JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 1961-1972

Microbial Sorption and Desorption of Chromium, Cadmium and Nickel from Aqueous Solution by Dried and Non Growing Biomasses of *Staphylococcus gallinarum* W-61

Mohammad Zubair Alam[1*], Shamim Ahmad[2], Esam I. Azhar[3,4], Absarul Haque[1] and Qamre Alam[1]

[1]King Fahd Medical Research Center, King Abdulaziz University, P. O. Box 80216, Jeddah 21589, Saudi Arabia.
[2]Microbiology Division, Institute of Ophthalmology, Faculty of Medicine, J.N. Medical College, Aligarh Muslim University, Aligarh 202 002 – India.
[3]Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, Saudi Arabia.
[4] Medical Laboratory Technology Department , Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.

(Received: 15 November 2013; accepted: 12 January 2014)

In the present work, *Staphylococcus gallinarum* W-61 was studied for the removal of Cd(II), Ni(II), Cr(VI), Cr(III) ions from aqueous solution. All experiments were conducted with the dried and non growing biomasses of *S. gallinarum* W-61 under varying conditions of pH, contact time, and initial concentration of the metal ion. The pH of the solution considerably altered the biosorption capacity of metal ions by the test isolate. Biosorption of Cd and Ni was maximum at pH 6.5, pH 4.5 was found optimum for Cr(III) whereas *S. gallinarum* W-61 adsorbed Cr(VI) maximum at pH 2.5. The removal of metal ions was conspicuously rapid; most of the total adsorption occurred within 30 min of reaction time. The sorption data was analyzed with the Langmuir and Freundlich isotherm models. The highest Q_{max} and K_f value was found for the biosorption of Cd(II) with 48.8 mg/g and 6.78 mg/g respectively when the experiment was conducted with the non growing biomass of *S. gallinarum* W-61. Recovery of metal ions (Cr(VI), Cr(III) Cd(II) and Ni(II)) through desorption was found better with the dried biomass compared with the non growing biomass of the isolate. The isolate was further tested for its bioaccumulation potential under actively growing conditions. .The results of bioaccumulation shows that *S. gallinarum* W-61 has accumulated varying amount of test metals intracellularly. The isolate could be employed for the removal of heavy metals from spent industrial effluents before discharging it into the environment.

Key words: Biosorption; Bioaccumulation; Metal; Bioremediation; Adsorption isotherms; *Staphylococcus gallinarum*

The heavy metals are major cause of environmental and health problems discharged into the environment by a large number of industries. . Several toxic heavy metals including chromium, cadmium, and nickel are released into the environment due to different industrial processes such as electroplating, metal finishing, porcelain enameling, metallurgy, leather working, photography etc. Chromium can exist in oxidation states ranging from ×2 to +6 but Cr(VI) and Cr(III) are the most persistent form of chromium in the environment[1]. Cadmium is exceptionally toxic and to date no biological function has been credited to it[2]. The health hazards associated with Ni(II) include allergic reactions, reproductive disorders and birth defects. Ni(II) is also considered as a

* To whom all correspondence should be addressed.
Tel.: +96626401000 Ext. 25185;F
Fax No : +966- 26952076;
E-mail: mohdzubairalam@yahoo.com

possible human carcinogen and high levels may lead to cancer of the bones, lungs and nose. The heavy metals are not biodegradable and tend to accumulate in living organisms, leading to various diseases and disorders[3]. Thus metals, an important resource, can also cause serious environmental pollution, threatening ecosystem and human health through their extreme toxicity. There are a number of conventional methods for the removal of heavy metal ions from wastewaters like ion-exchange, membrane filtration, oxidation-reduction, chemical precipitation, adsorption, evaporative recovery, and reverse osmosis. All these methods are cost-intensive. On the other hand, biosorption has emerged as an alternative method suitable for the heavy metal removal from wastewater. It is characterized by low cost and high removal of metals, particularly at low metal concentration in solution[4,5].

Metal accumulative bioprocesses generally fall into one of two categories, biosorptive uptake by non-living and non-growing biomass and bioaccumulation by living cells. Progressive metal accumulation within the microorganism produces significant toxic effects, which can lead to microbial cell death or reduced growth[6]. This makes the use of microorganisms an unsuitable strategy for the remediation of heavy metal polluted environments. However, the problem of metal toxicity to the microorganisms can be circumvented by the use of metal-resistant organisms, and hence a self-replenishing system can be left to run for extended periods. The use of microorganisms for removal of heavy metals could avoid the need for a separate biomass production process such as cultivating, harvesting, drying, etc., but requires having proper conditions to maintain cell growth. Inactivated microbial biomasses usually are able to sequester metals through surface bonding only[7]. Sorption behavior of inactivated biomass for metallic ions is a function of the chemical composition of the microbial cells forming the biomass. Mechanisms responsible for biosorption may be one or a combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation, and microprecipitation[8,9]. The aim of this work was to study the biosorption of metal ions Cr(VI), Cr(III), Cd(II), Ni(II) using biomass of both dead and living *Staphylococcus gallinarum* W-61.

MATERIAL AND METHODS

Isolation and identification of bacterial isolate

The isolation and identification of the bacterium is already described[10]. Briefly, a total of 198 bacteria were isolated from the tannery effluents and contaminated soil near Kanpur (Northern India). Bacterial isolates were identified by morphological and biochemical characteristics using standard methods[11,12]. Of these isolates, W-61 was selected for the present study due to its simultaneous high resistance toward multiple heavy metals. This isolate was further identified by 16S rDNA analysis using the primers 27f (52 -AGA GTT TGA TCC TGG CTC AG-32) and 1492r (52 -GGT TAC CTT GTT ACG ACTT-32) as *Staphylococcus gallinarum* and designated as W-61. The GenBank accession number of the 16S rDNA sequence of the isolate is EU706285.

Biosorption of chromium, cadmium and nickel

S. gallinarum W-61 was cultured overnight on Luria agar (Hi Media, Mumbai, India) plates at 28° C without metals. Confluent bacterial lawns were collected with the help of a sterilized spatula and suspended in deionized water for further testing as follows:

a) Non growing biomass: Biosorption of Cr(VI), Cr(III), Cd(II) and Ni(II) by the living but non growing cells was carried out by dispensing 100 mg cells (dry weight) in 100 ml of deionized water. The quantity of non growing biomass that would weigh 100 mg after drying was determined earlier. These non growing cells were termed as the non growing biomass (NB).

b) Dried biomass: Biosorption by the dead cells was conducted after drying the cells overnight in an oven at 80°C and then crushing them into a fine powder by a mortar and pestle. This type of biomass was termed as the dried biomass (DB). Here again 100 mg of DB was used for the biosorption experiment.

