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Biosorption of Metal Ions from Aqueous Solution and Electroplating Industry Wastewater by *Aspergillus japonicus:* Phytotoxicity Studies

A. R. Binupriya,[1] M. Sathishkumar,[2] K. Swaminathan,[1] E. S. Jeong,[2] S. E. Yun,[2] S. Pattabi[3]

[1] Microbial Biotechnology Division, Department of Biotechnology, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India
[2] Department of Food Science and Technology, Institute of Agricultural Science and Technology, Chonbuk National University, Chonju 561-756, Republic of Korea
[3] Department of Environmental Sciences, PSG College of Arts and Science, Coimbatore 641 014, Tamil Nadu, India

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Chromium, nickel, iron and mercury are some of the most widely used metals in industries, especially in electroplating industry. Cr(VI) contaminated wastewaters have become a recognized hazard, as Cr(VI) is a known carcinogen and mutagen. Epigastric pain, nausea, vomiting, severe diarrhoea and haemorrhage were observed when chromium compounds were ingested (Ranganathan, 1993). Symptoms of Iron toxicity are vomiting, diarrhea, and damage to the intestine. Iron may also get precipitated in the gills of fishes thus affecting the aquatic life (Ranganathan, 1993). Nickel is also a potent carcinogen and causes cancer in lungs, nose, stomach and bones. Mercury causes damage of the central nervous system and chromosomes, impairment of pulmonary functions, chest pain and dyspoea. Chronic exposure to mercury leads to mental retardation. The toxicity of metals makes its removal highly imperative. But, physico-chemical methods presently in use have several disadvantages like unpredictable metal ion removal, high reagent requirements, formation of sludge and their disposal, high installation and operational costs etc. (Kapoor et al., 1999). Adsorption by activated carbon appears to be particularly competitive and effective in the removal of metals in trace levels. There are still problems with its use; namely, activated carbon is expensive and higher the quality, greater the cost. Biosorption is an alternative technology in which an increased amount of study is being focused. Extensive investigations are being carried out to identify suitable and cheap adsorbent capable of removing the metals. The use of microbial biomass as potential sorbent for removal of metals from industrial and municipal wastewater has been proposed as a promising alternative to conventional metal management strategies in past decades.

Coimbatore (11° 00' N Lat. 76° 58' E Long.) is a major industrial city in the state of Tamil Nadu, India. In this city, effluents from industrial units involved in textile dyeing and electroplating are directly discharged into wetlands without any pretreatment (Mohanraj et al., 2000). Metals commonly used in electroplating include chromium, nickel, zinc, and mercury. But their concentration and presence highly depends upon the type of method used for plating (Selvakumari et al., 2002). The electroplating effluent contains considerable amount of iron in addition to the metals used for plating. In this study, an attempt was made to

Correspondence to: M. Sathishkumar

remove metals from aqueous solutions and electroplating industry effluent using the mycelial biomass of the fungus, *Aspergillus japonicus* as adsorbent. The toxicity of the treated effluent was tested against *Zea mays* plant system.

MATERIALS AND METHODS

For the preparation of adsorbent, 1 ml (10^6 spores) of *A. japonicus* spore suspension was inoculated into Czapex-Dox broth in 250 ml Erlenmeyer flasks and incubated at room temperature ($27^o \pm 3^oC$) for 5 days in an orbital shaker at 125 rpm. At the end of fifth day, the mycelial pellets were separated by filtration through Whatman No.1 filter paper (Sathishkumar et. al., 2004). Biomass was then washed with generous amount of deionized water until free from the media components. The washed, live mycelial pellets were used as adsorbent as such after squeezing out the excess water with the use of filter paper (Sathishkumar et. al., 2004). All the chemicals used were of analytical grade procured from Merck and Glaxo.

Adsorption experiments were conducted using separate solutions containing Fe(II), Ni(II), Cr(VI) and Hg(II) added in the form of $FeSO_4$, $NiSO_4$, $K_2Cr_2O_7$ and $HgCl_2$. To determine the effect of agitation time and adsorbate concentration on removal of heavy metals studied, 1g (wet weight) of the adsorbent was added to 100 ml of 25, 50, 75 and 100 mg/L solutions of respective metals and agitated in a rotary shaker at 150 rpm for predetermined time intervals at 30^oC. The adsorbate and adsorbent were separated by centrifugation at 10,000 rpm for 20 min. The remaining metals in the adsorbate were analyzed spectrometrically, where the detection is 0.05 mg/L (APHA, 1995) (Shimadzu, UV-1601, Japan). The same spectrophotometric method was used in the subsequent experiments. The study was carried out with different dosages of adsorbent (250-2250 mg/100 ml) for an equilibrium time to determine the effect of adsorbent dose on removal of heavy metals studied. The effect of pH on removal of heavy metals were studied by using 100 ml of 100 mg/L solution of respective metals, adjusted to an initial pH of 2-10 mixed with optimum dosage of adsorbent and agitated for equilibrium time. The experiments were done in triplicate and the mean values were used for further studies. Control experiments were carried out in absence of the adsorbent in order to find out whether there is any adsorption on the container walls. No adsorption on to the container walls was observed. From the data, Langmuir plot was drawn and adsorption constant (Q_o) was calculated from the slope of the curve.

