

Full Length Research Paper

Mycoremediation of Cu(II) and Ni(II)

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In the present study, Cu(II) and Ni(II) removal capacity of two filamentous fungi namely, *Aspergillus niger* van. Tieghem and *Rhizopus arrhizus* Fischer was evaluated through batch adsorption assays. Batch sorption experiments were performed at 30°C, pH 5.0 and 150 rpm taking biomass amount of 0.2 g. Adsorption trials were carried out as function of initial metal ion concentration within range of 100 to 500 mg/L⁻¹ for both single and binary metal solutions. Results revealed the biosorption capacity of both fungal species significantly increased as the initial concentration of metal ions was raised in the medium. Whereas, adsorption capacity of fungal species significantly decline in binary metal in comparison with single metal solution, desorption and reusability of the biosorbents biomass demonstrated a significantly higher desorption capability of *R. arrhizus* (> 65%) as compared to *A. niger* (> 60%) for both metal ions after 4th adsorption/desorption cycle. Batch experiments conducted with real industrial effluents revealed 5 to 10% reduction in sorption potential of test fungi in comparison to capacity obtained with synthetic solution of metal ions.

Key words: Fungi, biosorption, metals, copper(II), nickel(II).

INTRODUCTION

Biosorption exploring various biological materials is an effective and economic technology for the removal and recovery of heavy metal ions from wastewater streams (Javaid et al., 2011). Among the diversity of adsorbents, the group fungi offer additional benefits like reasonable priced, environmentally safe and technically sound, increased the social acceptability of fungal biomasses as adsorbents (Kumar et al., 2009). Cell wall components of fungi contribute key role in the metal adsorption. Varieties of polysaccharides in cell wall are complexed with proteins, lipids and other substances are major metal binding sites. Additionally, component of outer layer of the fungal cell wall like glucans, mannans, or galactans and the inner layer for example chitin chains provided additional sites for metal bindings (Alpat et al., 2010). Many fungal species such as *Rhizopus arrhizus* (Subudhi and Kar, 2008; Bajwa et al., 2009), *Rhizopus cohnii* (Jin-mong et al., 2011), *Aspergillus niger* (Tsekova et al., 2010), *Circinella* sp. (Alpat et al., 2010) and *Aspergillus terreus* (Varshney et al., 2011) have been used for

treatment of metal-loaded water. Generally, dead biomass is preferred over live for adsorption of metal ions owing to toxicity limitations, storage property for extended time period and easy desorption of adsorbed metal ions (Ahmet et al., 2005; Awofolu et al., 2006; Javaid et al., 2011). Whereas, among the number of physical methods, heat treatment impart better sorption potential to the biosorbents (Ahmet et al., 2005) as it significantly enhanced metal binding properties of biomass by eroding the cell surface integrity (Bayramoglu et al., 2002).

The protocols of biosorption would be more effective and economic by utilizing the same fungal biomass in number of repeated adsorption-desorption cycles. Regeneration of the biosorbent may be remarkably essential for observing the process costs down. The desorption process should therefore yield the metals in a concentrated form; restore the biosorbent close to the original condition for effective reuse with undiminished metal uptake and with no physical changes or damage to the biosorbent (Gupta et al., 2000). Generally, HCl and HNO₃ were used in desorption of metal ions from biomass (Al-Garni et al., 2009; Bajwa et al., 2009; Alpat et al., 2010). Another crucial problem in biosorption application is that, this technique has applied effectively

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with single-metal-ion solutions. Whereas, single toxic metallic species rarely exist in wastewaters and occurrence of multiple metal ions often causes an interactive effect. The combined effects of two or more metal ions in the solution are dependent on the number of metal-ion species competing for binding sites, as well as the metal-ion concentration of each species (Sheng et al., 2007). Thus, the current study was aimed at investigating biosorption potential of *A. niger* and *R. arrhizus* for removal of Ni(II) and Cu(II) from aqueous solution. Influence of initial concentration of metal ions and reutilization and desorption capabilities of fungal biomasses were also evaluated.

A. niger and *R. arrhizus* were chosen as both fungal species are cosmopolitan, easily available, fast growing and indigenous to Pakistan's environment. Metals uptake ability of both microfungi was also evaluated with real wastewater of electroplating.

MATERIALS AND METHODS

Adsorbent

The pure cultures of *A. niger* (FCBP 787) and *R. arrhizus* (FCBP 800) were acquired from First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences, Punjab University. Mycelial biomass of test fungi was prepared in pre autoclaved liquid medium (2% ME, 20 g malt extract 1000 L⁻¹), incubated for 6 to 7 days under controlled temperature of 25 ± 1°C at 150 rpm.

