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Bio-Separation of Toxic Arsenate Ions from Dilute Solutions by Native and Pretreated Biomass of *Aspergillus fumigatus* in Batch and Column Mode: Effect of Biomass Pretreatment

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Abstract The sorption of As(V) from aqueous solution onto live and pretreated biomass of *Aspergillus fumigatus* was studied. The studies on optimization of contact time, adsorbent dosage and pH showed that the $FeCl_3$ treated and $FeSO_4$ treated biomass had the maximum capacity to adsorb As(V) while acid treated biomass was found to be minimum. Adsorption parameters were determined using both Langmuir and Freundlich isotherm models. The maximum adsorption capacity of 0.054 mg/g was observed in $FeCl_3$ treated and $FeSO_4$ treated biomasses. Column mode studies were conducted using $FeSO_4$ treated biomass to compare its efficacy with batch mode to adsorb As(V). Batch mode experiments proved to be efficient. Desorption studies were also carried out with dilute sodium hydroxide to recover both the adsorbent and adsorbate.

Keywords *Aspergillus fumigatus* · Arsenic · Pretreatment · Adsorption

Arsenic is one of the non-metal with great consideration today, because of its acute toxicity to terrestrial and aquatic organisms. In soils, it is known that presence of phosphates decreases the adsorption of arsenic on to soil particles thereby increasing the bioavailability of arsenic both in soil and groundwater probably due to the fact that arsenate and phosphate compete for common adsorption sites within the soil (Benedí et al. 2005). But in the present study we did not examine the competitive adsorption of arsenate and phosphate, instead we concentrated only on arsenate which is considered to be more hazardous to health. Biswas et al. (1998) reported high rate of arsenic related diseases among the people consuming arsenic contaminated groundwater. They include skin carcinoma, black foot disease, diffused melanosis, spotted melanosis, diffused keratosis, spotted keratosis, hyperkeratosis, nonpitting oedema, bowen's disease, gangrene etc. Groundwater contamination of arsenic in many parts of the world is well documented (Biswas et al. 1998; Acharyya 2002). Several methods applied for removal of metal and non-metal ions include ion exchange, reverse osmosis, electrodialysis, chemical precipitation, adsorption using activated carbon or other appropriate sorbents (alumina, zeolites, goethite etc.) (Sathishkumar et al. 2004). But these technologies suffer certain drawbacks such as operational complexity, high cost, low efficiency and other difficulties. Moreover these technologies are either ineffective or expensive in removing metal and non-metal ions at trace levels (Murugesan 2002). Studies on removal of As(V) from aqueous solutions and wastewaters have been extensively studied using various adsorbents (Gupta and Chen 1978; Hodi et al. 1995; Senthilkumar 1998; Altundogan et al. 2002). Use of these biosorbents in treatment of potable water is not widely studied. But using microbial biomass for such purpose will prove to be effective and highly economical as the presently existing technologies

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like reverse osmosis, electrodialysis, ion exchange etc. are very expensive to treat water containing trace amounts of As(V). The aims defined in our investigation was to test the feasibility of live and pretreated biomass of *Aspergillus fumigatus* to remove As(V) from aqueous solution.

Materials and Methods

For the preparation of adsorbent, 1 mL (10^6 spores) of *A. fumigatus* spore suspension was inoculated into Czapex-Dox broth in 250 mL Erlenmeyer flasks and incubated at room temperature (27 ± 3°C) 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. Biomass was then washed with generous amount of deionized water until free from the media components. The washed, live mycelial pellets were squeezed gently with filter paper and used as adsorbent as such (Sathishkumar et al. 2004). The biomass was also subjected to various pretreatments to test the efficacy of it to adsorb As(V). Before each pretreatment the biomass was washed with generous amount of deionized water until free from the media components. The pretreatment are as follows:

- Autoclaved biomass: the biomass was autoclaved for 30 min at 121°C at 18 psi.
- Acid treated biomass: the biomass was boiled for 15 min in 500 mL of 10% (vol/vol) *o*-phosphoric acid.
- Alkali treated biomass: the biomass was boiled for 15 min in 500 mL of 0.5 N Sodium hydroxide solution.
- Formaldehyde treated biomass: the biomass was boiled for 15 min in 500 mL of 15% (vol/vol) formaldehyde solution.
- $FeSO_4$ treated biomass: the biomass was autoclaved and agitated with $FeSO_4$ solution containing 15 mg/L of Fe for 90 min.
- $FeCl_3$ treated biomass: the biomass was autoclaved and agitated with $FeCl_3$ solution containing 15 mg/L of Fe for 90 min.

