mucor hiemalis* NCIM 873,

obtained from National Chemical Laboratory, Pune, were cultured individually in potato dextrose broth (g/L: potato 200, dextrose 25, yeast extract 0.1, pH 5.0) containing 0.25% tween 80 (to prevent sporulation) under static conditions at $28 \pm 2^\circ C$. The biomasses were harvested after seven days, washed thoroughly with distilled water and dried at $80^\circ C$ in an oven for 24 h. Dried biomasses were ground in a mortar and pestle and sieved through a 150 mesh sieve to give uniform particle size.

Physico-chemical treatment of the biomasses: One g of *Rhizopus arrhizus* biomass was contacted with 100 mL 0.5 N HNO_3 for 24h at $28 \pm 2^\circ C$ with shaking at 120 rpm followed by physical treatment of autoclaving at 10 lbs for 30 min (Bai & Abraham 2002). Pre-autoclaved (10 lbs for 30 min) and dried biomass of *Mucor hiemalis* was treated with 0.5 N Na_2CO_3 in a manner exactly similar to that of acid treatment (Yan & Viraraghavan 2000). Both the physico-chemically pretreated biomasses were vacuum filtered, washed and dried at $60^\circ C$ for 6 h followed by re-sieving through a 150 mesh sieve.

Batch mode experiments for single metal removal:

Biosorption experiments were carried out in batch in 250-mL Erlenmeyer flasks to elucidate the sorption potential of both the untreated biomasses of *Rhizopus arrhizus* and *Mucor hiemalis* for both chromium and nickel over a pH range of 2.0-8.0. Dried biomass each of *Rhizopus arrhizus* and *Mucor hiemalis* (50 mg) was added to 100 mL of solutions containing 50 mg/L of Cr (VI) and Ni (II) individually. These flasks were kept at $28 \pm 2^\circ C$ on a rotary shaker at 150 rpm. After agitating the solutions for 1 h, they were filtered using Whatman No. 1 filter paper and the filtrate was then analyzed for chromium (VI) and nickel (II) concentrations (Tewari et al. 2005). Effectiveness of the physico-chemically treated biomasses of *Rhizopus arrhizus* and *Mucor hiemalis* was also evaluated in the biosorption of the metals for which they were found to be effective at the optimum pH.

Batch mode experiments for bi-metal removal:

The sorption of chromium (VI) and nickel (II) in a binary system was studied in batch experiments to evaluate the effect of each metal on the biosorption of the other. 50 mg each of the physico-chemically pretreated biomasses of *Rhizopus arrhizus*, *Mucor hiemalis* and a combination of both mixed in a 1:1 ratio was employed as biosorbent to optimize the pH of the binary system (50 mg/L of each metal) as described above (Aksu et al. 2002, Munir et al. 2010). The removal of the metals in a binary system was compared to the removal of individual metals in a single metal system.

Application of mono-component adsorption models to the biosorption of Cr (VI) and Ni (II):

The kinetic equilibrium studies for both the metal ions were carried out by

using the best biosorbent as optimized in the above step, at the optimum pH for each metal ion in a binary system. The effect of time on the biosorption of the binary metals was studied in order to demonstrate the kinetic modelling of the reaction. Sorbents (50 mg) optimized in the above step were added to 100 mL of binary metals solution (50 mg/L of each metal) at optimum pH for binary metals in 250-mL Erlenmeyer flasks under shaking conditions as described above. Samples were analyzed for concentration of both the metals after every 15 min for 300 min. The rate of biosorption of ions by the biosorbents was determined by plotting a graph of percent removal of Cr (VI)/Ni (II) ions v/s time. Biosorption potential of both the biosorbents was predicted by using isotherms based equilibrium modelling (Aksu et al. 2002, Munir et al. 2010).

Measurement of heavy metal concentration: The concentration of the chromium (VI) ions was determined spectrophotometrically at 540nm using 1, 5-diphenylcarbazide (DPC) to produce a red-violet coloured compound (APHA 1986). The concentration of Ni (II) ions was determined spectrophotometrically by dimethylglyoxime (DMG) at 366 nm (Armit & Harden 1906, Morgan 1960).

