Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Hg(II) Removal from Aqueous Solution by Dead Fungal Biomass of Marine *Aspergillus niger*: Kinetic Studies

Yasmin Khambhaty[a]; Kalpana Mody[a]; Shaik Basha[a]; Bhavanath Jha[a]
[a] Marine Biotechnology and Ecology Discipline, Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

To cite this Article Khambhaty, Yasmin , Mody, Kalpana , Basha, Shaik and Jha, Bhavanath(2008) 'Hg(II) Removal from Aqueous Solution by Dead Fungal Biomass of Marine *Aspergillus niger*: Kinetic Studies', Separation Science and Technology, 43: 5, 1221 — 1238
To link to this Article: DOI: 10.1080/01496390801888235
URL: http://dx.doi.org/10.1080/01496390801888235

Separation Science and Technology, 43: 1221–1238, 2008

ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390801888235

Hg(II) Removal from Aqueous Solution by Dead Fungal Biomass of Marine *Aspergillus niger*: Kinetic Studies

Yasmin Khambhaty, Kalpana Mody, Shaik Basha, and Bhavanath Jha

Marine Biotechnology and Ecology Discipline, Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

Abstract: Mercury removal from wastewater is a recognized pollution control challenge today. In the present investigation, the biosorption of Hg(II) onto the dead biomass of four different species of marine *Aspergillus*, prepared by alkaline treatment, was studied. Among the cultures studied, *A. niger* was found to be the most efficient for Hg(II) removal. The effects of initial Hg(II) concentration, contact time, pH, temperature, and biosorbent dosage on biosorption were also investigated. It was observed that biosorption equilibriums were established in about 2 h. Under the optimum conditions (pH: 3.0, Hg(II) concentration: 250 mg/L, biomass dose: 0.8 g/L, temperature: 40°C and contact time: 2 h), 40.53 mg Hg(II) was biosorbed per gram of dead biomass of *A. niger*. Kinetic studies based on fractional power, zero order, first order, pseudo first order, Elovich, second order, and second order rate expressions have also been carried out where the pseudo second order model exhibited best fit to experimental data. The intra-particle diffusion study revealed that film diffusion is the rate-limiting sorption process for Hg(II) on *A. niger*. The nature of the possible cell–metal ion interactions was evaluated by FTIR, SEM, and EDAX analysis. These examinations indicated the involvement of -OH and $-NH_2^+$ groups in the biosorption process present on the surface of the dead fungal biomass. Here, Hg(II) ions were deposited on the surface of the biomass as a film like structure.

Keywords: *Aspergillus niger*, biosorption, inorganic mercury, kinetics, marine fungi

Received 22 August 2007, Accepted 14 November 2007

Address correspondence to Kalpana Mody, Marine Biotechnology and Ecology Discipline, Central Salt & Marine Chemicals Research Institute, Bhavnagar 364 002, Gujarat, India. Tel.: +91-278-2561354; Fax: +91-278-2570885/2567562; E-mail: khmody@csmcri.org

INTRODUCTION

An ever increasing global demand for metals has resulted in increased mining, metal processing, and related activities leading to the contamination of the environment with heavy metals (1). Among these, mercury is one of the most toxic heavy metal ions to all living organisms. Mercury contamination of the environment is caused by natural as well as manmade sources. Mercury in any form, introduced to the natural environment, is converted into more toxic volatile forms (i.e. methylmercury chloride, ethylmercury chloride and phenylmercury chloride) by microorganisms and abiotic processes, forming more toxic alkylmercury. Bioaccumulation of alkylmercury through food chains causes a potential risk to consumers of fish and shellfish (2, 3). It is also known to affect the metabolism of sodium and potassium by increasing the latter's permeability, destroys the mitochondrial apparatus, causes swelling of cells leading to lysis and decreased DNA content in the cells (4).

Conventional treatments to remove mercury from contaminated water are mostly based on biosorption to material such as ion exchange resins (5). Biosorption of heavy metals by biomaterials has been suggested as a potential alternative to the existing physicochemical technologies for detoxification and recovery of toxic and valuable metals from wastewaters (6). Fungal biomass can also take up considerable quantities of heavy metals from solution by adsorption or related process, even in the absence of physiological activity (7). Many fungal species have been extensively studied for mercury biosorption and the process mechanism seems to be species dependent (8–10). Due to lack of adequate reports available on biosorption of mercury using marine fungi, detailed studies are required to be conducted before establishing their similar applications. In this paper, the possible use of dead biomass of marine *A. niger* as a biosorbent for removal of inorganic mercury from aqueous solutions was studied. This study was also aimed to understand the mechanism of biosorption, evaluate the biosorption kinetics as well as to establish the kinetic rate coefficients.

EXPERIMENTAL

Collection of Samples and Isolation of Fungi

Seawater and sediment samples were collected in sterile containers along the Gujarat coast (West coast of India) and brought to the laboratory. These samples were inoculated onto potato dextrose agar medium containing 500, 20, and 30 g of boiled and smashed potato, dextrose, and agar, respectively, in 1 L of seawater, and incubated at 30 $\pm$ 2°C. The fungi were purified on the same medium. The cultures were routinely maintained at 4°C on potato dextrose agar slants. The isolated fungi were identified at the Aghakar Research Institute, Pune, India. The various isolated, fungal biomasses were

cultivated in liquid medium containing (g/L), 250 and 20 g of boiled and smashed potato and dextrose respectively, and incubated at 30 ± 2°C. All reagents were from Himedia, or Merck (Mumbai, India). The pH of the growth medium was adjusted to 5.8–6.0 by the addition of 0.1 N HCl prior to autoclaving. After inoculation, the flasks were kept under static condition for 7 days at 33 ± 2°C. After 7 days of incubation, the live fungal mat was recovered by filtration, killed by boiling in 0.5 N NaOH solution for 15 min and washed with deionized water till the pH of the wash solution was in the neutral range. The biomass was then dried at 60°C for 20–24 h and powdered in a mortar. The powdered biomass residue was used for further studies.