Adsorption experiment

Biosorption experiments were conducted using separate solutions containing Ni(II) and Cu(II) added in the form of Ni(NO₃)₂, 6H₂O and CuCl₂, 2H₂O, respectively. Desired concentration of metal solution was prepared by diluting stock solution with double distilled water. A series of vials contained 0.2 g of biomass and 100 ml of heavy metal solutions of known concentration and the contents were shaken at 25°C for 2 h in a rotating shaker (150 rpm). After the experiment, mycelial biomass was filtered and the supernatant liquid was checked for metal analysis by atomic absorption spectrometer.

Competitive biosorption

The adsorption experiment for binary heavy metal mixtures was carried out in a similar fashion to the single metal cases. Serial dilutions of standard solution of Cu(II) and Ni(II) were prepared using distilled water to give solutions ranging in concentration from 100 to 500 mg/L⁻¹. The conditions of the sorption system, particularly pH were maintained periodically.

Regeneration and reuse

To evaluate the desorption efficiency, the metal loaded biomass was dried at 60°C for 24 h. The dried biomass was stirred at 200 rpm with 10 mM HCl for 60 min at 25 ± 1°C and allowed metal to be released from the biomass. After each cycle of adsorption-desorption, biosorbent was washed with double distilled deionized water till pH 5.0 to 6.0 of washed solution and reconditioned for

adsorption in succeeding cycle. The weight of biomass was measured after each elution and regeneration to examine the integrity of the powdered biomass. The eluted metal per gram of biomass (q_{des}) from the concentration of metal desorbed (C_{des}) in the solution was calculated by the following equation:

$$q_{des} = C_{des} \frac{V}{W},$$

Where, V = volume of the solution and W = weight of the biosorbent.

Percentage of desorption ratio was established by comparing metal released or desorbed (q_{des}) to the amount of metal adsorbed (q_a) to the biomass. Desorption percentage ratio was calculated from the following equation:

$$\% \text{ desorption ratio} = \frac{q_{des}}{q_a} \times 100$$

Trials with real metal-effluents

Biosorption experiments were performed with wastewater of electroplating in similar way as were indicated in adsorption experiment. The physico-chemical characterizations of electroplating effluents are given in Table 1.

Measurement of metal uptake

The amount of metallic ion biosorbed per gram of biomass (q) and the efficiency of biosorption (E) were calculated using the following equations:

$$q = \left(\frac{C_i - C_f}{m} \right) V; E = \left(\frac{C_i - C_f}{C_i} \right) * 100$$

Where, C_i = initial concentration of the metallic ion (mg/L⁻¹); C_f = final concentration of metallic ion (mg/L⁻¹); m = dried mass of the biosorbent in the reaction mixture (g); V = volume of reaction mixture (ml).

The data pertaining effect of initial concentrations of metal ion on biosorption capacity of fungi was calculated by using equilibrium isotherms that is, Langmuir and Freundlich adsorption isotherm:

$$\text{Langmuir model } \frac{q_{eq} = q_{max} b C_{eq}}{1 + b C_{eq}};$$

$$\text{Freundlich model } q_e = K_F (C_e)^{1/n}$$

Where, q_{eq} = metallic ions adsorbed per unit of weight of adsorbents at equilibrium (mg g⁻¹); q_{max} = maximum possible amount of metallic ions adsorbed per unit of weight of adsorbents (mg/g⁻¹); b = constant related to the affinity of binding sites for metal ions (L/mg⁻¹); C_{eq} = equilibrium concentration (mg/L⁻¹); K_F and n = Freundlich constant; represents the adsorption intensity (dimensionless).

Statistical analysis

The results obtained in various biosorption experiments were

Table 1. Physico-chemical characterization of electroplating effluents (EPE), Chaubergy Lahore.

Parameters	EPE current status	NEQS acceptable limits	WHO acceptable limits
Copper (II), mg/L ⁻¹	72.38	1.00	0.20
Nickel (II), mg/L ⁻¹	98.60	1.00	0.20
pH value (acidity/basicity)	4.5-5.0	6.0-10	No guideline
Temperature (°C)	25-26	40	No guideline

NEQS (1999): National Environmental Quality Standards for liquid Industrial effluents and WHO (2006): World Health Organization Standards for drinking water.