The amount of Cr (VI)/Ni (II) biosorbed per gram of dried biomass was calculated using the following equations:

The removal efficiency the of metal ion was calculated by using the formula,

$$\% \text{ Removal} = \frac{(C_i - C_f)}{C_i} \times 100$$

The sorption capacity of metal ion is the concentration of metal ion on the fungal biomass and can be calculated based on the mass balance principle,

$$q = \frac{V(C_i - C_f)}{m}$$

Where, in the two equations,

q = Chromium (VI) uptake (mg/g cell dry weight)

V = Volume of metal-bearing solution contacted (batch) with the biosorbent (L)

C_i = Initial concentration of metal in the solution (mg/L)

C_f = Final concentration of the metal in the solution (mg/L)

m = Dry weight of the biosorbent added (g)

Biosorption experiments were conducted in triplicates and average values were used in the analysis. Standard deviation is indicated wherever necessary. All the statistical analysis was carried out using Microsoft Excel 2007® to evaluate the results that are significant within the experimental error.

RESULTS AND DISCUSSION

Simultaneous biosorption of Cr (VI) and Ni (II) onto dried biomass of *Rhizopus arrhizus* and *Mucor hiemalis* from binary mixture was investigated and compared with single Cr (VI) and Ni (II) situation in a batch stirred system in this study. The single-component sorption phenomena were expressed by the mono-Langmuir and Freundlich adsorption models, which were developed at pH 2.0 and 5.0. The model parameters were determined by nonlinear regression. The equilibrium results were given as the units of adsorbed Cr (VI) or Ni (II) quantity per gram of dried biomass (q_{eq} ; mg/g) and unadsorbed Cr (VI) or Ni (II) concentration (C_{eq} ; mg/dm) at the equilibrium.

Effect of initial pH on the biosorption of Cr (VI) and Ni (II) in a binary system: The process of biosorption is microorganism-dependent and depends on the type of the biomass and the type of biomass preparation (Volesky 2001). Difference in cell wall composition and different adsorptive sites among different microorganisms can cause significant differences in the type and amount of metal ion binding to them. Another important parameter in the biosorptive process is pH which affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of the metallic ions (Ahalya et al. 2003). Different metal ions may have different pH optima, possibly due to the different solution chemistry of the species. Change in the pH of the solution affects the surface charge of the adsorbents, speciation of different pollutants, the degree of ionization, as well as the adsorptive process through dissociation of functional groups on the adsorbent surface active sites. The adsorption of various anionic and cationic species on such adsorbents has been explained on the basis of the competitive adsorption of H^+ and metal ions with the adsorbates. It is a common observation that the surface adsorbs anions favourably at lower pH due to the presence of H^+ ions, whereas, the surface is active for the adsorption of cations at higher pH due to the deposition of ions (Abdullah et al. 2010). Hence, pH was optimized for the sorption of the metal ions in single metal as well as in a binary metals solution.

Biomasses prepared from *Rhizopus arrhizus* and *Mucor hiemalis* were evaluated for their ability to remove both Cr (VI) and Ni (II) from solutions over a pH range of 2.0-8.0 (Table 1). *Rhizopus arrhizus* was more efficient in the removal of Cr (VI) ions (20.78±0.98%) at pH 2.0 compared to *Mucor hiemalis* (11.42±0.42%) showing 81.96% more sorption of Cr (VI) while *Mucor hiemalis* sorbed Ni (II) more efficiently (5.35±0.23%) at pH 8.0 which was 52.85% more as compared to *Rhizopus arrhizus* (Table 1). Both the

biomasses showed decreasing sorption of Cr (VI) with increase in pH showing negligible sorption at pH 8.0. The interaction of the matrix with the Cr (VI) ions is determined by the extent of protonation of the cell wall functional groups, which in turn depends upon the pH of the solution. The increased binding of Cr (VI) ions at low pH can be explained due to the electrostatic binding to positively charged groups such as amines of chitosan in *Rhizopus* cell wall (Bai & Abraham 2001). It is well-known that the dominant form of Cr (VI) at this pH value is the acid chromate ion species (HCrO_4^-) and increasing the pH will shift the concentration of HCrO_4^- to other forms, CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$.