Biosorption of Hg(II)

1.354 g of mercuric chloride ($HgCl_2$) was dissolved in 1 L of distilled water to obtain a stock solution having 1000 mg/L of Hg ion. The stock solution was diluted to obtain test solutions of desired strength. To screen efficient fungal species, 4.0 g/L of dead biomass of all the fungi were mixed with 25 mL of solution containing 15 mg/L of Hg(II) at pH 5 and agitated on a mechanical shaker (200 rpm) at 30 ± 2°C for different time intervals. The fungi, which exhibited maximum biosorption capacity was selected for further studies. In order to find the effect of the initial Hg(II) concentration on biosorption, solutions containing 50, 100, 150, 200, and 250 mg/L of mercury were used. The effect of pH on biosorption was investigated in the pH range of 1 to 9. The pH of the solution was adjusted with 0.1 N HCl or NaOH solution. The effect of temperature on the biosorption of Hg(II) was studied at five different temperatures viz. 10, 20, 30, 40, and 50°C with different concentrations of mercury such as 50, 100, 150, 200, and 250 mg/L using constant temperature (±2°C) water bath. The effect of biomass concentration on the removal of Hg(II) at 50 mg/L concentration was studied employing 0.8, 2.4, 4.0, 5.6, 8.0, 10.0, 12.0, 14.0, and 16.0 g/L of biomass. After attaining equilibrium, the aqueous phases were separated from the biosorbents by filtration through 0.45 μm Whatman filter paper and the concentration of Hg(II) ions in the filtrate was determined.

Kinetic experiments were conducted with 25 mL of 50 mg/L solution at pH 3.0 and 30 ± 2°C and samples were drawn at regular time intervals. The pH of the solution was monitored continuously with a pH electrode and adjusted with HCl or NaOH solution, if deviations were observed. All the experiments were repeated twice to confirm the results.

Mercury Analysis

Hg(II) was analyzed in the filtrate by Inductively Coupled Plasma Optical Emission Spectrometry (ICP, Perkin Elmer, Optima 2000 DV) after required dilutions of the filtrate.

Kinetic Models

Fractional power (11), zero order (12), first order (13), pseudo-first-order (14, 15), Elovich (16, 17), second order (18, 19) pseudo-second-order (20), and intraparticle diffusion (21, 22) rate equations have been used for modeling the kinetics of Hg(II) biosorption by *A. niger* (Table 1).

Non-linear Regression Analysis

All the model parameters were evaluated by non-linear regression using DATAFIT® software (Oakdale Engineering, USA). The optimization procedure requires an error function to be defined in order to be able to evaluate the fit of the equation to the experimental data (23). Apart from the correlation coefficient (r^2), the residual or sum of squares error (SSE) and the standard error (SE) of the estimate were also used to measure the goodness-of-fit. SSE can be defined as:

$$\mathrm{SSE} = \sum_{i=1}^{m} (Q_i - q_i)^2 \tag{1}$$

Standard error (SE) of the estimate, SE can be defined as:

$$\mathrm{SE} = \sqrt{\frac{1}{m-p} \sum_{i=1}^{m} (Q_i - q_i)^2} \tag{2}$$

Table 1. Kinetic sorption models

Sr. no.	Kinetic model	Equation	Reference
1	Fractional power	$q_t = k\ t^v$	(11)
2	Zero-order	$q_t = q_e - k_0\ t$	(12)
3	First-order	$q_t = q_e - \exp(k_1 t)$	(13)
4	Pseudo-first order	$q_t = q_e[1 - \exp(-k_1 t)]$	(14, 15)
5	Elovich	$q_t = (1/\beta_E) \ln(1 + \alpha_E\ \beta_E t)$	(16, 17)
6	Second-order	$q_t = \dfrac{q_e}{1 + q_e k_2 t}$	(18, 19)
7	Pseudo-second-order	$q_t = \dfrac{k_{2p} q_e^2 t}{(1 + k_{2p} q_e t)}$	(20)
8	Intraparticle diffusion	$q_t = k_p\ t^{0.5}$	(21, 22)

where q_i is the observation from the batch experiment i, Q_i is the estimate from the isotherm for corresponding q_i, m is number of observations in the experimental isotherm, and p number of parameters in the regression model. The smaller SE value indicates the better curve fitting.

BET Analysis

The surface area, pore volume and pore size of *A. niger* were measured by surface area analyzer (Micromeritics, ASAP 2010) and is given in Table 2.

FTIR, SEM, and EDAX Studies

Infrared spectra of unloaded and Hg(II) loaded biomass of *A. niger* was obtained using a Fourier Transform Infrared Spectrometer (FTIR GX 2000, Perkin-Elmer). For the FTIR study, 30 mg of finely ground biomass was pelleted with 300 mg of KBr (Sigma) in order to prepare translucent sample disks.

The surface structure of biosorbent was analyzed by scanning electron microscopy (SEM) coupled with energy dispersive X-ray analysis (EDAX)

Table 2. Characteristics of marine fungi *Aspergillus niger*

Parameter	Fungal biomass
Surface area	
Single point surface area, m^2/g	3.40
BET surface area, m^2/g	3.95 $\pm$ 0.03
BJH adsorption cumulative surface area of pores between 17.000000 and 150.000000Å diameter, m^2/g	4.03
BJH desorption cumulative surface area of pores between 17.000000 and 150.000000Å diameter, m^2/g	4.12
Pore volume	
Single point adsorption total pore volume of pores, cm^3/g	0.005
Pore size	
Adsorption average pore diameter (4 V/A by single point), Å	57.84
BJH adsorption average pore diameter (4 V/A by single point), Å	91.17
BJH desorption average pore diameter (4 V/A by single point), Å	82.86

using JEOL 560 LV SEM. Unloaded and metal-loaded *A. niger* biomass samples were mounted on a stainless steel stab with a double-stick tape followed by coating with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs.