Biosorption of selected metal ions was studied in batch experiments twice in duplicate. Out of total four readings, best three were considered in results. One hundred milligrams of biomass (on dry weight basis) of the test isolate was added to 100 ml deionized water in 250 ml Erlenmeyer flasks containing desired metal concentration and incubated at 28°C with shaking at 100 rpm. At different time intervals, 1 ml

suspension of biomass was separated by centrifugation at 8000 g for 10 min. The supernatant was filtered, and double distilled water was added to make up the volume of the filtrate to 100 ml. The metal content of the samples were then determined by an Atomic Absorption Spectrophotometer (AAS), (Model: GBC, 932 plus, Australia).

Effect of pH, concentration and contact time

The effect of varying pH on biosorption of Cr(VI), Cr(III), Cd(II) and Ni(II) ions was investigated at 100 mg/l metal concentration. The pH of the deionized water was adjusted to the desired value using 0.1 M NaOH or 0.1M HCl. The effect of metal concentrations on biosorption of Cr(VI), Cr(III), Cd(II) and Ni(II) was determined at the most favorable pH for each metal ion. The effect of contact time on biosorption was studied at the most favorable pH value with 100 mg/l metal concentration. All experiments were conducted at 28°C.

Desorption of adsorbed metal ions

Desorption of adsorbed Cr(VI) ions was carried out using 100 ml of 0.1M NaOH whereas 0.1M HCl was used to desorb Cr(III), Cd(II) and Ni(II). Desorption of metal ions was investigated at 100 mg/l metal concentration from the same experiment used for the determination of the effect of contact time on metal biosorption. On completion of the biosorption experiment at 120 min, biomass was separated from solution by centrifugation at 8,000 *g* for 10 min and the supernatant was discarded. The pellet was transferred to 25 ml of desorbing solution (100 ml of 0.1M NaOH/HCl in deionized water), mixed and vortexed for 5 min and again centrifuged at 8,000 *g* for 10 min. The resulting supernatant was collected in a flask. The above process was further repeated with 25 and 50 ml desorbing solution respectively. Finally, the collected desorbing solution (100 ml) was analyzed for metal recovery on AAS.

Bioaccumulation of chromium, cadmium and nickel

Bioaccumulation of Cr(VI), Cr(III), Cd(II) and Ni(II) ions was performed by growing *S. gallinarum* W-61 in 100 ml nutrient broth (Hi Media, Mumbai, India) amended with different concentrations of metals ranges from 25 to 200 mg/l for Cr(VI), Cr(III) and Ni(II) and 5 and 10 mg/l for Cd(II), up to the late exponential phase of growth. At the end of exponential growth, the culture was centrifuged at 8,000 *g* for 10 min to collect the pellet. The pellet was washed twice with acidified water (100 ml of 0.1 M HCl in deionized water) to remove any metal precipitated or adsorbed onto the cell surface. The pellet was dried, weighed, and then digested with HNO_3 and perchloric acid for determination of metal contents through AAS. A control blank (nutrient broth with metal but without test bacterium *S. gallinarum* W-61) was run simultaneously.

Calculation of biosorption and bioaccumulation

The amount of Cr(VI), Cr(III), Cd(II) and Ni(II) ions adsorbed at equilibrium, q_e (mg/g), which represents the metal uptake, was calculated from the difference in metal concentrations in the aqueous phase before and after biosorption, as per the following equation:

$$q_{eq} = V(C_i - C_{eq})/W \quad ...(1)$$

Where V is the volume of metal solution, C_i and C_e are the initial and equilibrium concentrations of metal in solution (mg/l), respectively, and W is the mass of biosorbent (dry weight). Amount of metal bioaccumulation (mg/g) was calculated by dividing the amount of metal accumulated by the dry weight of bacteria at the end of the experiment.

Adsorption isotherms

Data on biosorption of Cr(VI), Cr(III), Cd(II) and Ni(II) were analyzed using Langmuir and Freundlich adsorption isotherm models. The adsorption data were fitted to the Langmuir equation

$$q_{eq} = Q_{max}\, bC_{eq}/1 + bC_{eq} \quad ...(2)$$

Where q_{eq} is the amount adsorbed (mg/g), C_{eq} the equilibrium concentration of the adsorbate (mg/l), Q_{max} the Langmuir constant related to maximum monolayer adsorption capacity (mg/g) and b is the constant related to the free energy.

The data were also fitted to the Freundlich equation. The logarithmic form of the Freundlich equation is given as:

$$\ln q_e = \ln K_f + 1/n \ln C_e \quad ...(3)$$

The Freundlich isotherm constants K_f and *n* correspond to adsorption capacity and intensity respectively.

RESULTS

Effect of solution pH on biosorption

Highest biosorption by *Staphylococcus gallinarum* W-61 was observed at pH 2.5 for Cr(VI), pH 4.5 for Cr(III) and pH 6.5 for Cd(II) and Ni(II). Biosorption of cadmium was highest (46.8 mg Cd/g) at pH 6.5 with the non growing biomass of *S. gallinarum* W-61 (Fig. 1). The biosorption of Ni(II) (15.6 mg/g dry weight) by the non growing biomass was maximum at pH 6.5. The dried biomass of *S. gallinarum* W-61 adsorbed most Cd (38.2 mg/g) and Ni (11.5 mg/g) at pH 6.5. Hexavalent chromium biosorption was found highest at pH 2.5 whereas pH 4.5 was found best for Cr(III). The non growing biomass of *S. gallinarum* W-61 adsorbed 22.2 mg/g Cr(VI) whereas 15.8 mg Cr(VI)/g by its dried biomass at pH 2.5. When pH of the solution was raised to 5.5, the adsorption of Cr(VI) ions slumped to 5.4 mg/g and 3.2 mg/g by its non growing and dried biomasses respectively.