Biosorption of Ni (II) by both the biomasses showed exactly the opposite trend showing an increase in sorption capacity with the rise in pH exhibiting maximum sorption at pH 8.0. The increase in Ni (II) biosorption at higher pH values (5.0-8.0) may be explained by the ionization of functional groups on the cell surface which serves as the binding sites for the metal ions. Heavy metal ions such as Ni (II) have a strong affinity for proteins of the cell wall. At pH values above the isoelectric point, there is a net negative charge on the cell surface and the ionic state of the ligands such as carboxyl, phosphoryl, sulphhydryl, hydroxyl and amino groups will be such that the reaction with the metal cations is promoted. Effect of pH may be further explained in relation to the competition effect between the hydronium ions and metal ions. Nickel (II) ions that associate with the cell wall ligands have to compete with the hydronium ions for the active sites. At low pH values, the concentration of H_3O^+ far exceeds that of Ni (II) ions and hence, these are bound to the cell walls, leaving Ni (II) ions unbound. When the pH is increased, the competing effect of hydronium ions decreases and the positively charged Ni (II) ions adsorb on the free binding sites (Padmavathy et al. 2003).

Performance of the biosorbent, thus, depends on the ionic state of the biomass and hence, like the synthetic resins, biosorbents can be prepared with different ionic forms such as protonated (H^+ forms) or saturated with cations such as Na^+ , Ca^{2+} , Mg^{2+} , etc. by pre-treating the biomass with mineral acids, bases and/or salts. The treatment varies with the biomass type and the metal species to be biosorbed (Gupta et al. 2000). Hence, in the present investigation both the biomasses were subjected to physico-chemical pretreatments to further enhance the biosorption of the respective metals. The enhancement in the biosorption of metals after the physico-chemical pretreatments may be attributed to removal of surface impurities, rupture of cell membrane, replacement with a natural mix of ionic species bound on the cell wall with various functional groups and exposure of available binding sites for metal bioadsorption. Cabuk et al. (2005)

reported that autoclaving the biomass leads to exposure of some more active metal binding sites embedded in the cell wall of the biomass.

Acid-treatment cleans the cell wall and replaces the natural mix of ionic species bound on the cell wall of the biomass with protons and sulphates. Hence, *Rhizopus arrhizus* biomass was physico-chemically pretreated with 0.5N HNO_3 followed by autoclaving at 10 lbs for 30 min. The *Rhizopus* biomass contains abundant chitin-chitosan units, which serve as a matrix of -COOH and - NH_2 groups, which in turn takes part in binding of Cr (VI). Bai & Abraham (2002) also reported similar results showing increased sorption after acid hydrolysis which yields relatively pure amino sugar, D-glucosamine. This sugar is more easily protonated at lower adsorption pH (pH 2.0), thus, exposing more binding sites for the sorbate ions. In the present study acid treatment coupled with autoclaving resulted in increase in biosorption to $35.85 \pm 1.09\%$.

El-Morsy (2004) and Tunali et al. (2005) have reported favourable effects of alkali pretreatments for cation biosorption. The improved biosorption potential of *Aspergillus niger* after alkali treatment for cations has been reported by Luef et al. (1991) and Fourest et al. (1992). Yan & Viraraghavan (2000) also reported enhancement of nickel (II) bioadsorption using other alkali chemicals like NaOH, Na_2CO_3 and NaHCO_3 . Alkali pretreatments cause hydrolysis of the protein constituents and also deacetylation of chitin. The increased biosorption is attributed to the exposure of certain chemical groups by alkali treatment, which enhances the binding of cationic metal contaminants. In the present investigation, treatment of the autoclaved *Mucor hiemalis* with 0.5 N Na_2CO_3 enhanced the percent biosorption of Ni (II) ions to $14.88 \pm 0.67\%$.

In the present study, initial pH of biosorption solution played a critical role in the binary metals system as well, where pH seemed to influence the electrostatic binding of the ions to the corresponding functional groups. Both the biomasses singly and as a mixture (1:1) were employed to study the biosorption in a binary system. The efficiency of sorption for Cr (VI) was *Rhizopus arrhizus* > mixture of *Rhizopus arrhizus* and *Mucor hiemalis* > *Mucor hiemalis* over the range of pH from 2.0 -5.0. However, above, pH 5.0 all the three biomasses showed equivalent sorption. The optimum pH of the binary solutions when compared to that of single metal system for Cr (VI) remained the same (2.0). Cr (VI) ions were more effectively adsorbed by all the biomasses than Ni (II) at low pH values. As the pH is lowered, the overall surface charge on the cells becomes positive, which inhibits the approach of positively charged metal cations. The Cr (VI) ions being in the anionic form of HCrO_4^- in the aqua solu-