RESULTS AND DISCUSSION

Screening of Different Fungal Species for Hg(II) Removal

In order to screen potential fungal species for Hg(II) removal, time dependent concentration of Hg(II) was measured in a batch system containing four different species of *Aspergillus* (Figure not shown). It was observed that all the four species of fungi showed good capacity to biosorb Hg(II) however, the initial removal rate of Hg(II) depended on the species of *Aspergillus*; the order was *A. niger* > *A. terreus* > *A. oryzae* > *A. wentii*. The purpose of the screening experiment was to identify efficient fungal species having maximum biosorption capacity with respect to Hg(II). This was exhibited by *A. niger* where 96.98% of Hg(II) was biosorbed within 24 h as compared to 86.46, 85.10, and 84.33% by *A. terreus, A. oryzae* and *A. wentii* respectively. Since *A. niger* exhibited maximum potential for Hg(II) sorption, therefore, it was used for further studies.

Effect of Initial Concentration

The initial concentration of Hg ions in the solution remarkably influenced the equilibrium uptake of Hg(II). It was noted that as the initial concentration increased, the sorption of Hg(II) increased as it is generally expected due to the equilibrium process, finally reaching a saturation value. When the initial concentration of Hg(II) was increased from 50–250 mg/L the uptake capacity increased from 10.43 to 39.38 mg/g of biomass (Fig. 1). But simultaneously, the percent biosorption was found to decrease from 90.12 to 63.01 (Figure not shown). The amount of Hg(II) ions adsorbed per unit mass of the biosorbent (i.e., biosorption capacity) increased with the initial concentration which might be due to higher availability of Hg(II) ions in the solution. Moreover, a higher initial concentration provides increased driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phases resulting in higher probability of collision between Hg(II) ions and sorbents. The reduction in percent biosorption might be due to the lack of available binding sites in the biomass and an increase in the number of ions competing for the binding sites. It was also observed that equilibrium was established within 2 h for all the concentrations studied.

Table 3 shows the sorption capacity of other biosorbents to uptake Hg(II) from aqueous solutions. It was observed that Hg(II) sorption capacity of

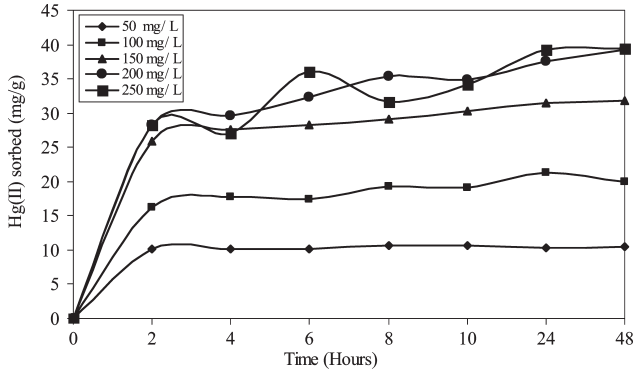

Figure 1. Hg(II) uptake at varying initial concentration of Hg(II) ion (biomass: 4.0 g/L; pH 5.0; temperature 30 ± 2°C) by *A. niger*.

A. niger in the present study was found to be relatively higher than the other biosorbents like bacteria, plant material and seaweed and lower than other fungi like *Phanerochaete chrysosporium and P. sajur-caju.*

Effect of pH

It is well known that metal biosorption on both non specific and specific sorbents is pH dependent (24). The pH of the medium affects the solubility of metal ions and the ionisation of the functional groups (i.e. carboxylate, phosphate, and amino groups) on the fungal cell wall (25). A high pH may result in the formation of stable metal complexes, e.g., hydroxides, oxides,

Table 3. Biosorption characteristics of various biosorbents for Hg(II) removal

Biosorbent	Type	Uptake capacity (mg/g)	pH	Reference
A. niger	Marine Fungi	40.53	3.0	Present study
A. niger	Fungi	3.2	5.2	(9)
Phanerochaete chrysosporium	Fungi	83.10	6.0	(28)
P. sajur-caju	Fungi	102.15	5.5	(8)
Saccharomyces cerevisiae	Yeast	64.2	5.5	(45, 46)
E. coli	Bacteria	17.6	3.0	(47)
Coriandrum sativum	Plant	24	6.0	(48)
Ulva lactuca	Seaweed	27.24	3.5	(49)

and carbonates, making the heavy metals less available to ion exchange resins or biosorbents. A low pH may increase the mobility of heavy metals and therefore may enhance their availability. In order to establish the effect of pH on the biosorption of Hg(II) ions on *A. niger*, batch equilibrium studies were conducted by varying pH from 1 to 9. The biosorption of Hg(II) on *A. niger* increased with pH up to 3 and then declined slightly with further increase in pH (Fig. 2). But, at the same time, the biosorption capacity was found to reduce by almost 50% when pH was reduced from 3.0 to 1.0. The highest uptake capacity of Hg(II) is observed at pH 3.0. It has been proposed that the groups responsible for metal binding are carboxyl groups, which have p*K*a's between 3 and 4 (26). The increase in binding that occurred at pH 3.0 can be attributed to these deprotonated and negatively charged species (27), whereas the decrease in biosorption at pH may be due to protonation. Svecova et al. (10) have reported maximum biosorption of mercury by *Penicillium* sp at pH-5.

Effect of Biomass Dosage

The influence of biosorbent dosage on percentage biosorption and uptake capacity by *A. niger* is depicted in Fig. 3. The increase in the sorbent dose from 0.8 to 16.0 g/L resulted in a rapid increase in biosorption of Hg ions from 40.19 to 89.4%. This is because of the availability of more binding sites for complexation of Hg ions. Further increment in sorbent dose did not cause significant improvement in biosorption capacity. This may be due to the binding of almost all ions to the sorbent and the establishment of equilibrium between the ions bound to the sorbent and those remaining unadsorbed in the solution. However, Hg uptake values showed a reverse trend, as it is a measure of the amount of Hg ions bound by unit weight of biomass and therefore, its magnitude decreased with increase in biomass dose. In the present investigation, a vast reduction from 21.8 to 2.77 mg/g in Hg

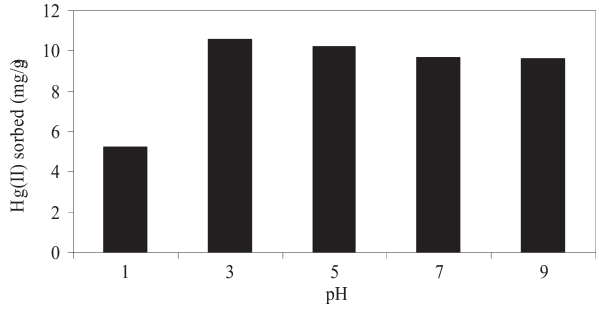

Figure 2. Effect of varying pH on percent biosorption and Hg(II) uptake capacity by *A. niger* (Hg(II) conc 50 mg/L, biomass: 4.0 g/L; temperature 30 ± 2°C contact time: 6 h).