The biomass after each pretreatment was washed with deionized water until the pH of the wash solution was in near neutral range (pH 6.8–7.2). All the chemicals used were of analytical grade procured from Merck, sd fine and Glaxo.

To determine the effect of agitation time and adsorbate concentration on removal of As(V), 1000 mg (wet biomass) of the adsorbent was added to 50 mL of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L solutions of As(V) and agitated in a rotary shaker at 150 rpm for predetermined time intervals at 30°C. The adsorbate and adsorbent were separated by centrifugation at 10,000 rpm for 20 min. The remaining As(V) in the adsorbate were analyzed spectrometrically (Palanivelu and Ramakrishna 1990) (Shimadzu, UV-1601, Japan). The minimum detection limit of this analysis technique was 0.05 mg/L and for very high concentrations, the sample was dilutes prior to analysis and the dilution factor was included during the final calculations. The same spectrophotometric method was used in the subsequent experiments. The study was carried out with different dosages of adsorbent (250–2250 mg/50 mL) for an equilibrium time to determine the effect of adsorbent dose on As(V) removal. The effect of pH on As(V) removal was studied by using 50 mL of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L solutions of As(V), adjusted to an initial pH of 2–10 mixed with optimum dosage of adsorbent and agitated for equilibrium time. 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. All the above experiments were carried out with all the pretreated fungal biomass. From the data, Langmuir plot was drawn and adsorption constant (Q_o) was calculated from the slope of the curve. Freundlich isotherm was also studied using the above equilibrium data.

The pretreated biomass with maximum adsorption capacity was used as adsorbent in column studies. To study the effect of flow rate, 2.5 cm bed height of the adsorbent was packed in a glass column (45 × 2.2 cm) and the flow rate of 1.5 mg/L As(V) solution was adjusted to 2.5–10 mL/min. Fractions were collected at regular intervals and analysed for As(V). The effect of bed volume on removal of As(V) was studied in 1.5 mg/L As(V) solution by varying the height of the bed (2.5–10 cm). The study was carried out with different concentration of As(V) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) to determine the effect of initial As(V) concentration on removal of As(V). Data obtained were used to plot of BDST curves and adsorption rate constant (N_o) was calculated from the plot.

After adsorption studies with 1.5 mg/L As(V) solution and optimum dosage of adsorbent in batch mode, the As(V) laden adsorbent was separated out by filtration and the filtrate was discarded. The adsorbent was then given a gentle wash with double distilled water to remove any unadsorbed As(V) molecules on the adsorbent surface or entrapped between adsorbent particles. Desorption studies were carried out using those adsorbent samples agitated with 50 mL of 0.1 N NaOH for predetermined intervals. Later with the equilibrium time, the adsorbent samples were agitated with 50 mL of NaOH of various strengths (0.1–0.5 N).

FTIR analysis was done to the native and pretreated biomasses, for which the dried biomasses were ground in a mortar and pestle. A small amount of finely ground biomass was mixed with about 100 times its weight of powdered potassium bromide. The mixture was then thoroughly ground in a mortar and pestle, which was then passed under high pressure (18 psi) in a pelletizer to form a small pellet of about 1–2 mm thick and 1 cm in diameter. The resulting pellet was transparent and was used to test the surface functional groups by IR spectroscopy (Shimadzu 8201PC, Japan) where it was scanned between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1}.

Results and Discussion

The infrared spectrum was highly complex, reflecting the nature of the examined biomass. Despite the complexity certain characteristics peak could be assigned. FT-IR spectrum of Fe^{3+} modified biomass is shown in Fig. 1. The trough observed at 3411.8 cm^{-1} result from the NH_2 asymmetric stretch mode of amines. The trough observed at 2925.8 cm^{-1} is indicating of C–H groups (Loukidou et al. 2003). The 1649.0 cm^{-1} band is a result of CO stretching mode, conjugate to a NH deformation mode and is indicative of amide 1 band. The band present at 1519.8 cm^{-1} indicates the presence of amide 2 and results from NH deformation mode conjugated to C=N. The trough at 1375.2 cm^{-1} indicates the presence of amide 3 or sulfamide band. The 1033.8 cm^{-1} and 1710.7 cm^{-1} bands were due to C–O and C=O stretching in carbonyl and carboxyl groups. The FT-IR analyses of modified biomasses indicated enhancement at 3300–3400 cm^{-1} and at 1649 cm^{-1} and suggested increased amino and amide stretching and bending. The observed band at 1236.3 cm^{-1} was attributed to C–N stretching (Won et al. 2006). Therefore, it can be noted that the FT-IR spectrum of supports the presence of amine groups, which has many binding sites including carboxyl.