The electroplating industry wastewater was collected from a local electroplating unit and characterized. The wastewater was diluted to 25, 50 and 75 % using distilled water. Effect of contact time and adsorbent dosage were studied and optimized in the wastewater. Toxicity of treated and untreated wastewaters at different concentrations (25, 50, 75 and 100%) was studied against *Zea mays* seeds. Ten seeds were placed in Petri dishes lined with germination sheets soaked in different concentrations of treated and untreated effluents. Plates were

Table 1. Equilibrium time for removal of metal ions.

Metal concentration (mg/L)	Equilibrium time (min)			
	Fe(II)	Cr(VI)	Ni(II)	Hg(II)
25	60	60	70	70
50	80	70	90	80
75	80	70	90	80
100	90	70	90	80

incubated in an incubator at 27° ± 2°C (ISTA, 1966). Percent germination and vigour index were calculated from germinating seeds (Abdul-Baki and Anderson, 1973). To determine phytotoxicity of effluents, twenty *Z. mays* seedlings were raised separately in sand trays irrigated with different concentrations of treated and untreated effluents. After ten days, growth parameters, viz., root length, shoot length, root biomass, shoot biomass and total biomass were calculated. From the data, effluent tolerant index and phytotoxicity were calculated (Turner and Marshal, 1972). Data were analysed using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Uptake of metal ions increased with increase in contact time but remained constant after an equilibrium time for all metal ions studied. Similar results have been reported for the removal of metal ions by other adsorbents (Ranganathan, 1993; Selvi et al., 2001; Selvakumari et al., 2002; Deepa et. al., 2006). Equilibrium time and metal ion uptake varied with different metal ions which may be due to difference in affinity of adsorbent for metal ions. The maximum equilibrium time of respective metal ion was considered for further studies, which is given in Table 1. The equilibrium time required by the adsorbent to remove metal ions was less when compared to other reported adsorbents, where the equilibrium time ranged between 100 to 180 min (Viraraghavan and Dronamraju, 1993; Selvi et al., 2001; Selvakumari et al., 2002; Deepa et al., 2006). This result is interesting because equilibrium time is one of the important considerations for economical water and wastewater treatment applications (Deepa et al., 2006). The rate constant of adsorption of metal ions studied on *A. japonicus* biomass followed the first order rate (Lagergren, 1898), where, q and q_e are amount of metal ion adsorbed (mg/g) at time t (min) and at equilibrium time, respectively, and K_{ad} is the rate constant for adsorption. The linear plots of $\log(q_e - q)$ vs t for the metal ion studied at different concentrations for the adsorbent, which indicate the applicability of eqn (1). The values of K_{ad} were calculated from the slope of the linear plots for all the metals and are given in Table 2. Langmuir isotherm was applied for the present study to estimate the adsorption capacity of the adsorbent used, where, C_e, is the equilibrium concentration (mg/L) and q_e, is the amount adsorbed at equilibrium time (mg/g) and Q_o and b are Langmuir constants related to adsorption capacity and energy of adsorption, respectively. Q_o and b values were calculated from the plots of C_e/q_e Vs C_e. The linear plots by the adsorbents

Table 2. Lagergren and Langmuir constants for removal of metal ions.

Metal	Lagergren constants				Langmuir constants	
	$K_{ad} \times 10^{-2}$ (min^{-1})				Q_o (mg/g)	b
	25	50	75	100		
Fe(II)	6.52	3.92	3.66	3.43	1.34	5.17
Cr(VI)	3.55	3.80	5.53	3.32	1.18	0.07
Ni(II)	3.20	3.64	5.53	3.20	1.89	0.06
Hg(II)	3.11	3.64	6.01	3.22	1.23	0.08

studied show that adsorption of metal ions follows Langmuir isotherm model. The values of Q_o and b were calculated from the slope and intercept of the Langmuir plot. The values of Q_o and b for the metal ions studied are listed in Table 2. The Q_o values for the adsorption of metal ions with various adsorbents are summarized in Table 3 for comparison. Removal of metal ions studied by *A. japonicus* showed better adsorption when compared to some chemical adsorbents. The experiment was carried out with different adsorbent dosage up to equilibrium time. It was noted that the rate of adsorption increased with an increase in adsorbent dosage, which may be due to the availability of more surface functional groups at higher adsorbent dosage (Deepa et al., 2006). But after the adsorbent dosage level of 1250 mg/100 ml, adsorption was either nil or pace of increase in adsorption was very less, consequently, this adsorbent dosage level (1250 mg/100 ml) was selected for further studies.