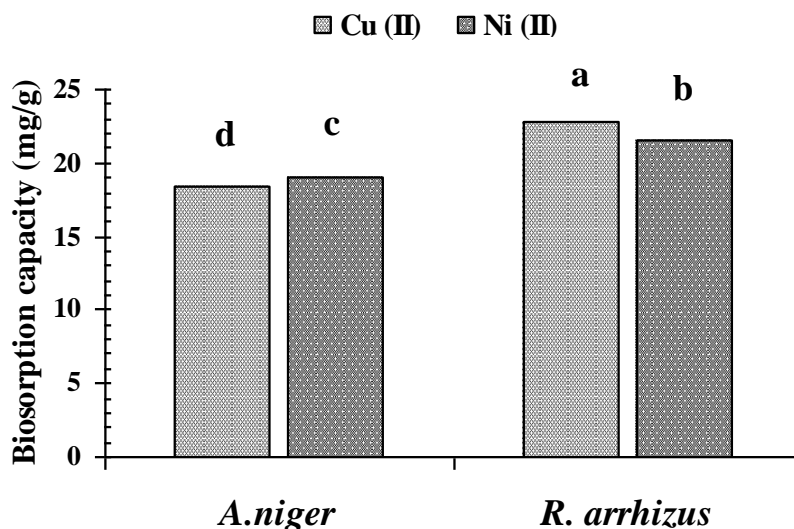


Figure 1. Assessment of metal removal capacity of fungal species. Initial concentrations of each Cu(II) and Ni(II) ions, 100 mg/L⁻¹. Biosorption conditions: biosorbent concentration, 0.2 g 100 ml⁻¹; pH, 5.0; 150 rpm and 25°C for 4 h. Value with different letters at their top show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

subjected to Duncan's Multiple Range Test (Steel and Torrie, 1980) to evaluate the effect and the interaction among the investigated factors.

RESULTS

Comparative biosorption assays

Comparative analysis of data acquired exhibited a slightly better species specific metal removal potential of *R. arrhizus* than *A. niger* for Cu(II) and Ni(II) (Figure 1). The preference for metal ions was not very great. Both biosorbents absorbed Ni(II) at 19 to 21 mg/g⁻¹ and Cu(II) at 18 to 23 mg/g⁻¹, displaying almost parallel capacity.

Single metal isotherm studies

Adsorption capacity of *A. niger* was 18.33 mg/g⁻¹ at concentration of 100 mg/L⁻¹ for Cu(II). However, there

was significant increase of up to 30 to 80% in capacity on increasing concentration from 200 to 500 mg/L⁻¹ as compared to values obtained at 100 mg/L⁻¹. For Ni(II), adsorption capacity of *A. niger* was 19 mg/g⁻¹ at 100 mg/L⁻¹, whereas capacity raised up to 50 to 100% at superior grades of concentrations that is 200 to 500 mg/L⁻¹ (Table 2). Adsorption capacity of *R. arrhizus* was 22.83 and 21.5 mg/g⁻¹ increased significantly up to 30 to 70% and 30 to 100% for Cu(II) and Ni(II), respectively on increasing concentration from 200 to 500 mg/L⁻¹ (Table 3).

Langmuir and Freundlich adsorption isotherm for single metal

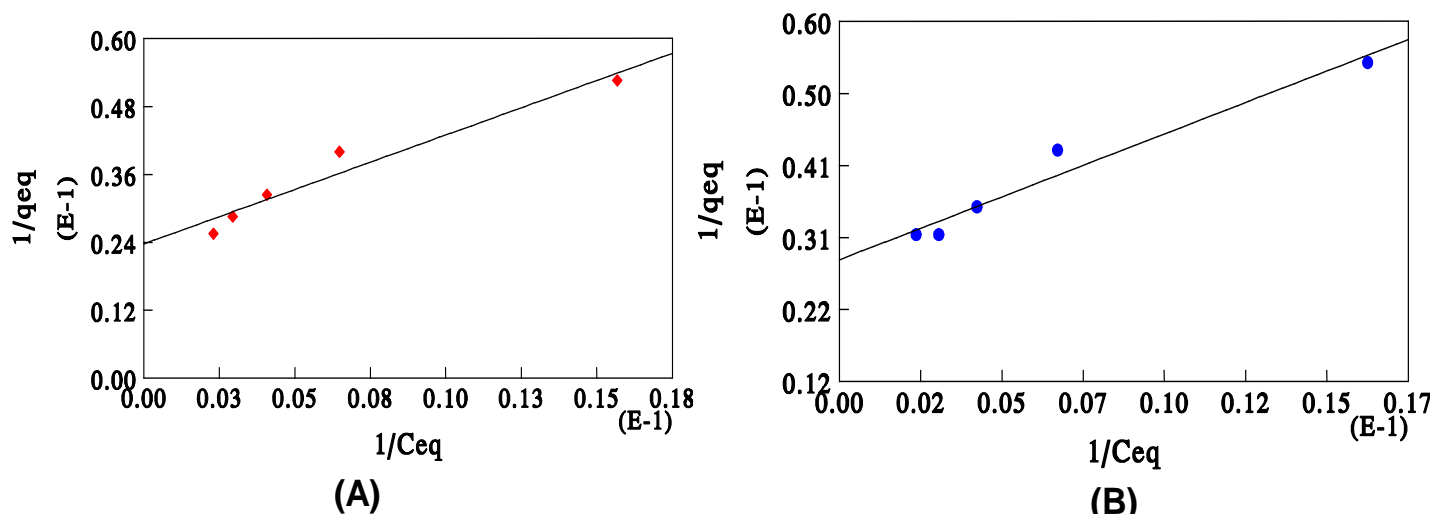
Calculated q_m values from Langmuir model for Cu(II) and Ni(II) ions for both test fungal species were very close to the experiment having significantly greater degree of $R^2 > 0.96$. In the Langmuir isotherm "b", the stability complex formed between metals ions and fungal cell wall under

Table 2. Biosorption of metal ions in single and binary metal systems.