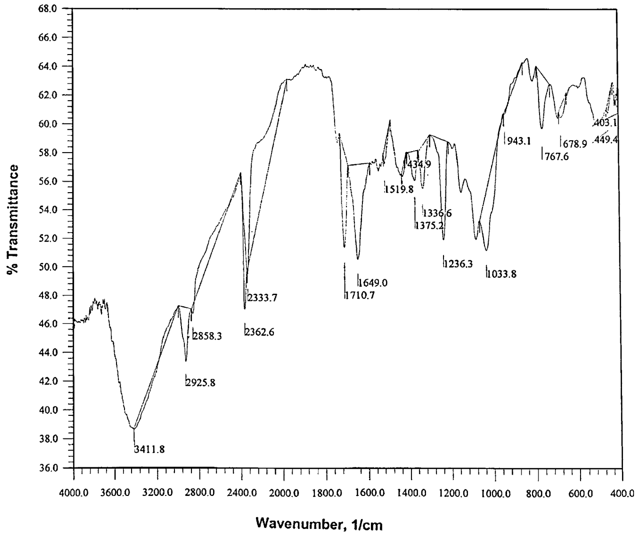

Fig. 1 FT-IR spectrum of $FeCl_3$ modified biomass

Table 1 shows the analytical data for adsorption of As(V) by autoclaved biomass with respect to contact time and initial As(V) concentration. As(V) uptake increased with increase in contact time but remained constant after an equilibrium time for all the adsorbents studied. Similar results have been reported for the removal of arsenic and some metal ions (Senthilkumar 1998; Selvakumari et al. 2002; Murugesan 2002). Equilibrium time varied with initial As(V) concentration and different adsorbent for As(V) removal. $FeCl_3$ treated and $FeSO_4$ treated adsorbent showed the maximum removal of As(V) and the acid treated adsorbent showed the minimum removal. The maximum equilibrium time of respective adsorbent was considered for further studies, which was 170 min for live, autoclaved, alkali treated, formaldehyde treated, $FeCl_3$ treated and $FeSO_4$ treated adsorbent and 165 min for acid treated adsorbent. The equilibrium time required by the adsorbent to remove As(V) is very less when compared to other reported adsorbents (Selvakumari et al. 2002). This result is interesting because equilibrium time is one of the important considerations for economical water and wastewater treatment applications (Sathishkumar et al. 2004).

The rate constant of As(V) adsorption on live and all other pretreated *A. fumigatus* biomass followed the first order rate expression given by Lagergren and Svenka (1898). The linear plots of $\log(q_e - q)$ vs. t for As(V) at different concentrations indicate the applicability Lagergren model. The values of K_{ad} were calculated from the slope of the linear plots for all the pretreated adsorbents and are given in Table 2. The rate constant was higher at lower concentrations. These are comparable with the K_{ad} values reported by Senthilkumar (1998). 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 and after the adsorbent dosage level of 2 g/50 mL, adsorption was either constant or the pace of adsorption was very less, which may be due to the availability of more surface functional groups at higher adsorbent dosage (Binupriya et al. 2008). Consequently, this adsorbent dosage level was selected for further studies. Acid treated adsorbent showed a very poor removal when compared to other adsorbents. 2 g of $FeSO_4$ treated adsorbent showed 91.16, 76.33, 66.34, 55.22 and 44.28% removal of As(V) from 50 mL water at a concentration of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L.

Langmuir isotherm was applied for the present study to estimate the adsorption capacity of the adsorbents used. Langmuir isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites (Langmuir 1918). The linear plots of C_e/q_e vs. C_e for the removal of As(V) by native and pretreated treated

Table 1 Effect of contact time and initial concentration of As(V) on adsorption of As(V) from aqueous solution by autoclaved fungal biomass