Effect of pH on removal of metal ions was observed over a pH range of 2-10. Dilute HNO_3 and NaOH was used to adjust the pH. Results reveal that increase in pH decreases the removal of Cr(VI) both in presence and absence of the adsorbent. In case of Fe(II) and Hg(II) maximum removal was observed in near neutral pH. But for Ni(II), maximum removal was in alkaline pH (Fig. 1). In all the metal ions studied metal removal by adsorption is more than precipitation. This suggests that the adsorbent is capable of removing metal ions in any pH range, irrespective of precipitation.

The removal of metal ions from industrial wastewater depends upon its composition, nature and pH. In the effluent collected 12 mg/L Fe (II), 44 mg/L of Cr(VI) and 90 mg/L of Ni(II) were found. Hg(II) was nil. Equilibrium for Fe(II) removal was attained at 90 min, whereas, in the case of Ni(II) and Cr(VI) at 105 min. The contact time for the rest of the batch experiments were fixed at 105 min. Langmuir isotherm was applied only for Ni(II) and Cr(VI) adsorption; Fe(II) was completely adsorbed, where this isotherm is not applicable (Fig. 2). The Q_o value was calculated to be 1.16 mg/g and 2.57 mg/g for Ni(II) and Cr(VI), respectively. These values varied from that of aqueous solutions, which may be due to initial metal concentration and the interference of other ions in the solution (Murugesan, 2002). Adsorbent dosage of 2250 mg/100 ml (wet weight) was found to be optimum for all metal ions. The work was carried out at natural pH (2.1) of the wastewater because it will not be economical to treat the effluent in large quantity by changing the pH. Comparing to aqueous solution, removal of metal ions from

Table 3. Comparison of adsorption capacity of *Aspergillus japonicus* biomass with other adsorbents.

Adsorbent	Q_o (mg/g)				Reference
	Fe(II)	Cr(VI)	Ni(II)	Hg(II)	
Granular activated carbon	-	-	-	0.8	Huang and Blarikenship, 1984
Wollastonite	0.433	-	-	-	Singh et al., 1988
Feldspar	-	0.0913	-	-	Singh et al., 1992
Fly ash at 21°C	-	-	0.116	-	Viraraghavan and Dronamraju, 1993
Fe(III)/Cr(III) hydroxide	34.48	1.43	21.00	-	Ranganathan, 1993
Fly ash	-	-	-	0.73	Kapoor and Viraraghavan, 1994
Granular activated carbon	-	-	1.49	-	Periasamy and Namasivayam, 1995
Live *Aspergillus niger*	-	-	3.85	-	Kapoor et al., 1999
Pretreated *Aspergillus niger*	-	-	2.20	-	Kapoor et al., 1999
Carbonized coconut tree saw dust	-	3.64	-	-	Selvi et al., 2001
Autoclaved biomass of *Medusomyces gisevii*	0.931	-	-	-	Murugesan, 2002
Acid treated biomass of *Medusomyces gisevii*	0.072	-	-	-	Murugesan, 2002
Maize cob carbon	-	66.67	57.53	-	Selvakumari et al., 2002
Autoclaved *Aspergillus flavus*	-	0.335	-	-	Deepa et al., 2006
Aspergillus japonicus	1.34	1.18	1.89	1.23	This work

wastewater is less, which may be due to the interference of some other ions present in the wastewater. But the experimental result of the removal of metal ions from industrial wastewater confirms the validity of the result obtained in the batch mode studies, i.e. *A. japonicus* can be used effectively for the removal of metal ions from industrial wastewater.

The results of phytotoxicity studies are shown in Table. 4. The phytotoxicity of the effluent treated with *A. japonicus* was reduced. The biomass production in seedlings irrigated with untreated effluent diluted to 25% was 62.8 mg but in 25% diluted-fungal treated effluent, it was increased to 69.2 mg. In untreated effluent-

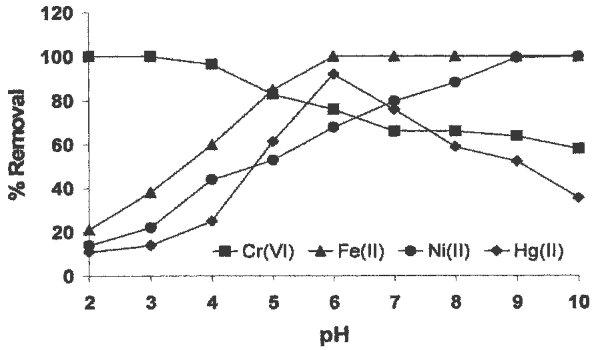

Figure 1. Effect of pH on removal of metal ions from aqueous solutions (Contact time: 90 min; Adsorbent dosage: 1250 mg/100 ml; Metal ion concentration: 25 mg/L)

