Removal of arsenic (III) ions from aqueous solution using *Aspergillus flavus* isolated from arsenic contaminated site  

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The biosorption of As(III) ions onto the dry biomass of *Aspergillus flavus* isolated from the arsenic contaminated soil has been studied. The arsenic resistant fungal mycelia of *Aspergillus flavus* is used for the removal of As (III) ions from aqueous solution in the concentrations range of 50-500 mg/L. The effect of initial As (III) ions concentration (50-500 mg/L), pH (3, 4, 5) and temperature (25, 30, 35ºC) on arsenic removal has been investigated. The biosorption of As (III) ions by the biosorbent at different pH and temperature is increased as the initial concentration of As (III) ions is increased in the medium. The biosorption equalization is established in about 240 min and the amount of adsorbed As (III) ions do not change further with time. The maximum biosorption of As (III) ions by *Aspergillus flavus* is obtained at pH – 5. The maximum biosorption occurred at 35ºC. The As (III) ions adsorption data were analyzed using the first and the second order kinetic models. The experimental results fitted that the second-order kinetic model is the most appropriate equation to predict the biosorption capacity of *Aspergillus flavus*. Langmuir and Freundlich isotherms are used to evaluate the data and the regression constants are derived. Biosorption equilibrium data are best described by Freundlich isotherm model followed by Langmuir model. 

**Keywords:** *Aspergillus flavus*, As (III) ion, Biosorption, Kinetic models, Sorption performance, Adsorption isotherms

Metals have a high degree of toxicity, which can be deleterious for both the human beings and the environment. Inorganic micro pollutants are of considerable concern because they are non-biodegradable, highly toxic and have a carcinogenic effect\(^1\). Arsenic occurs naturally in a wide range of minerals and also the wide spread use of arsenic in copper smelting industries, metallurgical activities, pigments and insecticides are the major sources of arsenic in soil and natural waters. Arsenic is an extremely toxic metal that adversely affects human health which can cause a variety of diseases including arsenical dermatitis, heart disease and skin cancer\(^3\). Remediation of heavy metals contaminated sites involves physical, chemical and biological approaches that may achieve the partial or complete removal of heavy metals from soil or the reduction of its bioavailability in order to minimize toxicity. The removal of heavy metals from soil and industrial wastes by biological treatment systems has continued to be of interest\(^5\). Bioremediation is an emerging form of technology in that microbes are used to remove or stabilize the contaminants which offer a low cost and ecologically valuable means for the mitigation of arsenic compounds toxicity in the environment. Arsenic in soil undergoes biological transformation resulting in the formation of organo arsenicals and other compounds. Arsenic can undergo microbially mediated biochemical transformation and the pathway of As (V) methylation initially involves the reduction of As (V) to As (III) with subsequent methylation of As (III) to dimethylarsine by co-enzyme S-adenosylmethionine\(^4\). Methylation is enhanced by sulfate reducing bacteria and several fungal species. Arsenic becomes methylated during transformation and therefore methylation is considered as major detoxifying processes for these microorganisms\(^5\). The biotransformation has been reported to occur in various aquatic systems mediated by bacteria\(^6\), algae\(^7\) and cyanobacteria\(^8\). Many fungi thrive in hostile environments by converting harmful organic toxins to relatively harmless chemical forms. Fungi have a large repertoire of enzymes for this purpose. The response of fungal physiology to challenges by heavy metals is also well documented\(^9,10\). Fungi cell wall contains large quantity of polysaccharides and proteins which offer many functional groups (such as carboxyl, hydroxyl, sulphate, phosphate and amino groups) for binding metal ions\(^11\). In this investigation the fungal biomass of *A. flavus* was isolated from the

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arsenic contaminated sites were evaluated for their sorption efficiencies of As (III) ions from aqueous solution using batch systems. The initial concentration, pH and temperature were also studied. The biosorption equilibrium and kinetics data are fitted using different models and parameters were evaluated.

**Experimental Procedure**

**Sampling and analytical method**

Soil sample was collected from the arsenic contaminated sites in Thoothukudi district. Soil sample was collected at different sites of the field by using sterile scalpel and transferred to sterile polythene bags for further analysis. The collected sample was acid digested\(^{12}\) and the residual arsenic content was analyzed by AAS. The arsenic content in the contaminated soil was found to be 145 mg/g.