Effect of initial metal concentration

Biosorption of Cr(VI), Cr(III), Cd(II) and Ni(II) at different initial concentrations at most favorable pH for each metal ion, is shown in Fig. 2. The amount of metal adsorption by both types of biomasses increased rapidly when initial metal concentration was increased from 25 to 100 mg/l and then either become constant or increased slightly with further increase in concentration to 150 and 200 mg/l. Greater amount of metals were biosorbed by the non growing biomass compared with the dried biomass. The non growing biomass of *S. gallinarum* W-61 exhibited biosorption of

Table 1. Langmuir and Freundlich isotherm parameters for the biosorption of metal ions by *Staphylococcus gallinarum* W-61

Metal ion	Biosorbent	Langmuir isotherm			Freundlich isotherm			
	Type	Q_{max} (mg/g)	*b* (l/mg)	r^2	K_f (mg/g)	1/*n*	*n*	r^2
Cr(VI)	DB	21.2	0.069	0.9424	1.18	0.661	1.513	0.9749
	NB	24.9	0.233	0.9939	2.07	0.632	1.582	0.8014
Cr(III)	DB	8.3	0.546	0.9802	1.36	0.545	1.835	0.8787
	NB	10.3	0.355	0.9565	1.32	0.618	1.618	0.9027
Cd(II)	DB	39.8	0.490	0.9975	3.21	0.651	1.536	0.6791
	NB	48.8	0.535	0.9980	6.78	0.478	2.092	0.4786
Ni(II)	DB	12.0	0.599	0.9939	2.42	0.453	2.208	0.6345
	NB	16.9	1.027	0.9992	3.29	0.494	3.024	0.5731

Experiment was performed at pH 6.5 for Cd(II) and Ni(II), pH 4.5 for Cr(III); pH 2.5 for Cr(VI); biomass conc. 1 mg/ml; contact time 120 min. DB = Dried biomass; NB = Non growing biomass; Qmax = maximum metal uptake; Kf = adsorption capacity, b = constant related to free energy; n = adsorption intensity; r2 = correlation coefficient

Table 2. Desorption of adsorbed metal ions from the dried and non growing biomass of *S. gallinarum* W-61

Metal Ion	Dried biomass			Non growing biomass		
	Biosorption (mg/g)	Metal recovered on desorption (mg/l)	Desorption (%)	Biosorption (mg/g)	Metal recovered on desorption(mg/l)	Desorption (%)
Cr(VI)	15.8 ± 1.10	15.0 ± 0.69	95.8	22.2 ± 1.60	15.7 ± 1.97	70.7
Cr(III)	8.80 ± 0.58	8.50 ± 1.22	97.1	10.3 ± 0.49	8.70 ± 2.98	84.8
Cd(II)	38.2 ± 1.70	35.9 ± 1.38	93.9	46.8 ± 1.83	34.9 ± 2.45	74.6
Ni(II)	11.5 ± 1.12	10.8 ± 1.09	94.3	15.7 ± 1.21	10.6 ± 1.14	67.8

Desorption was carried out after completion of biosorption experiment with 100 mg/l metal concentration at pH 6.5 for Cd(II) and Ni(II); pH 4.5 for Cr(III); pH 2.5 for Cr(VI), Desorption of metal ions were carried out by separating the biomass from solution trough centrifugation at 8000 g, dispensing the biomass (pellet) in 25 ml of desorbing solution, mixed vortexed and centrifuged at 8000 g for 10 min. The resulting supernatant was collected in a flask. The above process of desorption was repeated again with 25 and 50 ml desorbing solution respectively

Table 3. Bioaccumulation of metal ions by growing cells of S. gallinarum W-61

Metal ion	Conc. (mg/l)	Bioaccumulation (mg/g)
Cr(VI)	25	3.74 ± 0.20
	50	5.27 ± 0.23
	100	6.37 ± 0.18
	200	6.75 ± 0.24
Cr(III)	25	0.68 ± 0.02
	50	0.93 ± 0.01
	100	1.09 ± 0.01
	200	1.17 ± 0.02
Cd(II)	5	2.4 ± 0.03
	10	2.6 ± 0.02
Ni(II)	25	0.75 ± 0.03
	50	1.36 ± 0.04
	100	1.51 ± 0.05
	200	1.68 ± 0.09

Values are given as mean ± SD of three replicates Nutrient broth with metal but without test bacterium (S. gallinarum W-61) served as control.

18.9 mg Cd/g (dry weight) when the initial concentration was 25 mg Cd/l, that increased to 46.8 mg Cd/g (dry weight) when the concentration was raised to 100 mg/l. Biosorption of nickel also increased in similar fashion. The dried biomass of *S. gallinarum* W-61 adsorbed 15.8 mg Cr(VI)/g at 100 mg/l initial concentration. The non growing biomass of test bacterium adsorbed 3.8 mg/g Cr(III) ions at 25 mg/l that rose to 10.3 mg/g at 100 mg/l trivalent chromium concentration. The dried and non growing biomasses of *S. gallinarum* W-61 were found to adsorb 11.5 and 15.6 mg/g Ni(II) respectively at 100 mg/l initial nickel concentration.

Effect of contact time on biosorption

Biosorption of Cr(VI), Cr(III), Cd(II) and Ni(II) by the dried biomass of *S. Gallinarum* W-61 was rapid during the first 15 min of reaction. Only a slight increase in the amount of metal adsorption was observed at 30 and 60 min, reaching equilibrium at 120 min. In few cases, a small amount of adsorbed

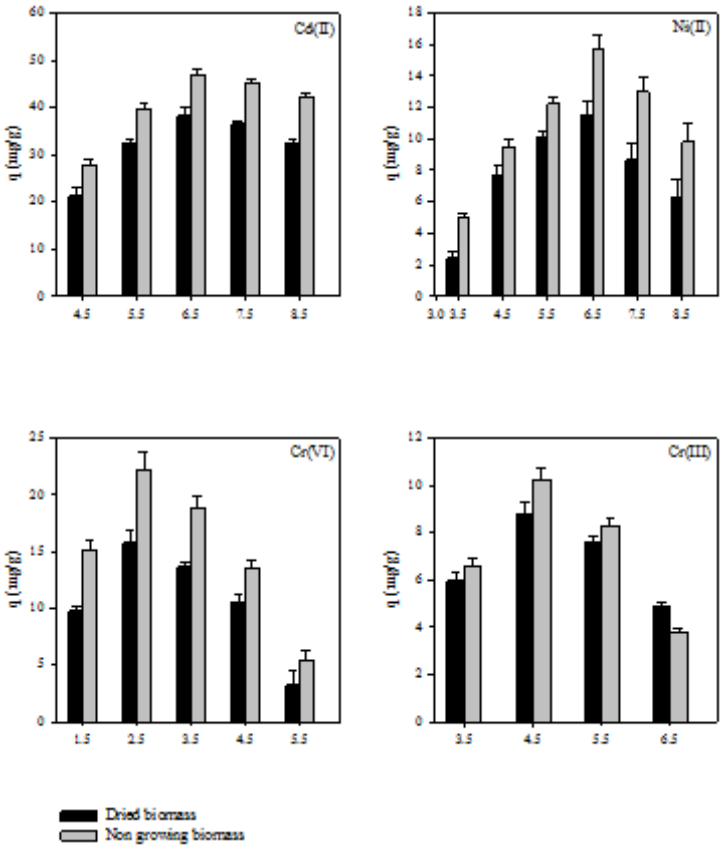

Fig 1. Effect of pH on biosorption of metal ions by dried and non growing biomass of *S. gallinarum* W-61 (1mg/ml), initial metal concentration of solution was 100 mg/l; contact time was 120 min.