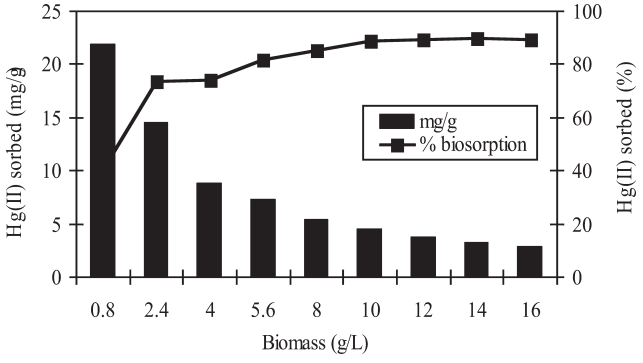

Figure 3. Effect of biomass dose on percent biosorption and Hg(II) uptake capacity by *A. niger* (Hg(II) conc 50 mg/L, pH 3.0; temperature 30 $\pm$ 2°C contact time: 6 h).

sorption capacity was observed when biomass dosage was increased from 0.8 to 16.0 g/L (Fig. 3).

Effect of Temperature

The temperature of the biosorption medium could be important for energy dependent mechanisms in metal biosorption by microorganisms. Energy independent mechanisms are less likely to be affected by temperature since the process responsible for biosorption is largely physicochemical in nature. Therefore, experiments were performed to examine the temperature dependency of Hg(II) uptake by the dead fungal biomass of marine *A. niger*. In the present investigation, the biosorption of Hg(II) appeared to be independent of temperature since an increase in temperature from 10–50°C did not substantially increase the Hg(II) biosorption capacity (Fig. 4). However,

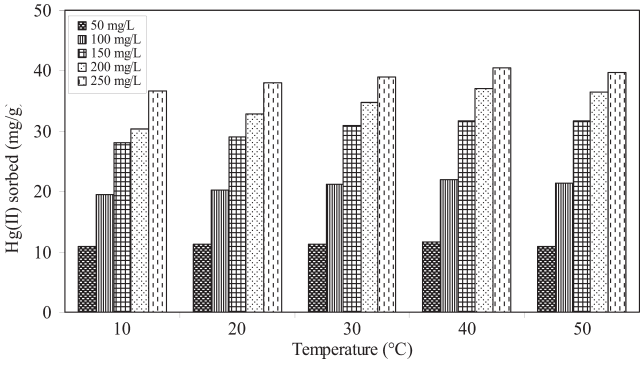

Figure 4. Effect of temperature on Hg(II) uptake at various initial concentration of Hg(II) ion (pH 3.0, biomass 4.0 g/L; contact time: 6 h) by *A. niger*.

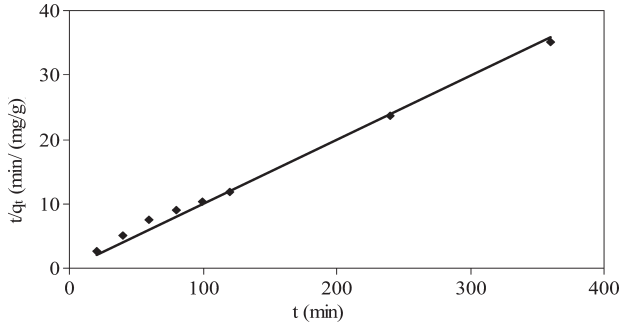

Figure 5. Pseudo second order model for the sorption of Hg(II) on *A. niger*.

maximum Hg(II) binding was observed at 40°C. Similar results have been observed by Saglam et al. (28) and Arica et al. (8).

Kinetic Studies

A simple kinetic analysis for sorption of Hg(II) on to *A. niger* has been tested according to fractional power model and Table 4 shows the estimated parameters of the model. The results indicate that the functional power model was unable to

Table 4. Kinetic parameters for the sorption of Hg(II) on *Aspergillus niger*

Models	r^2	SSE	SE	Constants
Fractional power	0.829	0.0189	0.0561	$k = 1.13$ (mg/g) $v = 1.6441$ (mg/min)
Zero order	0.617	3.3834	0.7509	$k_0 = -0.00763$ (mg/ml/min) $q_e = 8.0853$ (mg/g)
First order	0.605	0.0082	0.0371	$k_1 = -0.0008$ (1/min) $q_e = 8.0755$ (mg/g)
Pseudo first order	0.716	0.0004	0.0081	$k_{1p} = 7.8686$ (1/min) $q_e = 10.071$ (mg/g)
Elovich	0.827	1.5210	0.5034	$\alpha_E = 47.3459$ (mg/g/min) $\beta_E = 0.9111$ (g/mg)
Second order	0.592	0.0005	0.0097	$k_2 = -0.00094$ (g/mg/min) $q_e = 8.065$ (mg/g)
Pseudo second order	0.998	1.4113	0.4850	$q_e = 10.62891$ (mg/g) $k_{2p} = 0.007574$ (g/mg/min) $h = 0.85566$ (mg/g/min)
Intraparticle diffussion	0.934	0.0012	0.0346	$k_p = 0.0031$ (mg/(g/min)1/2)

describe the time-dependent Hg(II) sorption by *A. niger* as the value of the correlation coefficient was 0.829 and constant 'v' was greater than 1, though the values of SSE and SE were low. The kinetic constants, k_0, k_1, k_{1p}, α_E and β_E, and k_2 of zero order, first order, pseudo first order, Elovich and second order equations, respectively, for the sorption of Hg(II) by *A. niger* are also presented in Table 4. The results demonstrated that there is no significant relationship between the kinetic data (Figures not shown) with low correlation coefficients (<0.827) indicating that these models are not applicable in the present case. However, the value of q_e, in case of pseudo first order model, is almost closer to experimental value (10.22 mg/g). In most of the cases, first-order and Elovich models would seem not to be appropriate for most heterogeneous systems, since multiple sorption sites exist (18, 29).