Concentrations (mg/L ⁻¹)	Biosorption capacity of <i>A. niger</i> for metal ions (mg g ⁻¹)			
	Cu(II)		Ni(II)	
	Single metal system	Binary metal system	Single metal system	Binary metal system
100	18.33 ⁱ	16.01 ^j	19.0g ^h	16.83 ^l
200	23.32 ^f	20.83 ^g	25.02 ^e	24.33e ^f
300	28.01 ^d	23.0 ^c	30.83 ^c	28.35 ^d
400	31.66 ^c	25.13 ^e	35.01 ^b	31.70 ^c
500	31.67 ^c	26.16 ^d	39.16 ^a	35.00 ^b

Table 3. Biosorption of metal ions in single and binary metal systems.

Concentrations (mg/L ⁻¹)	Biosorption capacity of <i>R. arrhizus</i> for metal ions (mg/g ⁻¹)			
	Cu(II)		Ni(II)	
	Single metal system	Binary metal system	Single metal system	Binary metal system
100	22.83 ^h	19.01 ⁱ	21.5 ^{hi}	17.50 ^j
200	29.16e ^f	24.05 ^{gh}	27.0 ^f	22.66 ^h
300	33.00 ^d	27.06 ^f	34.17 ^d	25.83 ^g
400	37.50 ^c	30.50 ^{ef}	38.33 ^{bc}	31.66 ^e
500	39.35 ^b	31.83	45.0 ^a	33.0 ^d

**Figure 2.** The linearized Langmuir adsorption isotherm for Cu(II) (A) and Ni(II) (B) biosorption by *A. niger*.

specific experimental conditions clearly demonstrated the small values. The magnitude of intercept K_F and n (Freundlich constant) were calculated from Freundlich plots. The value of n , which is related to the distribution of bonded ions on the sorbent surface was greater than unity. The magnitude of Freundlich constant expresses easy separation of metal ions from aqueous medium and indicates favorable adsorption (Figures 2 to 5 and Table 4).

Adsorption in binary metal system

Coexistence of Ni(II) and Cu(II) reduced the maximum capacities of biosorption for both metals. At 100 mg/L⁻¹ of solutions containing both metal ions, biomass of *A. niger* showed 16.01 and 16.83 mg/g⁻¹ biosorption capacity, whereas at 500 mg/L⁻¹ capacity increased up to 80 and 100% for Cu(II) and Ni(II), respectively. However, capacity of *A. niger* was declined about 10 to 23% for

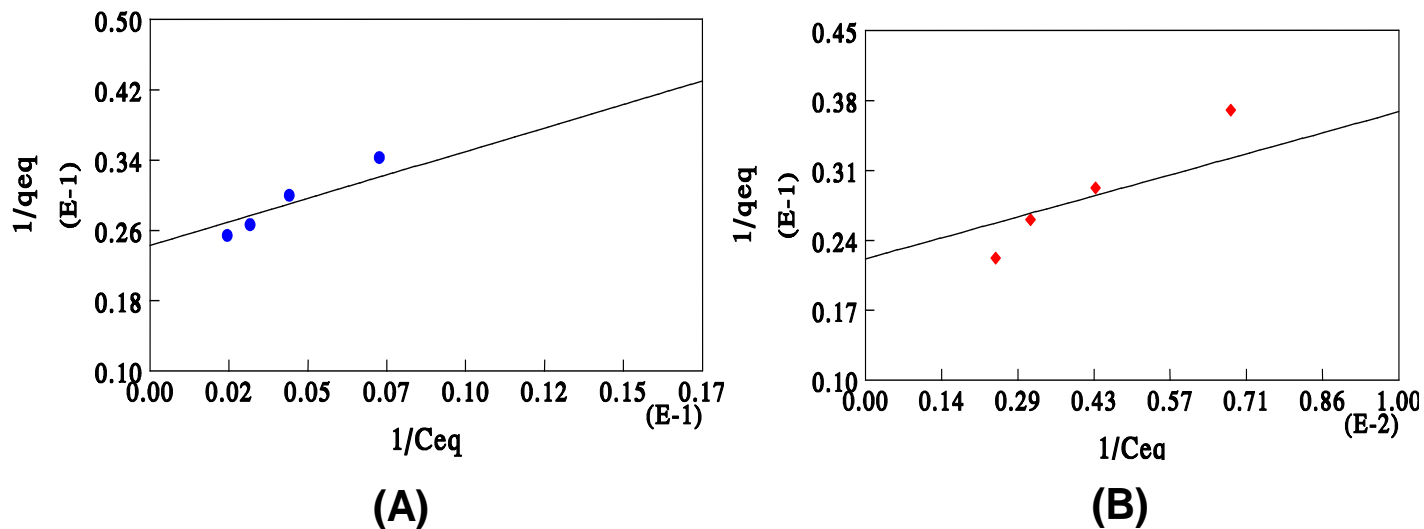


Figure 3. The linearized Langmuir adsorption isotherm for Cu(II) (A) and Ni(II) (B) biosorption by *R. arrhizus*.