tion interact with the cell surface in this case. The uptake of free ionic Ni (II) increased with increasing pH and was the greatest at pH 5.0 decreasing further up to a pH 8.0. Thus, in the binary system the optimum pH for the sorption of Ni (II) decreased from 8.0 to 5.0. The efficiency of Ni (II) sorption exhibited by the biomasses was *Mucor hiemalis* \geq mixture of *Rhizopus arrhizus* and *Mucor hiemalis* $>$ *Rhizopus arrhizus* (Table 2). Singh et al. (2001) studied the multi-metals combination between Ni (II) and Cr (VI) by *Microcystis* sp. Contrary to the results obtained in this study, they found that Ni (II) sorption capacity by this biomass was higher than that of Cr (VI) as the binding sites in the biomass had a greater affinity for Ni (II). This difference can be due to the inherent characteristics of the biomasses employed in these studies (Apiratikul et al. 2004).

The mechanism of multi-metal ions uptake by microorganisms is quite complex. There are possible interactive effects of different species in solution and potential interactions with the surface. Competition among the different metal ions for the surface binding sites will certainly occur and depend on some of the ionic characteristics. In general, a mixture of metal ions can produce three possible types of behaviours: synergism, antagonism and non-interaction (Aksu & Donmez 2006). In the present investigation, it was observed that though the binary system, showed preferential uptake of Cr (VI) at pH 2.0 and that of Ni (II) at pH 5.0, the removal of both the metals decreased at all pH levels, in comparison to single metal systems exhibiting antagonistic effect on removal of each other. 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, causing change in preference of metal adsorption (Romera et al. 2008).

In a binary system, the effect of Ni (II) on Cr (VI) sorption was more pronounced showing a decrease of 64.42% as compared to the effect of Cr (VI) on Ni (II) sorption where the sorption reduced by 41.98%. Similar results showing antagonistic effect of Ni (II) on Cr (VI) sorption (42.98% reduction) were reported by Narvekar & Vaidya (2008). They attributed this decrease in sorption during co-removal of chromium (VI) and nickel (II) in the binary metals ion solution to the complex formation of nickel (II) chromate (NiCrO_4) with the sorbents in which chromate ions acted as a ligand. NiCrO_4 is chemically a stable compound and customarily classed as insoluble. The observed sharp decrease in the sorption of both chromium (VI) and nickel (II) could be due to the low solubility of NiCrO_4 showing high resistance to sorption. Contradictory results were reported by Munir et al. (2010) showing enhanced bimetal removal in the pH ranging from 3.0 to 6.0. They attributed this to the fact that the ionic forms of chromium (CrO_4^-) and Ni (II)

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. The results thus indicated that the initial pH of wastewater could provide selectivity for the removal of the desired component in the mixture of Cr (VI) and Ni (II) as observed in the simultaneous removal of these components at these two initial pH values.

Biosorption of Cr (VI) and Ni (II): application of mono-component adsorption models: In a multi-ion situation, which is generally encountered in effluents, a realistic approach would be to infer results in mixed metal solutions at optimum pH (Gupta et al. 2000). Biosorption isotherm data explain 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). Adsorption isotherms are critical in optimizing the use of adsorbents. Adsorption efficiency is generally determined by the extent of solute removal from the solution and the mathematical models can be used to describe and characterize the adsorption process (Oliveira et al. 2005).

Two most commonly used adsorption models, namely Langmuir and Freundlich were applied to evaluate the feasibility of adsorbate-adsorbent (metal ions to biomass) interaction. Both the isotherm models have been shown to be suitable for describing short-term and mono-component adsorption of metal ions by different materials. Adsorption model constants, the values of which express the surface properties and affinity of the biosorbent, can be used to compare the adsorptive capacity of biosorbent for different components. The individual Langmuir and Freundlich adsorption isotherms of Cr (VI) and Ni (II) ions obtained in a bimetal system at their respective optimum pH values viz., pH 2.0 for *Rhizopus arrhizus* and pH 5.0 for *Mucor hiemalis* are shown in Figs. 1 to 3 and 4 to 7 respectively. Their individual adsorption constants along with the linear regression coefficients are also listed in Table 3.