The results in Table 4 describes the sorption rate constant, k_{2p}, initial sorption rate, h, and equilibrium sorption capacity, q_e, of the pseudo-second-order model (Fig. 5). These results show a very good compliance with the pseudo-second-order equation with high correlation coefficient (0.998) and standard error <1. The equilibrium concentration, q_e obtained from this model is closely in line with the experimental value. The pseudo second order model has been widely used to describe non chemical equilibrium (13, 14, 30) and non physical equilibrium (31, 32). This model assumes that two reactions are occurring, the first one is fast and reaches equilibrium quickly and the second is a slower reaction that can continue for long time periods. The reactions can occur either in series or in parallel (33).

The initial sorption rate, h, has been widely used for evaluation of the sorption rates (34–36). In the present study the value of "h" is 0.85566 mg/g/min. Benhammoua et al. (37) obtained the initial rate of 11.26 and 1.493 mg/g/min for Hg(II) on Fe- and raw *Moroccan stevensite*, respectively. The intraparticle diffusion coefficient for the sorption of Hg(II) was calculated from the slope of the plot between the amount of Hg(II) sorbed, q_t (mg/g) vs $t^{1/2}$ ($min^{1/2}$). Based on these plots (Fig. 6), it was concluded that the sorption process of Hg(II) is comprised of two phases, suggesting that the intraparticle

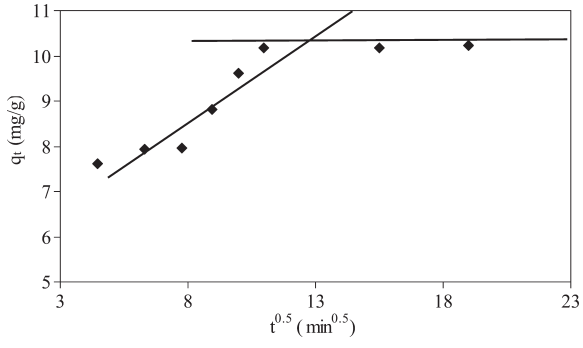

Figure 6. Intraparticle diffusion plot for the sorption of Hg(II) on *A. niger*.

diffusion is not the rate limiting step for the whole reaction (18). The initial portion of the plot indicated an external mass transfer whereas the second linear portion is due to intraparticle or pore diffusion. The intercept of the plot provides an estimation of the thickness of the boundary layer, i.e., the larger the intercept value the greater is the boundary layer effect (37). The slope of the second linear portion of the plot has been identified as the intraparticle diffusion rate constant k_p (mg/g/min). The kinetic data were further analyzed using the kinetic expression given by Boyd et al. (38) to check whether sorption proceeds via external diffusion or intraparticle diffusion mechanism, which is expressed as follows:

$$F = 1 - \frac{6}{\Pi^2} \exp(-B_b t) \tag{3}$$

where, Bb is a constant and F is the fractional attainment of equilibrium at time t given by

$$F = \frac{q_t}{q_e} \tag{4}$$

where q_e and q_t represent the amount of Hg(II) sorbed (mg/g) at equilibrium and any time t, respectively. To compute Bbt, Eq. (4) is substituted into Eq. (3) and the kinetic expression becomes

$$B_b\ t = -0.4977 - \ln\left(1 - \frac{q_t}{q_e}\right) \tag{5}$$

Thus, the value of $B_b t$ can be computed for each value of F, and then plotted against time (Fig. 7) to configure the so-called Boyd plots (38). The linearity of this plot is employed to distinguish between external-transport-(film diffusion) and intraparticle-transport-controlled rates of sorption (38). A straight line passing through the origin is indicative of sorption processes governed by particle-diffusion mechanisms; otherwise they are governed by film diffusion (38). In the present case, the plots were neither linear nor passed through the origin (Fig. 7). This indicates that, film diffusion is the

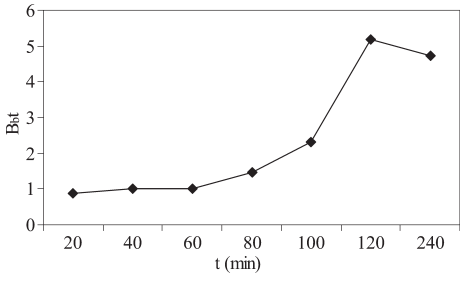

Figure 7. Boyd plot for the sorption of Hg(II) on *A. niger*.

rate-limiting sorption process for Hg(II). However, further studies are needed to establish whether film diffusion is the rate-limiting step in Hg(II) sorption onto *A. niger*. Similar results were obtained by Wang et al. (39) and El-Kamash et al. (40).

FT-IR, SEM, and EDAX Analysis

FTIR spectroscopy offers excellent information on the nature of the bonds present and allows identification of different functionalities on the cell surface. Numerous chemical groups on the cell surface of fungal biomass have been proposed to be responsible for the biosorption of metals. These include hydroxyl, amino and carboxyl groups. Their relative importance in metal sorption may depend on factors such as the quantity of sites, their accessibility, chemical state, and affinity between site and metal.

The FTIR spectra of raw *A. niger* (Fig. 8a) clearly demonstrate presence of different functional groups. The sharp band around 3425 cm^{-1} is attributed to -OH groups, whereas the band at 2926cm^{-1} is representative of -CH stretching, whereas bands at 2361cm^{-1} and 2341 cm^{-1} are indicative of miscellaneous groups like R-H. The wide band located around 1639 cm^{-1} corresponds to amide I band, the band at 1423 cm^{-1} could be attributed to amide III from proteins, whereas the band at 1037 cm^{-1} corresponded to -CN stretching (41–44).