metal was lost to solution. With the non growing biomass, biosorption was rapid for the first 15 min of reaction time and then a steady increase in the amount of metal biosorption was observed until equilibrium was attained at 120 min. The dried and non growing biomass of *S. gallinarum* W-61 adsorbed 29.5 and 19.5 mg/g Cd(II) respectively within 15 min of contact time (Fig. 3). The dried biomass of *S. gallinarum* W-61 adsorbed 89.6% (14.1 mg/g) out of the maximum adsorption of 15.8 mg/g hexavalent chromium at 120 min of contact time. On the other hand, the non growing biomass of the test isolate adsorbed 63.9% (14.2 mg/g) Cr(VI) in first 15 min of contact time, 74.3% (16.5) at 30 min, 84.7% (18.8 mg/g) at 60 min and 100% (22.2 mg/g) at 120 min of contact time. A similar trend was also observed for Ni(II) and Cr(III).

Langmuir and Freundlich adsorption isotherms

The biosorption data of Cr(VI), Cr(III), Cd(II) and Ni(II) were analyzed for maximum metal uptake (Q_{max}) and adsorption capacity (K_f) by Langmuir and Freundlich isotherm models respectively. In each case, Q_{max} values were found higher for the non growing biomass as compared with the dried biomass. Considering biosorption of Cd(II), Q_{max} and K_f values by the non growing biomass was found to be 48.8 mg/g and 6.78 mg/g respectively. The value of *b*, which is a constant related to the affinity of the metal ion to the binding sites was found to be 0.535 l/mg for the non growing biomass. In case of nickel, the non growing biomass of *S. gallinarum* W-61 had a K_f value of 3.29 mg/g (Table 1).

Desorption of adsorbed heavy metal ions

Adsorbed Cr(VI) ions were desorbed from the surface of *S. gallinarum* W-61 by treating it with 0.1 M NaOH. Desorption of Cr(III), Cd(II), and Ni(II) were carried out with 0.1 M HCl. Metal desorption was better from the dried biomass as compared to the non growing biomass. Metal

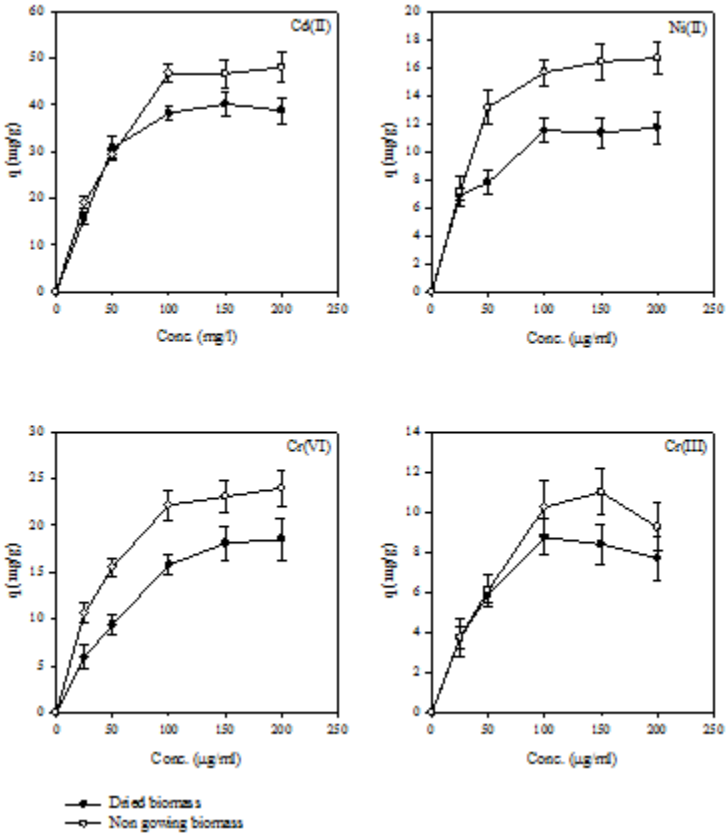

Fig 2. Effect of varying concentrations of metal ions on biosorption by dried and non growing biomass of *S. gallinarum* W-61 (1 mg/ml). Experiment was conducted at pH 6.5 for Cd(II) and Ni(II); at pH 4.5 for Cr(III); at pH 2.5 for Cr(VI); contact time was 120 min

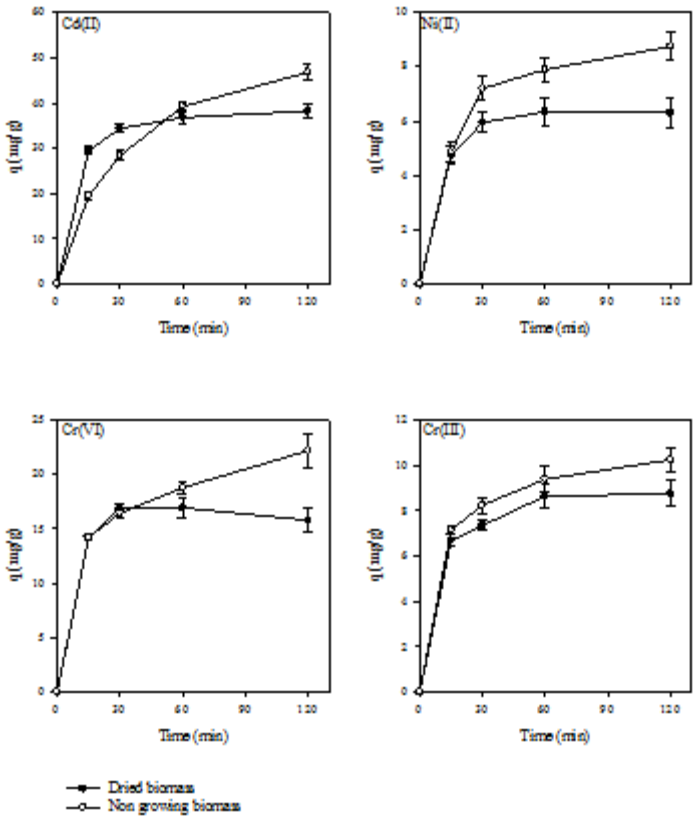

Fig. 3. Effect of contact time on biosorption of metal ions by dried and non growing biomass of *S. gallinarum* W-61 (1 mg/ml). Experiment was performed at pH 6.5 for Cd(II) and Ni(II); at pH 4.5 for Cr(III); at pH 2.5 for Cr(VI); initial metal concentration of solution was 100 mg/l.

recovery through desorption from the dried and non growing biomasses ranged from 97.1% for Cr(III) to 67.8% for Ni(II). The non growing and dried biomass of *S. gallinarum* W-61 had 70.7% and 95.8% desorption for Cr(VI) (Table 2).