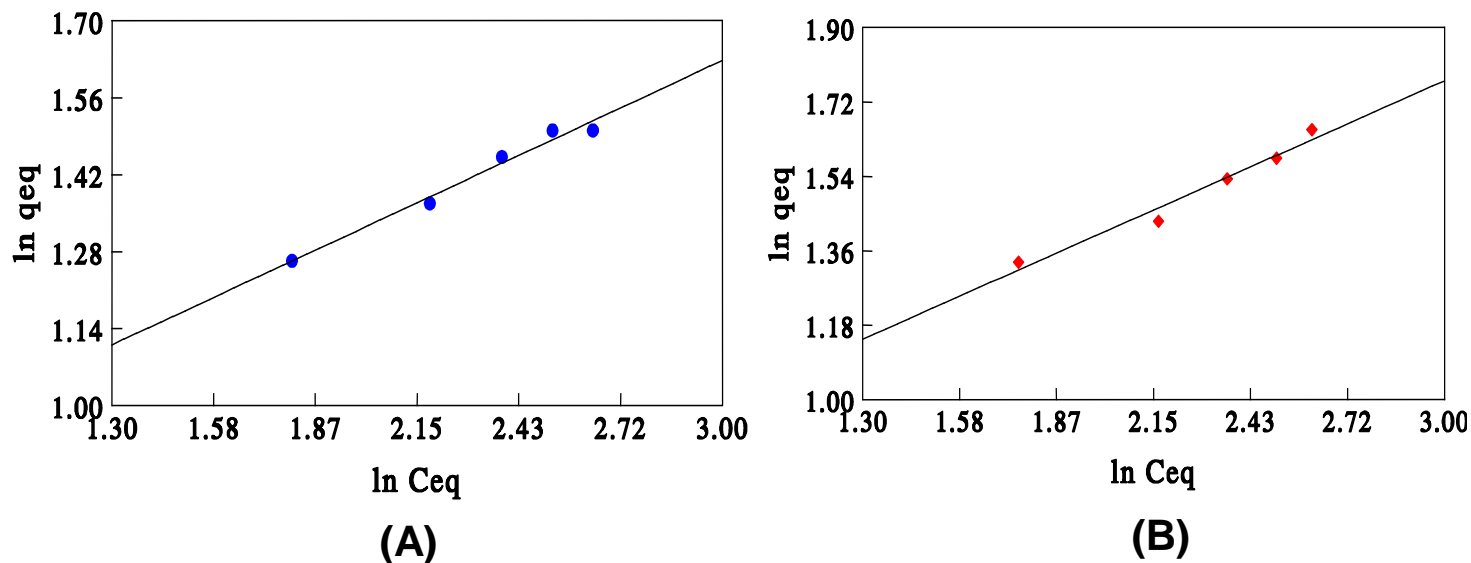


Figure 4. The linearized Freundlich adsorption isotherm for Cu(II) (A) and Ni(II) (B) biosorption by *A. niger*.

Cu(II) and 4 to 10% for Ni(II) in binary metal as compared to values acquired in single metal solution over investigated concentration range (100 to 500 mg/L⁻¹) (Table 2). *R. arrhizus* showed different preference for metal ions. In binary metal state, although rate of Ni(II) removal (19 to 31.83 mg/g⁻¹) was slightly greater than Cu(II) (19 to 33 mg/g⁻¹), but rate of Cu(II) was less affected by the presence of Ni(II). The capacity of *R. arrhizus* dropped from 17 to 24% for Cu(II) and 23 to 36% for Ni(II) in the presence of both metals in contrast to removal rate acquired in single metal solution at

employed concentration of 100 to 500 mg/L⁻¹ (Table 3).

Desorption/reusability test

The data acquired demonstrated a significantly higher desorption capability of *R. arrhizus* (> 65%) as compared to *A. niger* (> 60%) for both metal ions after each adsorption/desorption cycle (Figure 6). Comparison between metal ions showed that both fungi exhibited greater reduction in biosorption efficiency for Cu(II) (21%)