Figure 8(b) shows FT-IR spectrum of *A. niger* subjected to Hg(II) where significant reduction in the intensity of bands at 3437, 2925, 2356, 1639, and 1039 cm^{-1} was observed indicating the involvement of hydroxyl, $-CH_2$ and

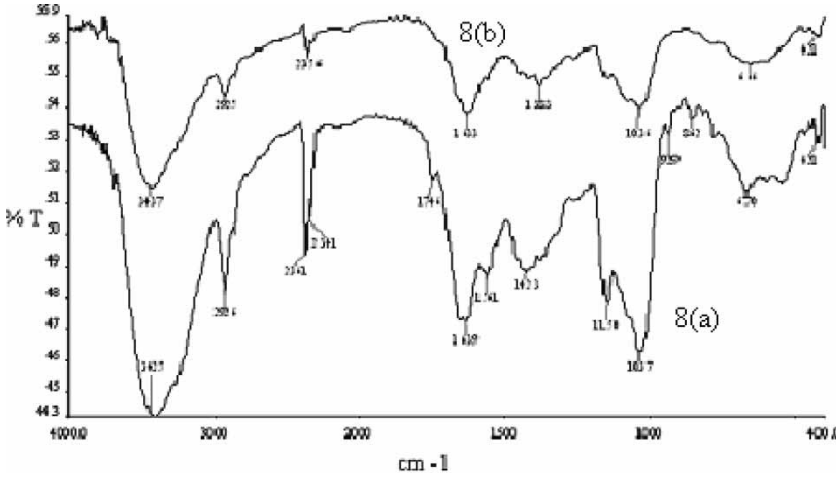

Figure 8. FTIR spectra of raw and Hg(II) loaded *A. niger*.

Table 5. Functional groups of Hg(II) loaded and unloaded biomass of *A. niger* and the corresponding infrared absorption wavelengths

Observed wavelength (cm^{-1})	Wavelength range (cm^{-1})	Assignment
3425–3437	3600–3200	Bonded hydroxyl groups (OH^-)
2925–2926	2960–2850	C-H stretching
2341–2361	2700–2250	NH_2^+, NH^+, NH
1633–1639	1650–1560	Amide I, NH_2 bending
1383–1423	1420–1330	Amide III
1036–1037	1050–1030	P-O alkyl (phosphorous compounds)
851–862	860–800	NO_3^-

amino groups for Hg(II) binding to *A. niger*. Svecova et al. (10) studied the biosorption of cadmium, lead and mercury on waste fungal biomass obtained from fermentation industry, where, FT-IR studies indicated disappearance of the band at 1580 cm^{-1} which tends to prove that the amine group was involved in the binding of the metals. They have also observed a decrease in the band at 1650–1660 cm^{-1} due to metal binding which was attributed to amide bands conjugated with -OH bending from water. Thus, our results match well with the reported literature (Table 5).

This study, although preliminary in nature, points to differences in binding affinity of the various functional groups to Hg(II) which indicates the feasibility of further studies on the relationship between composition of cell surface of fungus and FTIR behavior at different metal concentrations.

SEM micrographs and EDAX spectra of raw and Hg(II) loaded dead *A. niger* biomass are presented in Fig. 9 and Fig. 10, respectively. After metal binding, obvious morphological changes were seen in the cell wall

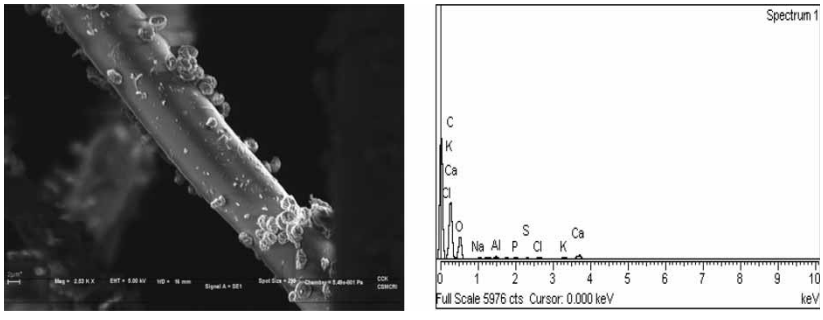

Figure 9. SEM and EDAX of raw *A. niger*.

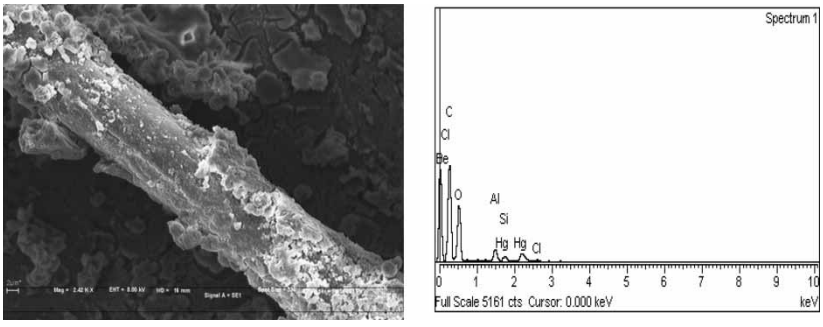

Figure 10. SEM and EDAX of Hg(II) loaded *A. niger*.

structure, where metal seems to be deposited on the surface of the biomass as a film like structure. Biosorption of Hg(II) was also confirmed by EDAX analysis which revealed the presence of Hg(II) signals on the metal loaded fungal biomass.

CONCLUSION

Studies pertaining to the assessment of the best biosorption parameters and quantitative analysis of Hg(II) uptake by *A. niger* revealed that under the optimum conditions (pH: 3.0, Hg(II) concentration: 250mg/L, biomass dose: 0.8 g/L, temperature: 40°C and contact time: 6 h), 40.53 mg Hg(II) could be adsorbed per gram of dead biomass of *A. niger*. Hence, it can be concluded that marine *A. niger* exhibited significant potential for its exploitation in the treatment of industrial effluents containing Hg(II). It was also seen that the pseudo second order model showed the best fit for kinetic of Hg(II) sorption by *A. niger*.