Bioaccumulation of heavy metal ions

Intracellular accumulation of Cr(VI), Cr(III), Ni(II) and Cd(II) was tested under growing conditions. The test strain accumulated 6.37 mg/g and 6.75 mg/g chromium when initial medium contained 100 mg/l and 200 mg/l hexavalent chromium concentration respectively. Trivalent chromium was found to be least accumulated by the test isolate (Table 3). *S. gallinarum* W-61 accumulated 2.6 mg Cd/g when the initial Cd(II) concentration in the medium was 10 mg/l.

DISCUSSION

The sorption process heavily depends on the pH of the solution because there is a competition between the metallic ions and the protons for the same active site occurs. Since the pH of the solution can significantly influence the removal of heavy metals, it is, therefore, an important condition for biosorption of Cr(VI), Cr(III), Cd(II) and Ni(II) ions. In the present investigation, biosorption of Cr(VI) was found maximum at pH 2.5. At neutral pH, the bacterial surface has negative charge. Chromate is also a negatively charged ion. Hence, in aqueous solutions it cannot bind to a negatively charged bacterial surface. At a more acidic pH, metal cations and protons (Hþ) compete for sorption sites on

the biomass, thus making the number of positively charged (protonated) sites more abundant. This creates a repulsive ionic environment, less favorable for binding. On the other hand, Cr(VI)þ, which occurs as an anionic metal complex, i.e., $CrO_4^{2\times}$ or $Cr_2O_7^{2\times}$ besides the $HCrO_4^{\times}$ can bind readily to the positively charged moieties of the biomass at lower pH ranges[13]. At a lower pH such as pH 2.5, the overall surface charge becomes positive promoting adsorption of anionic chromate. Zouboulis et al. 2004 observed pH 2.5 as optimum for Cr(VI) biosorption by *Bacillus laterosporous* and *Bacillus licheniformis*[14]. Higher chromate biosorption at more acidic pH has been reported by other investigators also[15,16]. In the pH range 2.0–6.0, $HCrO_4^{"}$ and CrO--$_4^{2"}$ ions are in equilibrium. At lower pH (pH < 2) values, $Cr_3O_{10}^{2"}$ and $Cr_4O_{10}^{3"}$ species are formed[17]. A decrease was observed in the biosorption with increase of pH, which may be due to the decrease in electrostatic force of attraction between the sorbent and sorbate ions. The high electrostatic force of attraction at lower pH ranges, the percentage of Cr(VI) removal is also high. At very low pH value, the surface of sorbent would also be surrounded by the hydronium ions which enhance the interaction of Cr(VI) with binding sites of the biosorbents by greater attractive forces. A sharp decrease in biosorption above pH 4 may be due to occupation of the biosorption sites by anionic species like $HCrO_4^{"}$, $Cr_2O_7^{2"}$, $CrO_4^{2"}$, which retards the approach of such ions further toward the sorbent surface[18].

The present investigation has revealed pH 4.5 as optimum for the Cr(III)þ biosorption. *Pseudomonas aeruginosa* AT18 adsorbed Cr(III)þ maximally at pH 6.0[19]. Rabbani et al. 2005 studied the biosorption behavior of Cr(III)þ for a bacterium and found pH 4.0 as optimum[20]. In a study, *Spirogyra* sp. was tested for the Cr(III)þ removal from aqueous solutions and a maximal Cr(III)þ biosorption of 30.2 mg/g was found at pH 5.0[21].

We found maximum biosorption at pH 6.5 for Cd(II) and Ni(II). Around pH 7.0 bacterial surfaces are negatively charged and this favors the adsorption of positively charged ions. At pH more than 9, cadmium precipitates as an insoluble hydroxide on the cell surface. Ziagova et al. (2007) investigated the influence of solution pH on Cd(II) biosorption by *Staphylococcus xylosus* and *Pseudomonas* sp and found optimum pH as 6.0 and 7.0, respectively[22]. In other study, pH above 5.5 is reported to be most favorable for Cd(II) biosorption by *Ulva lactuca*[23]. It has been reported that at low pH, protonation of the cell wall components decreases metal uptake by the biomass, whereas with increasing pH values the negative charge density increases due to deprotonation of the metal binding sites. At pH higher than 7.0 precipitation of metal can occur by the formation of metal hydroxides[24]. Nickel biosorption was also found highest at pH 6.5. At pH above 7.0, nickel precipitate as its hydroxides. In a study, biosorption of nickel and zinc by *Bacillus brevis* found maximum at pH 6.0[17]. Nickel removal by *Yarrowia lipolytica* has been reported maximum at pH 7.5[25].

The rate of biosorption by the dried biomass was very rapid during the first 15 min of contact and approached equilibrium during the next 15 min for each metal ion. Biosorption involves several passive mechanisms. These mechanisms are fast and reversible and associated with cell surfaces. The principal mechanism of passive biosorption is the exchange of heavy metals for counter ions like Ca(II)þ, Na(I)þ, H(I) bound to the weak acidic carboxyl, hydroxyl, or amino groups present in cellular walls, cellular membranes or in polysaccharides adhered to the cell wall[26]. Passive biosorption is often followed by much slower, irreversible, metal binding processes that include covalent bonding, surface precipitation, redox reactions, or crystallization on the cell surface[27]. Biosorption with the non growing biomasses initially was very fast and then the process slowed down reaching equilibrium at longer times compared to the dried biomasses. Here, biosorption consisted of two phases: Firstly the passive adsorption and then the active uptake (metabolically driven) which is usually slower and longer. This mode of metal uptake is widely reported to describe the metal uptake in living cells[28,29]. In a study it was found that the biosorption of Ni(II) ions by *Y. lipolytica* strains was rapid during the time frame of 5 – 15 min. The biosorption process was slower at later stages (15 – 2 h). There was no significant increment in the biosorption after 2 h[25]. Ozdemir et al. 2004 studied biosorption of Cr(VI), Cd(II), and Cu(II) using *Pantoea* sp. TEM18 and observed very rapid biosorption which reached equilibrium within 5 min of contact

time[30]. Reddy et al. 2012 reported a sharp increase in biosorption of Cd(II) and Ni(II) in the first 20 min of contact time and equilibrium biosorption was established within 50 min[31]. As contact time increased, metal uptake increased initially, and then become almost stable, denoting attainment of equilibrium. These changes in metal uptake may be due to the fact that, initially, all adsorbent sites were vacant and the solute concentration was high. After that period, only a very low increase in the metal uptake was observed. A similar observation of contact time effect on biosorption was also reported by other investigators[32,33,34].