Contact time (min)	As(V) concentration (mg/L)														
	0.5			1			1.5			2			2.5		
	% Removal	Arsenic adsorbed (mg/g)	$q_e - q$ (mg/g)	% Removal	Arsenic adsorbed (mg/g)	$q_e - q$ (mg/g)	% Removal	Arsenic adsorbed (mg/g)	$q_e - q$ (mg/g)	% Removal	Arsenic adsorbed (mg/g)	$q_e - q$ (mg/g)	% Removal	Arsenic adsorbed (mg/g)	$q_e - q$ (mg/g)
15	33.26	0.166	0.253	27.95	0.280	0.42	28.79	0.432	0.479	23.33	0.467	0.508	19.18	0.480	0.479
30	44.84	0.224	0.195	39.38	0.394	0.306	37.35	0.56	0.351	31.68	0.634	0.341	24.53	0.614	0.345
45	53.68	0.268	0.151	47.34	0.474	0.226	42.80	0.642	0.269	36.05	0.721	0.254	27.67	0.692	0.267
60	60.84	0.304	0.115	52.85	0.529	0.171	46.69	0.7	0.211	38.70	0.774	0.201	29.70	0.743	0.216
75	67.36	0.337	0.082	56.32	0.563	0.137	49.02	0.735	0.176	40.98	0.820	0.155	31.36	0.784	0.175
90	72.42	0.362	0.057	58.97	0.590	0.11	51.75	0.776	0.135	42.88	0.858	0.117	32.47	0.812	0.147
105	76.42	0.382	0.037	61.42	0.614	0.086	54.28	0.814	0.097	44.59	0.892	0.083	33.57	0.840	0.119
120	78.73	0.394	0.025	63.87	0.639	0.061	56.42	0.846	0.065	44.97	0.899	0.076	34.87	0.872	0.087
135	81.26	0.406	0.013	66.32	0.663	0.037	58.17	0.873	0.038	46.48	0.930	0.045	36.34	0.909	0.05
150	83.15	0.416	0.003	68.77	0.688	0.012	59.92	0.899	0.012	48.19	0.964	0.011	37.45	0.936	0.023
165	83.78	0.419	0	69.79	0.698	0.002	60.50	0.908	0.003	48.57	0.972	0.003	38.19	0.955	0.004
180	83.78	0.419	0	70	0.7	0	60.70	0.911	0	48.76	0.975	0	38.37	0.959	0
	q_e: 0.419 mg/g			q_e: 0.7 mg/g			q_e: 0.911 mg/g			q_e: 0.975 mg/g			q_e: 0.959 mg/g		

Table 2 Lagergren constant for As(V) removal

As(V) (mg/L)	$K_{ad} \times 10^{-2}$ (L/min)						
	Live	Autoclaved	Acid treated	Alkali treated	Formaldehyde treated	$FeCl_3$ treated	$FeSO_4$ treated
0.5	2.8	2.8	2.0	2.9	2.8	2.7	2.7
1.0	2.2	2.2	2.0	2.3	2.3	2.2	2.2
1.5	2.3	2.3	1.8	2.4	2.4	2.3	2.2
2.0	1.9	2.3	1.9	2.1	2.1	1.9	2.0
2.5	1.9	1.9	1.9	1.8	1.8	1.9	1.9

biomasses studied show that adsorption of As(V) 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 live and pretreated biomass of *A. fumigatus* are listed in Table 3. $FeSO_4$ treated biomass with Q_o value of 0.054 mg/g showed the maximum adsorption of As(V). The Q_o values for the adsorption of As(V) ions with various adsorbents are summarised in Table 4 for comparison. The Freundlich adsorption isotherm (Freundlich 1906) was also applied for the adsorption of As(V) by live and pretreated biomass of *A. fumigatus*. Linear plots of $\log_{10} C_e$ vs. $\log_{10} X/m$ shows that the adsorption of As(V) onto live and pretreated biomass of *A. fumigatus* also follows Freundlich isotherm model. It also indicates that the average energy of adsorption decreases with increasing adsorption density. Freundlich constants K_f and n were calculated from the plots are presented in Table 3. The removal of As(V) by native and pretreated adsorbents were observed over a pH range of 2–10. Dilute HNO_3 and NaOH was used to adjust the pH. Adsorption of As(V) remained almost the same in all pH range which shows that pH did not have a significant effect on As(V) removal. Zouboulis et al. (1993) and Senthilkumar (1998) reported similar results.