The most widely used model for equilibrium adsorption studies is the Langmuir adsorption model, based on the monolayer sorption on a homogeneous surface with a finite number of identical sites without interaction between sorbed species (Abdullah et al. 2010). Langmuir model assumes that the adsorptive forces are similar to the forces in the chemical interaction for a single solute and a constant temperature. The Langmuir adsorption isotherm equation can be written as:

$$1/q_e = 1/(X_{max} b) 1/C_e + 1/X_{max} \quad \dots(1)$$

Where, C_e is the equilibrium aqueous metal ions concentration (mg/L), q_e is the amount of metal ions adsorbed

Table 1: Effect of pH on the biosorption of Cr (VI) and Ni (II) by untreated biomasses of *Rhizopus arrhizus* and *Mucor hiemalis* in a single metal system.

pH	Sorption of Cr (VI) (%)		Sorption of Ni (II) (%)	
	<i>Rhizopus arrhizus</i>	<i>Mucor hiemalis</i>	<i>Rhizopus arrhizus</i>	<i>Mucor hiemalis</i>
2.0	20.78±0.98	11.42±0.42	1.34±0.05	1.62±0.05
3.0	15.10±0.85	7.81±0.35	1.74±0.08	2.75±0.13
4.0	5.76±0.12	2.96±0.12	2.17±0.10	3.02±0.15
5.0	3.98±0.15	1.66±0.06	2.45±0.09	3.26±0.14
6.0	2.13±0.11	1.02±0.04	2.89±0.13	3.96±0.17
7.0	1.75±0.03	0.71±0.03	3.18±0.11	4.86±0.21
8.0	0.67±0.03	0.35±0.01	3.52±0.16	5.35±0.23

Table 2: Optimization of pH for Cr (VI) and Ni (II) ions in a binary metals system.

pH	% Biosorption of Cr (VI) ions			% Biosorption of Ni (II) ions		
	<i>M. hiemalis</i>	<i>R. arrhizus</i>	<i>M. hiemalis</i> + <i>R. arrhizus</i> (1:1)	<i>M. hiemalis</i>	<i>R. arrhizus</i>	<i>M. hiemalis</i> + <i>R. arrhizus</i> (1:1)
2.0	11.24±0.40	12.75±0.48	11.43±0.44	3.93±0.11	3.11±0.16	3.52±0.11
3.0	4.59±0.15	8.68±0.37	6.59±0.23	4.74±0.19	4.50±0.18	4.74±0.15
4.0	2.65±0.13	4.97±0.20	4.47±0.22	6.45±0.25	6.27±0.22	6.45±0.26
5.0	2.11±0.07	2.56±0.09	2.45±0.09	8.63±0.38	6.73±0.17	7.29±0.32
6.0	2.03±0.10	2.31±0.10	2.27±0.08	5.084±0.17	3.63±0.09	4.66±0.12
7.0	2.14±0.10	2.25±0.11	2.14±0.10	4.64±0.12	2.08±0.03	4.12±0.18
8.0	1.49±0.08	1.85±0.09	1.65±0.04	3.55±0.09	0.94±0.01	3.29±0.14

Table 3: Langmuir and Freundlich adsorption constants associated with the simultaneous biosorption of Cr (VI) and Ni (II) by pretreated biomasses of *Rhizopus arrhizus* at pH 2.0 and *Mucor hiemalis* at pH 5.0.

Isotherms	Parameters	pH 2.0		pH 5.0	
		Cr (VI)	Ni (II)	Cr (VI)	Ni (II)
Langmuir	X_{max} (mg/g)	50000	3125	2000	Does not fit
	b (L/g)	0.00001	0.00016	0.00025	
	R_L	0.999	0.991	0.9877	
	R^2	1.0	1.0	1.0	
Freundlich	K_f (L/g)	1.9992	1.9982	1.9989	2.00001
	n	0.9998	0.9994	0.9995	1
	R^2	1.0	1.0	1.0	1.0

per gram of adsorbent at equilibrium (mg/g), X_{max} and b are constants related to the maximum adsorption capacity (mg/g) and the energy of adsorption (L/mg), respectively. X_{max} represents the practical limiting adsorption capacity when the surface is fully covered with sorbed species.