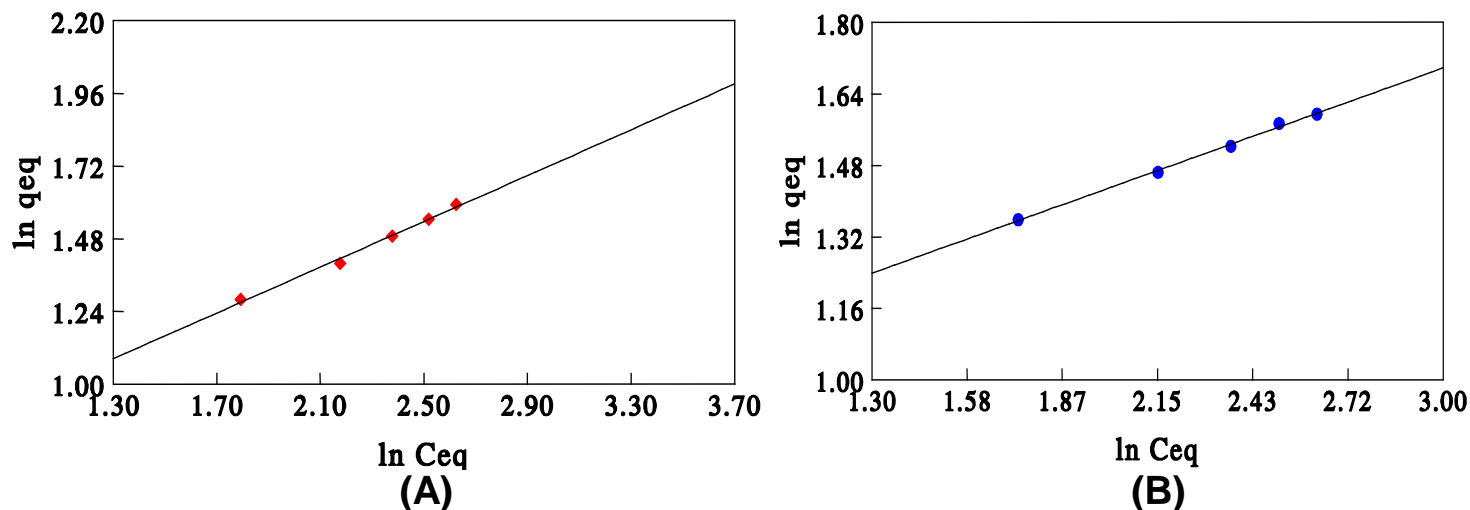


Figure 5. The linearized Freundlich adsorption isotherm for Cu(II) (A) and Ni(II) (B) biosorption by *R. arrhizus*.

Table 4. Isotherm model parameters for the biosorption of metal ions.

Fungal adsorbent	Metal	q_{exp} (mg g ⁻¹)	Langmuir			Freundlich		
			q_m (mg/g ⁻¹)	b (mg/L ⁻¹)	R^2	K_F	n	R^2
<i>Aspergillus niger</i>	Cu(II)	32.12	35.71	0.016	0.98	2.01	3.28	0.99
	Ni(II)	39.16	43.47	0.013	0.97	2.64	2.64	0.99
<i>Rhizopus arrhizus</i>	Cu(II)	39.31	41.12	0.021	0.98	1.86	3.70	0.99
	Ni(II)	45.00	45.45	0.015	0.95	2.32	2.78	0.98

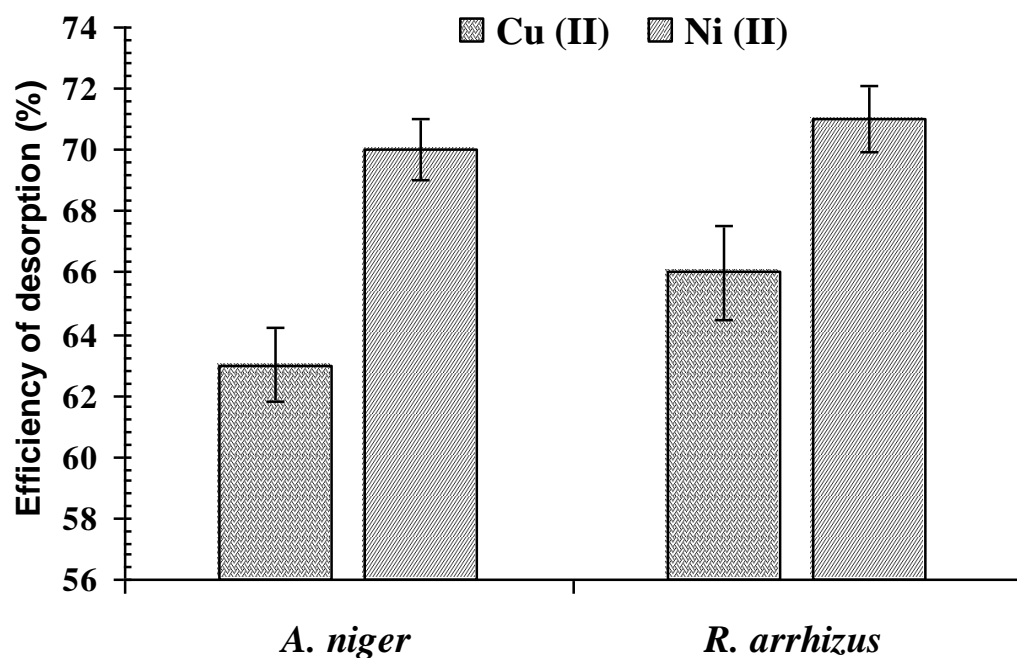


Figure 6. Comparison of desorption efficiency (%) of test fungi for Cu(II) and Ni(II) ions. Vertical bars show standard error of mean of three replicates.