Table 3 Langmuir and Freundlich constants for As(V) removal

Treatment	Langmuir constants		Freundlich constants	
	Q_o (mg/g)	b	K_f	n
Live	0.052	8.78	5.269	0.24
Autoclaved	0.053	8.33	5.497	0.24
Acid treated	0.022	5.64	5.725	0.08
Alkali treated	0.053	6.62	5.729	0.24
Formaldehyde treated	0.052	8.47	5.723	0.23
$FeCl_3$ treated	0.053	11.15	4.937	0.27
$FeSO_4$ treated	0.054	10.39	5.211	0.26

Column mode adsorption studies are considered to be very important in treatment application point of view because it is economical to treat any wastewater in continuous mode than in batch mode. Column mode adsorption studies were carried out to find the efficiency of the adsorbent for continuous removal of As(V). Column studies were carried out only with $FeSO_4$ treated adsorbent, which

Table 4 Comparison of adsorption capacity of *A. fumigatus* biomass with other adsorbents

Adsorbent	Q_o (mg/g)	Reference
Y(III) impregnated alumina	14.45	Wasay et al. (1996a)
Alumina	13.64	Wasay et al. (1996a)
La(III) impregnated alumina	12.88	Wasay et al. (1996a)
“Waste” Fe(III)/Cr(III) hydroxide	11.02	Senthilkumar (1998)
La(III) impregnated silica gel	8.85	Wasay et al. (1996b)
Activated alumina	5.02	Gupta and Chen (1978)
Activated bauxite	3.89	Gupta and Chen (1978)
Activated red mud at 70°C	1.327	Altundogan et al. (2002)
Activated carbon	1.05	Gupta and Chen (1978)
Activated red mud at 25°C	0.942	Altundogan et al. (2002)
Raw red mud at 70°C	0.809	Altundogan et al. (2002)
Raw red mud at 25°C	0.514	Altundogan et al. (2002)
$Al_2O_3/Fe(OH)_3$	0.09	Hodi et al. (1995)
$FeSO_4$ treated *Medusomyces gisevii* biomass	0.082	Murugesan (2002)
Acid treated *M. gisevii* biomass	0.075	Murugesan (2002)

showed the maximum adsorption of As(V) in batch mode studies. The efficiency of a column mode experiment depends on the flow rate at which it is operated. In order to examine the influence of flow rate on As(V) removal, four different flow rates (2.5, 5.0, 7.5 and 10.0 mL/min) were examined. Increasing the flow rate decreases the removal of As(V) and after a certain period removal is totally absent. This suggests that increase in flow rate decreases the contact time between the adsorbent and the adsorbate. Moreover after some time the available adsorption sites become less or totally nil, where As(V) removal stops. The rate of adsorption decreases as time is increased. Plots of Ct/C_o vs. effluent volume also suggests that increasing the flow rate decreases the adsorption of As(V). Effect on bed volume on removal of As(V) with four different bed volumes (2.5, 5.0, 7.5 and 10.0 cm) were studied. Results obtained show that increase in bed volume increases adsorption. This is again due to the availability of more surface functional groups on higher bed volumes. Bed-Depth-Service-Time model (BDST), proposed by Hutchins (1975) is a simpler method to correlate the service time, t with process variables in fixed bed adsorber. The linear plot suggests that fixed bed removal of As(V) by $FeSO_4$ treated biomass obey the BDST model. Adsorption rate constant k_a and N_o were calculated from the slope intercept of the BDST curves. N_o value for As(V) adsorption by $FeSO_4$ treated adsorbent was 4.00, 4.89, 5.84, 5.81 and 2.20×10^{-5} mg/g for 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L of As(V) respectively, which is lower than the Q_o value obtained from batch mode studies. This suggests that batch mode adsorption is more efficient in removing As(V) from aqueous solution than column mode.

Desorption process allows the recovery of precious metals from wastewater and regeneration of the adsorbent, which minimizes the cost of treatment. Moreover desorption studies help elucidate the adsorption mechanism (Binupriya et al. 2008). Effect of agitation time on desorption of As(V) under alkaline condition shows that after 75 min nearly 50% of As(V) ions were desorbed and further no desorption was noticed. Effect of NaOH concentrations on the desorption of As(V) was also studied. Increase in NaOH concentration increased the desorption of As(V) but after 0.4N the pace of desorption was very less. This is in agreement with the observation of Hodi et al. (1995).

The study leads to a conclusion that the $FeCl_3$ treated and $FeSO_4$ treated mycelial biomass of *A. fumigatus* is an effective adsorbent for the removal of As(V) from aqueous solutions. As the biomass of *A. fumigatus* is easily cultivable and also is available as waste in large quantities from certain fermentation industries, using it as a adsorbent will be economical and can be viewed as a waste management strategy. Moreover, as the biomass of *A. fumigatus* is easily biodegradable, the As(V) ions can be desorbed from the biomass after adsorption and the biomass can be taken for land filling or composting after several cycles of adsorption.

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