The Langmuir constants X_{max} and b can be determined from the linearised form of equation (1). A linear plot of $1/q_e$ against $1/C_e$ should give a straight line of slope $1/(X_{max} b)$ and an intercept $1/X_{max}$. This also represents the physical bondage between the adsorbate and the adsorbent. 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:

$$R_L = 1/(1 + bC_0) \quad \dots(2)$$

Where, b is the Langmuir constant and C_0 is the initial

concentration of Cr (VI)/Ni (II). R_L values between 0 and 1 indicate favourable adsorption (Ahalya et al. 2005). As seen in Table 3, the sorption of Cr (VI) and Ni (II) by *Rhizopus arrhizus* at pH 2.0 indicates favourable adsorption since the R_L values were between 0 and 1. These results suggest that Langmuir isotherm model fitted well for Cr (VI) ions at both pH 2.0 and 5.0, whereas it fitted well for Ni (II) ions only at pH 2.0. Although the data in case of Ni (II) ions at pH 5.0 did not fit well to Langmuir model, it helped in prediction of maximum biosorption capacity in the cases where the model fitted (Munir et al. 2010). The magnitude of X_{max} indicated that the amount of chromium (VI) ions per unit weight of biosorbent to form a complete monolayer on the surface was much higher than that of Ni (II) at pH 2.0 (Aksu et al. 2002). This model indicates that each binding site accepts only one Cr (VI)/Ni (II) molecule, sorbed molecules

are organized as a monolayer as the biomass is not fully saturated and all sites are energetically equivalent and there is no interaction between sorbed molecules and that the surface of the sorbent is homogenous (Tewari et al. 2005). The linear regression was unity in all cases.

The well-known Freundlich isotherm is a special case applied to non-ideal sorption on heterogeneous surfaces and to multilayer sorption, suggesting that binding sites are not equivalent and/or independent. The Freundlich model is based on the relationship between the metal uptake capacity Q (mg/g) of biomass and the residual (equilibrium) metal ion concentration C_e (mg/L). The Freundlich isotherm is represented by the equation (Freundlich 1907):

$$q = K_f C_{eq}^{1/n} \quad \dots(3)$$

Where, C_{eq} is the equilibrium concentration (mg/L) and q is the amount adsorbed (mg/g).

K_f and n are empirical constants incorporating all parameters affecting the adsorption process, such as adsorption capacity and intensity respectively. They are dependent on several environmental factors, and can be determined from the linear plot of $\ln qe$ versus $\ln C_e$. K_f is primarily related to the capacity of the adsorbent for the given ion; the higher the value of K_f , the larger is the capacity of sorption. The value of n , which is related to the distribution of bonded ions on the sorbent surface, between 1 and 10 represent beneficial adsorption (Ahalya et al. 2005). The magnitude of K_f ,

and n shows easy sorption of heavy metal ions from aqueous solutions and high adsorption capacity (Tunali et al. 2005).

The linearised form of Freundlich adsorption isotherm was used to evaluate the sorption data and is represented as:

$$\ln q = \ln K_f + 1/n \ln C_{eq} \quad \dots(4)$$

It was found that the results fitted well with the Freundlich isotherm model which yielded a straight line with the regression coefficient (R^2) value of unity in all cases (Table 3). For dead biomass of *Rhizopus arrhizus*, the value of n was found to be 0.9998, thus, indicating that adsorption of chromium (VI) was favourable at pH 2.0, while for *Mucor hiemalis* biomass n was unity at pH 5.0, indicating favourable sorption of Ni (II) at that pH. At pH 5.0, nickel (II) adsorption studies fitted only with the Freundlich model. The correlation of the linearised Freundlich isotherm suggests that a degree of heterogeneity is possible for the existing ionic species in the solution and the surface (Oliveira et al. 2005). Conformity to the Freundlich model suggests adsorption in multilayer. This indicated that the biomass was completely saturated and the metal ions were adsorbed onto the surface in multilayered pattern (Bai & Abraham 2002).

The conformity of Cr (VI) and Ni (II) ions, to both the adsorption models, in all but one case, can be explained on the basis of complex mechanism involved in the sorption of the metal ions by the *Rhizopus arrhizus* and *Mucor hiemalis*

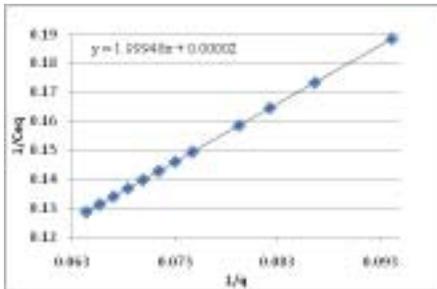


Fig 1: Langmuir adsorption isotherm of Cr (VI) at pH 2.0 in a binary metals system.