In the present investigations, biosorption of heavy metal ions by *S. gallinarum* W-61 increased when the concentration of metal ions in the solution was raised from 25 to 100 mg/l. However, when the metal concentration was further increased to 150 and 200 mg/, no marked increase in the amount of metal biosorption was observed in most of the cases. The non growing biomass of *S. gallinarum* W-61 performed better in terms of metal biosorption compared to its dried biomass. The living biomasses used in this study were not growing but were metabolically active. Therefore, it is possible that some Cr(VI), Ni(II) and Cd(II)þ ions may be internalized by living cells consequently they showed better biosorption results than dried biomass. We also reported similar observation previously[35]. However, the biosorption of Cr(III)þ for non growing and dried biomasses was identical. The bacterial membrane is usually impermeable to Cr(III)þ[36].

The present study shows that the maximum specific metal uptake (Q_{max}) and adsorption capacities (K_f) were higher for the non growing biomass of *S. gallinarum* W-61 as compared with the dried biomass for each metal ion tested. The non growing biomass used in this study was living but not dividing as the experiment was conducted in deionized water containing the desired metal concentration. The cell membrane contains various transport systems. It is possible that some metal ions crossed over the cell membrane and accumulated in the cytoplasm. This fact is also supported by the data from desorption experiment, where more metal ions were desorbed from dried biomass as compared to the non growing biomass. In other words, metal ions, which gained entry to the cells, cannot cross back through the cell membrane into the aqueous solution. Several investigators have reported maximum specific metal uptake and adsorption capacity for Cd(II), Ni(II), Cr(III), and Cr(VI) using Langmuir and Freundlich isotherm models. Qu et al. 2011 investigated biosorption of Ni(II) using *Leucobacter* sp. N-4 and found Q_{max} value for Ni(II) as 19.6 mg/g. They further reported K_f value for Ni(II) as 6.953 mg/g[37]. *Alternanthera philoxeroides* was reported to adsorb Ni(II) and Zn^{2+} with Q_{max} values at 20.16 mg/g and 18.23 mg/g respectively[38]. The maximum biosorption capacity for Cd(II) by *Nostoc commune* calculated from Langmuir biosorption isotherm was 126.32 mg/g[39]. The specific adsorption of Ni(II) by *Mucor hiemalis* is reported to be 21.49 mg/g[40]. A micro alga, *Nannochloris oculata*, has been reported to have Q_{max} as 31.7 mg/g and 37.7 mg/g whereas the adsorption capacities (K_f) were found to be 1.95 mg/g and 0.794 mg/g for Cr(III) and Cr(VI) respectively[41]. The Langmuir isotherm fit better to the biosorption of Cd(II) by *Bacillus jeotgali*, while the Freundlich model better represented Zn^{2+} biosorption[42]. The estimation of correlation coefficients (r^2) shows that the data on biosorption of Cr(VI), Cr(III), Cd(II) and Ni(II) were better represented by Langmuir isotherms than the Freundlich model.

Metal desorption is required when metal recovery is targeted or when the cost of the biosorbent limits the economy of the process[43]. In this study, the use of 0.1 M HCl/0.1M NaOH was efficient for desorption of adsorbed metal ions. This is in accordance with with (Chen et al., 2005) [44] and (Sprocati et al., 2006)[45] who reported successful metal ion removal through desorption. In another study, more than 90% of Cd(II) and Ni(II) were recovered through desorption[46]. More than 75% adsorbed Cr(VI) were desorbed from the biomass of *Oedogonium hatei* using 0.1M NaOH as desorbing solution[47]. A similar observation was also reported for the desorption of Cr(VI) from *Chlorella miniata*[48]. In the present study, desorption was higher with the dried biomass compared with the non growing biomass of *S. gallinarum* W-61 for each metal ion. The living cells can accumulate metal ions intracellularly, which are then practically impossible to recover through a desorption process[44,49].

In the present study, bioaccumulation of Cr(VI), Cr(III), Cd(II) and Ni(II) by growing *S.*

gallinarum W-61 in culture media was examined. To date no biological function has been ascribed to the cadmium, as it is extremely toxic. Cadmium accumulation by bacteria and fungi has been studied and reported by several researchers but in most of the cases, accumulation was examined in metabolically active cells under non-growing conditions over a short time period. Most of these studies were carried out in buffers or deionized water[50,51]. Therefore, comparison of the results on heavy metal bioaccumulation obtained in this study with other reports is very difficult. Deng et al. 2007 reported accumulation of Cd(II) by genetically engineered *E. coli* cells expressing the cadmium transport system and metallothionien[52]. The engineered cells accumulated 63.26 mg Cd/g dry weight on incubation for 1 h in Cd(II) amended, deionized water. In a study accumulation of 92% of Cr(VI)þ and 55% of Ni(II) at an initial concentration of 100 mg/l by *Micrococcus* sp has been demonstrated[53]. In other study, 10.54% of total chromium accumulation is reported by *Paecilomyces lilacinus* when initial hexavalent chromium concentration was 200 mg/l[54]. In the present study, the bioaccumulation experiments were conducted at 5, 10, and 20 mg/l for Cd(II) whereas for Cr(VI), Cr(III) and Ni(II) the experiment was carried out at higher concentrations. Metal accumulation increased with increasing concentration of metal ions in the culture media. Upon increasing the metal ion concentration, the growth of bacteria was slightly reduced. Consequently, the production of biomass was also reduced and therefore, on dividing the amount of metal accumulated by the weight of biomass produced, a higher value of metal accumulation was obtained. It can be 'falsely' concluded that increasing the concentration of metal ions would increase the level of metal accumulation. Therefore, in the present study, bioaccumulation of metal ions was tested at the concentration up to which no major decline in the production of biomass was observed when comparing it with the biomass at the lowest concentration tested.

CONCLUSION

The conclusion of the present study is that *Staphylococcus gallinarum* W-61 exhibited efficient removal of Cr(VI), Cr(III), Cd(II) and Ni(II) through biosorption. The non growing biomass performed better metal biosorption capability compared to the dried biomass. The metal recovery though desorption was, however, better with the dried biomass and hence the adsorbent can be used repeatedly. The bacterial strain studied was also found to remove metal ions through accumulation under in vitro growing conditions. The isolate could be used for the removal of metal ions from spent industrial effluents before discharging it into the environment.