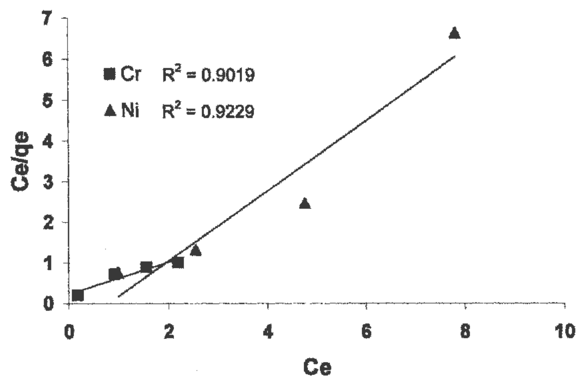

Figure 2. Langmuir plot for metal removal from wastewater

irrigated seedlings the values of phytotoxicity and effluent tolerant index were 42.57 and 0.574 respectively; but in 25% diluted-fungal treated effluent the phytotoxicity was reduced to 18.62 and effluent tolerant index was increased to 0.864. Heale and O'rmrod (1982) have found retarded growth of maple, dog wood, honey suckle and pine by application of Ni at 20 mg/L. Seed germination, plant growth, root nodulation and productivity of lentil plants were adversely affected by Ni(II) particularly at high concentrations (Khan et al., 1987). Vigour index denotes the ability of the seeds to germinate. The effluent diluted to 25% and treated with *A. japonicus* showed a vigour index higher than that of the untreated effluent and control. This shows that the nutrient content in 25% diluted-fungal treated effluent is at favorable level. Comparing the increase of vigour index in 25% diluted-fungal treated effluent with carbon treated effluent as reported by Selvakumari et al., (2002) shows that fungal treatment is able to remove metals and other chemicals from the effluent more efficiently than the carbon. Phytotoxicity implies the toxicity of the effluent to plant growth and the

Table 4. Effect of electroplating industry effluent on seed germination and seedling development of *Zea mays*.

Treatment / Effluent concentration (%)	Percent germination	Embryonic axis length (mm)	Shoot length (mm)	Root length (mm)	Shoot biomass (mg)	Root biomass (mg)	Total biomass (mg)	Vigour index	DMRT ranking	Phyto toxicity	Effluent tolerant index	DMRT ranking
Control	100	12.70	27.13	14.80	41.40	32.90	74.30	100.00	b,2	0.00	1.000	a,1
Untreated effluent												
25	100	9.60	19.10	8.50	38.50	24.30	62.80	96.00	c,3	42.57	0.574	d,4
50	100	5.30	10.30	5.20	15.00	8.00	23.00	53.00	e,5	64.86	0.351	e,5
75	100	3.10	0.00	0.00	0.00	0.00	0.00	31.00	h,8	100.00	0.000	g,7
100	100	1.20	0.00	0.00	0.00	0.00	0.00	12.00	i,9	100.00	0.000	g,7
Treated effluent												
25	100	12.30	23.30	9.10	40.80	28.40	69.20	123.00	a,1	18.62	0.864	b,2
50	100	7.80	12.50	7.20	17.00	9.00	26.00	78.00	d,4	28.38	0.487	c,3
75	100	5.20	4.10	0.90	10.00	2.00	12.00	52.00	f,6	48.65	0.061	f,6
100	100	3.60	0.00	0.00	0.00	0.00	0.00	36.00	g,7	91.22	0.000	g,7
CV :			1.2%	2.9%			0.40%	0.6%		0.10%	0.3%	
p :			0.010	0.010			0.01	0.010		0.01	0.0100	
SED :			0.110	0.120			0.11	0.31		0.067	0.001	
LSD(1%) :			0.31	0.34			0.31	0.8		0.193	0.0028	

effluent tolerant index denotes the ability of the plant to resist the effluent toxicity. More than 50% reduction of phytotoxicity in effluent diluted to 25% and treated with fungus shows the effectiveness of the treatment. Similarly a notable reduction in the effluent tolerant index of treated effluent when compared to untreated effluent shows the efficacy of the treatment. Improvement of effluent tolerant index in treated effluent was almost the same when compared with the result reported by Deepa et. al. (2006).

The study thereby implies that the mycelial pellets of *A. japonicus* may be used as biosorbent for the removal of metal ions from wastewaters containing dilute concentrations. As the biomass of *A. japonicus* is easily cultivable and also is available as waste in large quantities from certain fermentation industries, using it as an adsorbent will be economical and can be viewed as a waste management strategy. Moreover, as the biomass of *A. japonicus* is easily biodegradable, the metals can be desorbed from the biomass after adsorption and can be taken for land filling or composting after several cycles of adsorption.

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