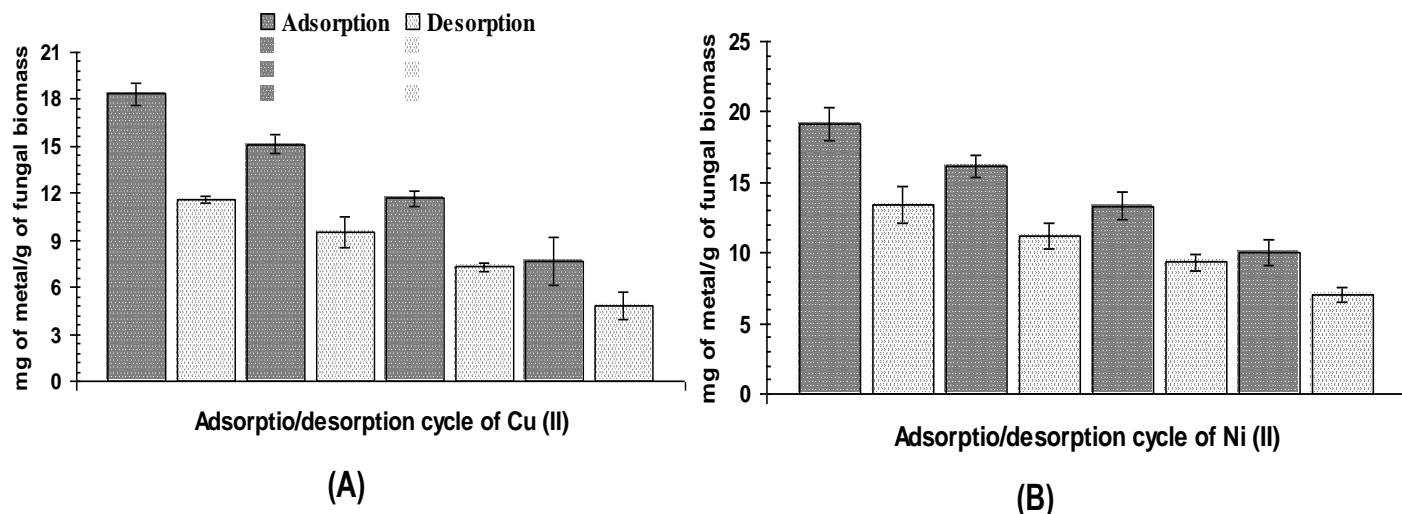


Figure 7. Adsorption/desorption cycles of *A. niger* for Cu(II) (A) and Ni(II) (B).

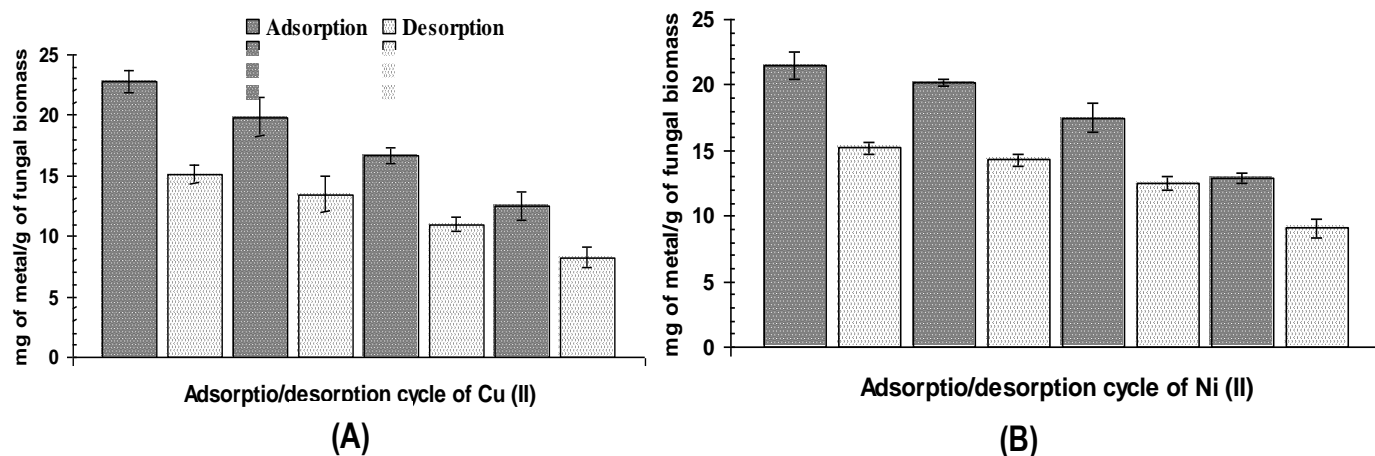


Figure 8. Adsorption/desorption cycles of *R. arrhizus* for Cu(II) (A) and Ni(II) (B).

than Ni(II) (17.5%) after 4th cycle (Figures 7 and 8).