NOTATIONS

B_b	Boyd Constant
F	Fractional attainment of equilibrium at time t
h	Initial sorption rate in pseudo-second model, mg/g/min
k	Fractional power model constant
k_0	Zero order model constant
k_p	Initial rate of the intraparticle diffusion, mg/g/min
k_1	Lagergren's biosorption isotherm rate constant, 1/min
k_{1p}	Pseudo first order model constant
k_2	Pseudo-second order sorption rate constant, g/mg/min
k_{2p}	Pseudo second order model constant, k_{2p}, g/mg/min

q_e	Amount of adsorbate adsorbed at equilibrium, mg/g
q_t	Amount of sorbate sorbed at time t, mg/g
Q_i	Estimated sorption capacity of batch experiment, i
r^2	Correlation coefficient
SE	Standard error
SSE	Sum of squares error
t	Sorption time, min
v	Fractional power model constant
α_E	Initial sorption rate in Elovich model, mg/g/min
β_E	Desorption constant in Elovich model, g/mg

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr P. K. Ghosh, Director, CSMCRI, Bhavnagar for constant support and encouragement and Council of Scientific and Industrial Research, New Delhi for financial support to Dr. Yasmin Khambhaty.

REFERENCES

1. Landis, W.G. and Yu, H.H. (1999) *Introduction to Environmental Toxicology: Impacts of Chemicals up on Ecological Systems*; 2nd (edn.); CRC press LLC.
2. Pak, K.R. and Bartha, R. (1998) Mercury methylation and dimethylation in anoxic lake sediments and by strictly anaerobic bacteria. *Appl. Environ. Microbiol.*, 64: 1013–1017.
3. Spry, D.J. and Wien, J.G. (1991) Metal bioavailability and toxicity to fish in low-alkalinity lakes: a clinical review. *Environ. Pollut.*, 71: 243–304.
4. Kurtland, L.T., Faro, S.N., and Sieldler, H. (1960) Minamata disease: the outbreak of a neurological disorder in Manamata, Japan, and its relationship to the ingestion of seafood contaminated mercuric compounds. *World Neurol.*, 1: 370–395.
5. Volesky, B. (2001) Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy*, 59: 203–216.
6. Gadd, G.M. and White, C. (1989) Removal of Thorium from simulated acid process steam by fungal biomass. *Biotechnol. Bioeng.*, 33: 592–597.
7. Osteen, A.B. and Bibler, J.P. (1991) Treatment of radioactive laboratory waste for mercury removal. *Water Air Soil Pollut.*, 56: 63–74.
8. Arica, M.Y., Arpa, C., Kaya, B., Bekta, S., Denizli, A., and Gençet, O. (2003) Comparative biosorption of mercuric ions from aquatic systems by immobilized live and heat inactivated *Trametes versicolor* and *Pleurotus sajur-caju. Biores. Technol.*, 89: 145–154.
9. Karunasagar, D., Arunachalam, J., Rashmi, K., Naveena Lavanya Latha, J., and Maruthi Mohan, P. (2003) Biosorption of inorganic and methyl mercury by a biosorbent from *Aspergillus niger*. *World J. Microbiol. Biotechnol.*, 19: 291–295.
10. Svecova, L., Spanelova, M., Kubal, M., and Guibal, E. (2006) Cadmium, lead and mercury biosorption on waste fungal biomass issued from fermentation industry. I. Equilibrium studies. *Sep. Purif. Technol.*, 52: 142–153.

11. Dalal, R.C. (1974) Desorption of phosphate by anion exchange resin. *Commun. Soil Sci. Plant Anal.*, 5: 531–538.
12. Covelo, E.F., Andrade, M.L., and Vega, F.A. (2004) Heavy metal biosorption by humic umbrisols: selectivity sequences an competitive sorption kinetics. *J. Colloid. Inter. Sci.*, 280: 1–8.
13. McCall, P.J. and Agin, G.L. (1985) Desorption of picloram as affected by residence time in the soil. *Environ. Toxicol. Chem.*, 4: 37–44.
14. Lagergren, S. (1898) Zur theorie der sogenannten adsorption geloster stoffe. *Vetenskapsakad Handl.*, 24: 1–39.
15. Karickhoff, S.W. (1980) In *Contaminant and Sediments*. *Ann Arbor Sci*; Baker, R.A. (ed.); Ann Arbor: MI., pp. 193–205.
16. Low, M.J.D. (1960) Kinetics of chemisorption of gases on solids. *Chem. Rev.*, 60: 267–312.
17. Cheung, C.W., Porter, J.F., and McKay, G. (2000) Elovich equation and modified second-order equation for sorption of cadmium ions onto bone char. *J. Chem. Technol. Biotechnol.*, 75: 963–970.
18. Ho, Y.S. and Ofomaja, A.E. (2005) Kinetics and thermodynamics of lead ion sorption on palm kernel fibre from aqueous solution. *Process Biochem.*, 40: 3455–3461.
19. Cheung, C.W., Porter, J.F., and McKay, G. (2001) Sorption kinetic analysis for the removal of cadmium ions from the effluent using bone char. *Water Res.*, 35: 605–612.
20. Ho, Y.S. (2006) Second-order kinetic model for the sorption of cadmium onto tree fern: A comparison of linear and non-linear methods. *Water Res.*, 40: 119–125.
21. Aharoni, C., Sparks, D.L., Levinson, S., and Ravina, I. (1991) Kinetics of Soil Chemical Reactions: Relationships between Empirical Equations and Diffusion Models. *Soil Sci. Soc. Am. J.*, 55: 1307–1312.
22. McKay, G., Otterburn, M.S., and Aga, J.A. (1987) Intraparticle diffusion process occurring during adsorption of dyestuffs. *Water Air Soil Pollut.*, 36: 381–390.
23. Ho, Y.S., Chiu, W.T., and Wang, C.C. (2005) Regression analysis for the sorption isotherms of basic dyes on sugarcane dust. *Bioresour. Technol.*, 96: 1285–1291.
24. Ho, Y.S. (1995) Adsorption of heavy metals from waste streams by peat. Ph.D. thesis, University of Birmingham, Birmingham, UK.
25. Hafez, N., Abdel-Razek, S., and Hafez, M.B. (1997) Accumulation of some heavy metals on *Aspergillus flavus*. *J. Chem. Tech. Biotechnol.*, 68: 19–22.
26. Okino, S., Iwasaki, K., Yagi, O., and Tanaka, H. (2000) Development of a biological mercury removal–recovery system. *Biotechnol. Lett.*, 22: 783–788.
27. Namasivayam, C. and Kadirvelu, K. (1999) Uptake of mercury (II) from wastewater by activated carbon from an unwanted agricultural solid byproduct: coirpith. *Carbon*, 37: 79–84.
28. Saglam, A., Yalcinkaya, Y., Denizli, A., Arica, M.Y., Genc, O., and Bektas, S. (2002) Biosorption of mercury by carboxymethylcellulose and immobilized *Phanerochaete chrysosporium*. *Microchem. J.*, 71: 73–8.
29. Ho, Y.S., Chiu, W.T., Hsu, C.S., and Huang, C.T. (2004) Sorption of lead ions from aqueous solution using tree fern as a sorbent. *Hydrometallurgy.*, 73: 55–61.
30. Hamaker, J.W. and Thompson, J.M. (1972) *Organic Chemicals in the Environment*; Goring, C.A.I. and Hamaker, J.W. (eds.); Dekker: New York, 39–151.
31. van Genuchten, M.Th. and Wagenet, R.J. (1989) Two-site/two-region models for pesticide transport and degradation: Theoretical development and analytical solutions. *Soil Sci. Soc. Am. J.*, 53: 1303–1310.