ACKNOWLEDGEMENTS

Financial support to MZ Alam from the Council of Scientific and Industrial Research, File No: 24(0271)/04/EMR-II, Government of India, is thankfully acknowledged. MZ Alam is thankful to Professor Javed Musarrat, former Chairman, Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India where the experimental work was originally carried out. MZ Alam also acknowledge the IT facilities provided by King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

REFERENCES

1. Cervantes, C., Campos-García, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., Torres-Guzmán, J.C., Moreno-Sánchez, R. Interactions of chromium with microorganisms and plants. *FEMS Microbiol. Rev.*, 2001; **25**(3): 335-347.
2. Gadd, G.M. Mycotransformation of organic and inorganic substrates. *Mycologist.*, 2004; **18**(2): 60-70.
3. Liao, X.P., Tang, W., Zhou, R.Q., Shi, B. Adsorption of metal anions of vanadium(V) and chromium(VI) on Zr(IV)-impregnated collagen fiber. *Adsorption.*, 2008; **14**(1): 55-64.
4. Volesky, B. Biosorption and me. *Water Res.*, 2007; **41**(18): 4017-4029.
5. Gadd, G.M. Biosorption: critical review of scientific rationale, environmental importance, and significance for pollution treatment. *J. Chem. Technol. Biotechnol.*, 2009; **84**(1): 13-28.
6. Sani, R.K., Peyton, B.M., Brown, L.T. Copper-Induced Inhibition of Growth of Desulfovibrio desulfuricans G20: Assessment of Its Toxicity and Correlation with Those of Zinc and Lead.

Appl. Environ. Microbiol., 2001; **67**(10): 4765-4772.

7. Preetha, B., Viruthagiri, T. Bioaccumulation of chromium(VI), copper(II) and nickel(II) ions by growing *Rhizopus arrhizus*. *Biochem. Eng. J.*, 2007; **34**(2): 131-135.
8. Wang, J., Chen, C. Biosorbents for heavy metals removal and their future. *Biotechnol. Adv.*, 2009; **27**(2): 195-226.
9. Lin, Z., Ye, Y., Li, Q., Xu, Z., Wang, M.A. Further insight into the biosorption mechanism of Au(III) by infrared spectrometry. *BMC Biotechnol.*, 2011; **11**(1): 98-98.
10. Alam, M.Z., Ahmad, S. Toxic chromate reduction by resistant and sensitive bacteria isolated from tannery effluent contaminated soil. *Ann. Microbiol.*, 2012; **62**(1): 113-121.
11. Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T. Bergey's manual of determinative bacteriology (9th edn.): Lippincott Williams andWilkins, Philadelphia, Pennsylvania 1994.
12. Cappuccino, J.G., Sherman, N. Microbiology: A laboratory manual (third edition). Pearson education, London, UK; 1995.
13. Singh, V.K., Tiwari, P.N. Removal and Recovery of Chromium(VI) from Industrial Waste Water. *J. Chem. Technol. Biotechnol.*, 1997; **69**(3): 376-382.
14. Zouboulis, A.I., Loukidou, M.X., Matis, K.A. Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. *Process Biochem.*, 2004; **39**(8): 909-916.
15. Yang, L., Chen, J.P. Biosorption of hexavalent chromium onto raw and chemically modified *Sargassum* sp. Bioresour. *Technol.*, 2008; **99**(2): 297-307.
16. González Bermúdez, Y., Rodríguez Rico, I.L., Guibal, E., Calero de Hoces, M., Martín-Lara, M.Á. Biosorption of hexavalent chromium from aqueous solution by *Sargassum muticum* brown alga. Application of statistical design for process optimization. *Chem. Eng. J.*, 2012; **183**: 68-76.
17. Kumar, R., Singh, R., Kumar, N., Bishnoi, K., Bishnoi, N.R. Response surface methodology approach for optimization of biosorption process for removal of Cr (VI), Ni (II) and Zn (II) ions by immobilized bacterial biomass sp. *Bacillus brevis*. *Chem. Eng. J.*, 2009; **146**(3): 401-407.
18. Dönmez, G., Aksu, Z. Removal of chromium(VI) from saline wastewaters by *Dunaliella* species. *Process Biochem.*, 2002; **38**(5): 751-762.
19. Pérez Silva, R.M., Ábalos Rodríguez, A., Gómez Montes De Oca, J.M., Cantero Moreno, D. Biosorption of chromium, copper, manganese and zinc by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum. *Bioresour. Technol.*, 2009; **100**(4): 1533-1538.
20. Rabbani M, G.H., Sadeghi, S., Nazeri, Y. Biosorption of Chromium(III) by New Bacterial Strain (NRC-BT-2), . *Int. Congr. Ser.*, 2005: 268 – 269.
21. Bishnoi, N.R., Kumar, R., Kumar, S., Rani, S. Biosorption of Cr(III) from aqueous solution using algal biomass *Spirogyra* spp. *J. Hazard. Mater.*, 2007; **145**(1–2): 142-147.
22. Ziagova, M., Dimitriadis, G., Aslanidou, D., Papaioannou, X., Litopoulou Tzannetaki, E., Liakopoulou-Kyriakides, M. Comparative study of Cd(II) and Cr(VI) biosorption on *Staphylococcus xylosus* and *Pseudomonas* sp. in single and binary mixtures. *Bioresour. Technol.*, 2007; **98**(15): 2859-2865.
23. Areco, M.M., Hanela, S., Duran, J., Afonso, M.D.S. Biosorption of Cu(II), Zn(II), Cd(II) and Pb(II) by dead biomasses of green alga *Ulva lactuca* and the development of a sustainable matrix for adsorption implementation. *J. Hazard. Mater*., 2012; **213-214**: 123-132.
24. Klimmek, S., Stan, H.J., Wilke, A., Bunke, G., Buchholz, R. Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. *Environ. Sci. Technol.*, 2001; **35**(21): 4283-4288.
25. Shinde, N.R., Bankar, A.V., Kumar, A.R., Zinjarde, S.S. Removal of Ni (II) ions from aqueous solutions by biosorption onto two strains of *Yarrowia lipolytica*. *J. Environ. Manag.*, 2012; **102**: 115-124.
26. Chojnacka, K., Chojnacki, A., Górecka, H. Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue-green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. *Chemosphere*, 2005; **59**(1): 75-84.
27. Muñoz, R., Alvarez, M.T., Muñoz, A., Terrazas, E., Guieysse, B., Mattiasson, B. Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. *Chemosphere*, 2006; **63**(6): 903-911.
28. Bai, H.J., Zhang, Z.M., Yang, G.E., Li, B.Z. Bioremediation of cadmium by growing *Rhodobacter sphaeroides*: Kinetic characteristic and mechanism studies. *Bioresour. Technol.*, 2008; **99**(16): 7716-7722.
29. Zhao, X.W., Zhou, M.H., Li, Q.B., Lu, Y.H., He, N., Sun, D.H., Deng, X. Simultaneous mercury bioaccumulation and cell propagation by genetically engineered *Escherichia coli*. *Process Biochem.*, 2005; **40**(5): 1611-1616.