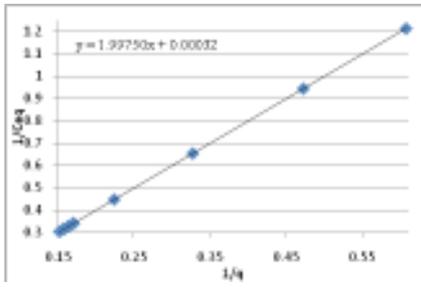


Fig 2: Langmuir adsorption isotherm of Ni (II) at pH 2.0 in a binary metals system.

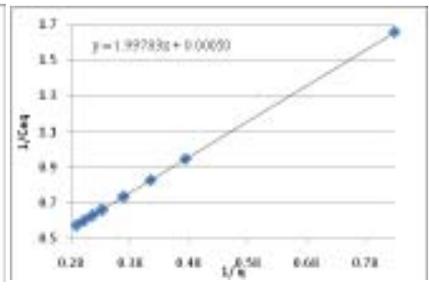


Fig 3: Langmuir adsorption isotherm of Cr (VI) at pH 5.0 in a binary metals system.

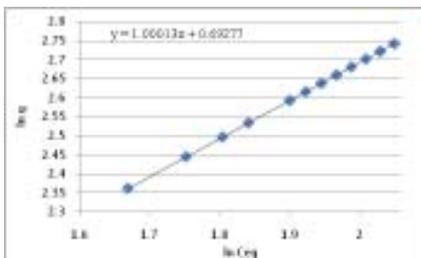


Fig 4: Freundlich adsorption isotherm of Cr (VI) at pH 2.0 in a binary metals system.

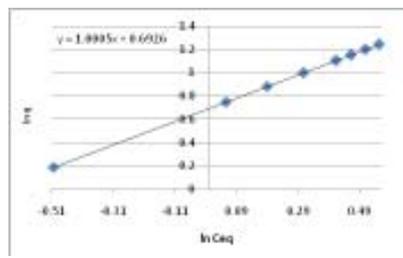


Fig 5: Freundlich adsorption isotherm of Cr (VI) at pH 5.0 in a binary metals system.

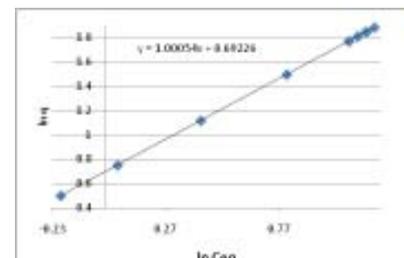


Fig 6: Freundlich adsorption isotherm of Ni (II) at pH 2.0 in a binary metals system.

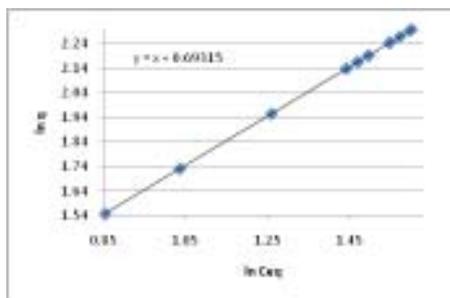


Fig 7: Freundlich adsorption isotherm of Ni (II) at pH 5.0 in a binary metals system.

biomasses. The overall sorption of metals on the biomass is complex, involving more than one mechanism, such as ion exchange, surface complexation and electrostatical attraction (Sag et al. 2001). Hence, it is not surprising that the biosorption data of the present study fitted both the models. This can be interpreted as indicating a complex adsorption process involving multilayer, interactive or multiple site type binding or some combination of these phenomena which are taking place simultaneously.

CONCLUSION

The applicability of mono-component Langmuir models at both the studied pH values indicated that the individual biosorption of Cr (VI) is favourable and could be characterized as a monolayer, single-site-type phenomenon with no interaction between sorbed components and the microbial surface. It may be concluded that the Langmuir model did not agree well for the sorption of Ni (II) at pH 5.0, whereas the Freundlich model agreed well with the biosorption of both Cr (VI) and Ni (II) at both the studied pH values. The present study revealed that the chemical interaction of multiple ions is complex and, thus, requires further analysis and research for development of a viable system for metal removal from wastewater containing multiple metal ions. This work could help to extrapolate the prediction of adsorption equilibria of the binary system if experimental data are not available for a certain level of bisolute concentrations.

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