**Isolation and identification of As (III) ions resistant fungi**

10 g soil sample were added into 90 mL sterile distilled water and agitated for uniform microbial suspension. Serial dilutions were performed by decimal dilutions were made up to \(10^{-7}\) from there dilution. 10 mL aliquots were poured into sterile petriplates and 15-20 mL sterile Sabourd Dextrose Agar medium (Hi-media, Mumbai, India) supplemented with chlorotetracycline (10 mg/L). Plates were incubated at room temperature for 3-5 days. Fungal isolates were identified using the characteristics structures seen in culture which includes colonial morphology, hyphae, a-sexual spores, reproductive bodies and conidia arrangements \(^{13}\). Slide culture techniques were used to observe morphological characteristics of fungi \(^{14}\).

**Biosorption studies**

The biosorption of As (III) ions experiment were conducted in Erlenmeyer flask containing 100 mL of heavy metal solution and 0.1 g of *Aspergillus flavus* were investigated in batch biosorption equilibrium experiments. The effects of the medium pH, temperature and the initial concentrations of heavy metal ion on the biosorption rate and capacity were studied.

The effect of pH on the biosorption rate in aqueous solution (100 mL) by the arsenic resistant fungi with As (III) ions were investigated at pH range (3, 4, 5) (which was adjusted with HCl or NaOH at the beginning of the experiment). The general experimental procedure were repeated for various values of temperature such as (25, 30, 35°C) respectively. The pH was maintained at 5 (optimum). The effect of the initial As (III) ions concentration on the biosorption medium (100 mL) was studied at different pH and temperatures described above except that the concentration of heavy metal ion in the adsorption medium was varied between 50 to 500 mg/L. After the desired incubation period the aqueous phases were separated from the fungal biomass by centrifugation. The concentrations of remaining metal ions in biosorption medium were determined by atomic absorption spectrophotometer.

**Data analysis**

The amount of adsorbed heavy metal ions per unit biosorbent (mg metal ions/g dry biosorbent) was obtained by using the following expression\(^ {15}\).

\[
 q = \left[ (C_o - C_t) \cdot V \right] / M
 \]

where \(q\) is the amount of heavy metal adsorbed onto the unit amount of the adsorbents (mg/g) and \(C_o\) and \(C_t\) are the concentrations of the metal ions in the solution (mg/L) before and after biosorption respectively; \(V\) is the volume of the aqueous phase and \(M\) is the amount of the adsorbents (g).

**Kinetic modeling**

The study of sorption kinetics describes the adsorbate uptake rate and evidently this rate controls the residence time of adsorbate at the solid-liquid interface\(^ {15}\). The kinetics of As (III) ions sorption on fungal adsorbents was analyzed using different kinetic models, these include, the pseudo first order\(^ {16}\) and pseudo second order. The agreement between the experimental data and the model predicted values for each model was expressed by the coefficient of determination \((r^2)\). A relatively high \(r^2\) value indicates that the model so examined successfully describes the kinetics of As (III) sorption onto the fungal biomass.

**Pseudo-first and second order equation**

The Pseudo first order equation\(^ {16}\) is generally expressed as follows,

\[
 dq_t / dt = k_1 (q_{eq} - q_t)
 \]

where \(k_1\) is the rate constant of pseudo-first-order biosorption \((\text{min}^{-1})\) and \(q_{eq}\) and \(q_t\) denote the amounts of biosorption equilibrium at time \(t\) (mg/g), respectively. After integrating by applying boundary conditions, \(q_t = 0\) at \(t = 0\) and \(q_t = q_{eq}\) at \(t = t\), gives
The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of a metal binding to a site on an adsorbent depends on whether or not the adjacent sites are occupied\(^{20,21}\). The Freundlich model is described by,

\[
q_e = K_F C_e^{\frac{1}{n}}
\]  

\(q_e\) is metal uptake at equilibrium concentration (mg/g); \(C_e\) is equilibrium metal ion concentration (mg/g); \(K_F\) is Freundlich’s constant of adsorption capacity; \(n\) is Freundlich’s constant of adsorption intensity. The \(K_F\) is estimated from the y-intercept and \(n\) is calculated from the slope.