30. Ozdemir, G., Ceyhan, N., Ozturk, T., Akirmak, F., Cosar, T. Biosorption of chromium(VI), cadmium(II) and copper(II) by *Pantoea* sp. TEM18. *Chem. Eng. J.*, 2004; **102**(3): 249-253.
31. Reddy, D.H.K., Seshaiah, K., Reddy, A.V.R., Lee, S.M. Optimization of Cd(II), Cu(II) and Ni(II) biosorption by chemically modified *Moringa oleifera* leaves powder. *Carbohydr. Polym.*, 2012; **88**(3): 1077-1086.
32. Bhattacharya, A.K., Mandal, S.N., Das, S.K. Adsorption of Zn(II) from aqueous solution by using different adsorbents. *Chem. Eng. J.*, 2006; **123**(1–2): 43-51.
33. Ibrahim, W.M. Biosorption of heavy metal ions from aqueous solution by red macroalgae. *J. Hazard. Mater.*, 2011; **192**(3): 1827-1835.
34. Pehlivan, E., Pehlivan, E., Tutar Kahraman, H. Hexavalent chromium removal by Osage Orange. *Food Chem.*, 2012; **133**(4): 1478-1484.
35. Alam, M.Z., Ahmad, S. Chromium Removal through Biosorption and Bioaccumulation by Bacteria from Tannery Effluents Contaminated Soil. CLEAN – *Soil, Air, Water.,* 2011; **39**(3): 226-237.
36. Zayed, A.M., Terry, N. Chromium in the environment: factors affecting biological remediation. *Plant Soil.*, 2003; **249**(1): 139-156.
37. Qu, Y., Li, H., Li, A., Ma, F., Zhou, J. Identification and characterization of *Leucobacter* sp. N-4 for Ni (II) biosorption by response surface methodology. *J. Hazard. Mater.*, 2011; **190**(1–3): 869-875.
38. Wang, X.S., Qin, Y. Removal of Ni(II), Zn(II) and Cr(VI) from aqueous solution by *Alternanthera philoxeroides* biomass. *J. Hazard. Mater*., 2006; **138**(3): 582-588.
39. Morsy, F.M., Hassan, S.H.A., Koutb, M. Biosorption of Cd(II) and Zn(II) by *Nostoc commune*: Isotherm and Kinetics Studies. CLEAN – *Soil, Air, Water.,* 2011; **39**(7):680-687.
40. Shroff, K.A., Vaidya, V.K. Kinetics and equilibrium studies on biosorption of nickel from aqueous solution by dead fungal biomass of *Mucor hiemalis*. *Chem. Eng. J.*, 2011; **171**(3): 1234-1245.
41. Kim, E.J., Park, S., Hong, H.-J., Choi, Y.-E., Yang, J.W. Biosorption of chromium (Cr(III)/ Cr(VI)) on the residual microalga *Nannochloris oculata* after lipid extraction for biodiesel production. *Bioresour. Technol.*, 2011; **102**(24): 11155-11160.
42. Green-Ruiz, C., Rodriguez-Tirado, V., Gomez-Gil, B. Cadmium and zinc removal from aqueous solutions by *Bacillus jeotgali*: pH, salinity and temperature effects. *Bioresour. Technol.*, 2008; **99**(9): 3864-3870.
43. Kratochvil, D., Pimentel, P., Volesky, B. Removal of Trivalent and Hexavalent Chromium by Seaweed Biosorbent. Environ, Sci, *Technol.*, 1998; **32**(18): 2693-2698.
44. Chen, X.C., Wang, Y.P., Lin, Q., Shi, J.Y., Wu, W.X., Chen, Y.X. Biosorption of copper(II) and zinc(II) from aqueous solution by *Pseudomonas putida* CZ1. Colloid. *Surf. B: Biointerfaces.*, 2005; **46**(2): 101-107.
45. Sprocati, A.R., Alisi, C., Segre, L., Tasso, F., Galletti, M., Cremisini, C. Investigating heavy metal resistance, bioaccumulation and metabolic profile of a metallophile microbial consortium native to an abandoned mine. *Sci. Total. Environ.*, 2006; **366**(2–3): 649-658.
46. Feng, N., Guo, X., Liang, S., Zhu, Y., Liu, J. Biosorption of heavy metals from aqueous solutions by chemically modified orange peel. *J. Hazard. Mater.*, 2011; **185**(1): 49-54.
47. Gupta, V.K., Rastogi, A. Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogonium hatei* from aqueous solutions. *J. Hazard. Mater.*, 2009; **163**(1): 396-402.
48. Han, X., Wong, Y.S., Wong, M.H., Tam, N.F.Y. Biosorption and bioreduction of Cr(VI) by a microalgal isolate, *Chlorella miniata*. *J. Hazard. Mater.*, 2007; **146**(1–2): 65-72.
49. Kaduková, J., Vircíková, E. Comparison of differences between copper bioaccumulation and biosorption. *Environ. Int.*, 2005; **31**(2): 227-232.
50. Saeed, A., Iqbal, M. Bioremoval of cadmium from aqueous solution by black gram husk (*Cicer arientinum*). *Water Res.*, 2003; **37**(14): 3472-3480.
51. Cruz, C.C.V., da Costa, A.C.A., Henriques, C.A., Luna, A.S. Kinetic modeling and equilibrium studies during cadmium biosorption by dead *Sargassum* sp. biomass. *Bioresour. Technol.*, 2004; **91**(3): 249-257.
52. Deng, X., Yi, X.E., Liu, G. Cadmium removal from aqueous solution by gene-modified *Escherichia coli* JM109. *J. Hazard. Mater.*, 2007; **139**(2): 340-344.
53. Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., Thamaraiselvi, K. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *J. Hazard. Mater.*, 2007; **146**(1–2): 270-277.
54. Sharma, S., Adholeya, A. Detoxification and accumulation of chromium from tannery effluent and spent chrome effluent by *Paecilomyces lilacinus* fungi. *Int. Biodeter. Biodegrad.*, 2011; **65**(2): 309-317.