Trials with real industrial effluents

Adsorption trial with real industrial effluents demonstrated higher sorption capacity of *R. arrhizus* for both metal ions as compared to *A. niger* (Table 5). Similarly, test fungi exhibited better removal potential for Ni(II) than Cu(II). Results acquired with real industrial effluents revealed almost 5% reduction in biosorption capacity of the test fungi for both metal ions in comparison to values obtained with synthetic solution of metal ions.

DISCUSSION

A. niger is filamentous ascomycetes and *R. arrhizus* is

filamentous zygomycetes. The difference in the removal ability of candidate fungi could be attributed to the larger surface area of *Rhizopus* biomass as mycelium grown in the form of suspended growth and high chitin and chitosan content of the cell walls (Carlile et al., 2001) for adsorption, while *Aspergillus* spp. generally grow in the form of pellets with a lower surface area having chitin and glucan in cell wall (Ahmed et al., 2005). In the current investigations, concentration range of 100 to 500 mg/L⁻¹ apparently exhibited significant elevation in uptake capacity of fungi. This assessment is in line with previously reported studies of Mukhopadhyay et al. (2007), Sheng et al. (2007) and Javaid et al. (2010, 2011). According to previous few researches, initial metal concentrations provide a driving force to overcome mass transfer resistance of metal ions between the aqueous and solid phases (Malkoc and Nuhoglu, 2005). Mukhopadhyay et al. (2007) states that this increase

Table 5. Comparative biosorptive capacity of fungal species with electroplating effluents.

Adsorbents	Adsorption capacity (mg g ⁻¹) of fungal biomass in contact with Electroplating effluents	
	Cu(II)	Ni(II)
<i>A. niger</i>	14.5 ^b	15 ^{ab}
<i>R. arrhizus</i>	15 ^{ab}	16 ^a

could be due to electrostatic interactions (relative to covalent interaction) involving sites of progressively lower affinity for metal ion. Differences in fungal preference toward metals could be governed by two attributes: 1) physical and chemical characteristics of Cu(II) and Ni(II) such as atomic number, atomic radius and the bonding of the ions with hydroxide groups on the surface of the adsorbent; 2) contribution and competition among specific functional groups on fungal cell wall in metal binding (Subudhi and Kar, 2008; Ma et al., 2009). The interference phenomenon for metal biosorption from binary mixture has been observed by many researchers (Subudhi and Kar, 2008; Zafar et al., 2007; Ma et al., 2009).

The capacity of the adsorption isotherm is elementary, and contribute essential role in the assessment of the maximum capacity of adsorbent. It also helps in explaining the efficiently by which a biosorbent will adsorb metal and allows an estimate of the economic viability of the biosorbents (Tumin et al., 2008). Presently, the Langmuir and Freundlich isotherms were found to best fit on the measured sorption data with high correlation coefficient $R^2 > 0.97$. It, therefore suggests that the binding of metal ion occurred as a monolayer consistent with specific and strong adsorption on to specific sites on biomass of biosorbents. As the exchange reaction between surface sites and previously adsorbed ions is only a monolayer or less, there may be an accumulation of matter at the solid-solution interface without creation of three-dimensional structure. On the other hand, magnitude of K_F and n (Freundlich constant) showed easy separation of metal ions from aqueous medium indicating favorable adsorption (Javaid et al., 2011). Several workers indicated the reuse of desorbed biomass for readsorption of metal ions for several times (Al-Garni et al., 2009; Jin-mong et al., 2011; Alpat et al., 2010; Javaid et al., 2011). Efficiency of HCl is based on the competition between the protons and the metal ions adsorbed by the biosorbent, which will be released if the eluant concentration is high enough and there is no steric impediment (Herrero et al., 2008). In current study, desorption efficiency was 60 to 70% which could be enhanced by increasing concentration of HCl. The reduction in metal uptake as a result to repeated elution by acid and readsorption may be due to the antagonistic effect of eluents on binding surface on the biomass (Al-Garni et al., 2009).

Similar to current investigation was reported by Javaid

et al. (2010, 2011) while investigating role of fungi in removing metal from electroplating effluents. According to them, impurities in form of other co-ions in electroplating effluents may compete for binding sites on the fungal cell walls. Secondly, electroplating effluent have low pH thus acidity may increase the H⁺ ions thus decrease binding of metal ions on biomass surface.

Conclusion

The current study concluded that both *A. niger* and *R. arrhizus* exhibited promising potential as biocontrol agents against target industrial heavy metals Cu(II) and Ni(II) of electroplating. Both fungal species can grow easily in bulk on low cost medium and are by-products of fermentation industries. Initial concentration of metal ions greatly influenced the uptake capacity of the biosorbent. Desorption studies showed that both metal biosorbed on fungal species could be desorbed effectively using 10 mM HCl.

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