32. Lee, L.S., Rao, P.S.C., Brusseau, M.L., and Ogwada, R.A. (1988) Nonequilibrium sorption of organic contaminants during flow through columns of aquifer materials. *Environ Toxicol Chem.*, 7: 779–793.
33. Brusseau, M.L. and Rao, P.S.C. (1984) Sorption nonideality during organic contaminant transport in porous media. *CRC Cri. Rev. Environ. Control*, 19: 33–99.
34. Ho, Y.S. and McKay, G. (1998) Sorption of dye from aqueous solution by peat. *Chem. Eng. J.*, 70: 115–124.
35. Benhammou, A., Yaacoubi, A., Nibou, L., and Tanouti, B. (2005) Study of the removal of mercury(II) and chromium (VI) from aqueous solutions by *Moroccan stevensite*. *J. Hazard. Mater.*, 117: 243–249.
36. Ho, Y.S. and McKay, G. (2000) The kinetics of sorption of divalent metal ions onto sphagnum moss peat. *Water Res.*, 34: 735–742.
37. Oubagaranadin, J.U.K., Sathyamurthy, N., and Murthy, Z.V.P. (2007) Evaluation of Fuller's earth for the adsorption of mercury from aqueous solutions: a comparative study with activated carbon. *J. Hazard. Mater.*, 142: 165–174.
38. Boyd, G.E., Adamson, A.W., and Myers, L.S. (1947) The exchange adsorption of ions from aqueous solutions by organic zeolites. *J. Am. Chem. Soc.*, 69: 2836–2848.
39. Wang, X.S., Qin, Y., and Li, Z.F. (2006) Biosorption of zinc from aqueous solutions by rice bran: kinetics and equilibrium studies. *Sep. Sci. Technol.*, 41: 747–756.
40. El-Kamash, A.M., Zaki, A.A., and Abed-El-Geleel, M. (2005) Modeling batch kinetics and thermodynamics of zinc and cadmium removal from waste solutions using synthetic zeolite A. *J. Hazard. Mater.*, 127: 211–220.
41. Schmitt, J. and Flemming, H.-C. (1998) FTIR-spectroscopy in microbial and material analysis. *Int. Biodeter. Biodegr.*, 41: 1–11.
42. Shah, R. and Devi, S. (1996) Preconcentration of mercury(II) on dithizone anchored poly(vinyl pyridine) support. *React. Funct. Polym.*, 31: 1–9.
43. Williams, D.H. and Fleming, I. (1991) *Spectroscopic Methods in Organic Chemistry*; Tata McGraw-Hill Book Company Ltd.: New Delhi, 40–76.
44. Ashkenazy, R., Gottlieb, L., and Yannai, S. (1997) Characterization of acetone-washed yeast biomass functional groups involved in lead biosorption. *Biotechnol. Bioeng.*, 55: 1–10.
45. Al-Saraj, M., Abdel-Latif, M.S., El-Nahal, I., and Baraka, R. (1999) Bioaccumulation of some hazardous metals by sol–gel entrapped microorganisms. *J. Non-Cryst. solids*, 248: 137–40.
46. Madrid, Y., Cabrera, C., Perez-Corona, T., and Camara, C. (1995) Speciation of methylmercury and Hg(II) using baker's yeast biomass (*Saccharomyces cerevisiae*). Determination by continuous flow mercury cold vapor generation atomic absorption spectrometry. *Anal. Chem.*, 67: 750–4.
47. Chen, S. and Wilson, D.B. (1997) Genetical engineering of bacteria and their potential for Hg^{+2} bioremediation. *Biodegradation*, 8: 97–103.
48. Karunasagar, D., Balarama Krishna, M.V., Rao, S.V., and Arunachalam, J. (2005) Removal and preconcentration of inorganic and methyl mercury from aqueous media using a sorbent prepared from the plant *Coriandrum sativum*. *J. Hazard. Mater.*, 118: 133–139.
49. Zeroual, Y., Moutaouakkil, Adnane, Dzairi, F.Z., Talbi, Mohamed, Chung, P.U., Lee, Kangmin, and Blaghen, M. (2003) Biosorption of mercury from aqueous solution by. *Ulva lactuca* biomass. *Biores. Technol.*, 90: 349–35.