Results and Discussion

Identification of arsenic resistant fungi

The present work was taken up to investigate to adsorb As (III) ions from aqueous solution by fungal biomass isolated from the arsenic contaminated soil. In this preliminary screening in total count of fungus in the soil sample was ranged from 123 ± 7.31 × 10\(^2\) to 3.7 ± 0.23 × 10\(^7\) cfu/g. Fungi with different colony morphology were selected, purified and stored in the Sabourd Dextrose Agar Medium (Hi-media, Mumbai, India) supplemented with chlorotetracycline (10 mg/mL). The dominant arsenic resistant isolate was grows well in 35°C and they show green brown shade with velvety texture on sabourd dextrose agar plate. Based on these characteristics the resistant fungus was identified as *Aspergillus flavus*. The resistant fungal mycelium was cultivated in fungal broth media amended with various concentration of As (III) ion. It appears the fungal mycelium was able to survive at metal concentrations as high as 300 mg/L of As (III) ions. *A. nidulans* isolated from an arsenic contaminated soil shows tolerance to the maximum concentration of 500 mg/L of As(III) ions\(^{22}\). *Aspergillus* Sp P37 is an arsenate hypotolerent fungus isolated from a river in Spain and it is able to grow in the presence of 0.2M arsenate\(^{23}\). *A. Clavatus* absorbs oxyanions of arsenic (0.25-100 mg/L) from aqueous solution. Lower sorption of arsenic by biomass\(^{24}\), which is in solution presented as negatively charged oxyanion may relate with repulse electrostatic interactions between negatively charged surface of biomass and AsO\(_4^–\). Arsenite enters the cell through specific (Pst) or unspecific (Pit) phosphate transporters, therefore the incorporation of arsenate is lower in phosphate rich media or environments. Arsenite uptake has also been
correlated to unspecific sugar transport such as hexose permease described in yeast.

**Biosorption rate of As (III) ions at different pH and temperature**

Biosorption of heavy metal ions onto microbial biomass is affected by several factors, intertwining surface properties of the microbial cell wall and the physicochemical properties of the adsorption medium such as metal ion concentration, pH, temperature and the amount of the biomass. The biosorption rate of heavy metal ions (100 mL) on *Aspergillus flavus* was obtained by following the decrease of the concentration of As (III) ions within the adsorption medium with time at different pH and temperatures. Figure 1 shows the biosorption rate of As (III) ions at different pH on *Aspergillus flavus* from solution containing 200 mg/L of As (III) ions. As seen in the figure the saturation level was obtained after about 240 min. Biosorption rate capacity of *Aspergillus flavus* increased at different pH (3, 4, and 5) with increasing the time. As seen from Fig. 1 the amount of biosorbed As (III) ions on pH - 5 was 134 mg/g after 240 min. Similarly Fig. 2 shows the biosorption rate of As (III) ions with different temperatures at pH - 5 and here also the saturation level obtained after 240 min. The amount of adsorbed As (III) ions on the *Aspergillus flavus* at pH – 5 was found to be 170 mg/g at 35°C. The biosorption equilibrium time of chromium (IV) on the dead and immobilized biomass of *R. arrhizus* was 2 h. The Pb biosorption rate on *P. chrysosporium* is fast and reached saturation value within 2 h. The uptake of cadmium by *Spirulina platensis* at a period of 24 h showed to maximum during the initial period of contact at 1.6 mg/L.

**Effect of pH on different As (III) ions concentration**

It is well known that metal ion adsorption on non-specific and specific sorbents is pH dependent. The medium pH affects the solubility of metal ions and the ionization state of the functional groups (i.e., carboxylic, phosphate and amino groups) on the fungal cell wall. In this study batch experimental system was used in the optimization of As (III) ion concentration by fungal biomass with respect to pH. Figure 3 shows the effect of pH on different As (III) ions concentration. Biosorption capacity of the fungal biomass increased with increasing initial concentration of As (III) ions in the medium and reached a saturated value at 300 mg/L of As (III) ions at different pH (3, 4, 5). As seen from Fig. 3 the amount of biosorbed As (III) ion on pH 3, 4 and 5 was found to be 187, 179 and 182 mg/g respectively. The metal biosorption hence depends on the protonation or deprotonation of these carboxyl groups, which have pKa between 3 and 4. The arsenic removal by pretreated waste tea fungal biomass was obtained at pH - 3 and 5. Measurement of final pH represented the simultaneous release of H⁺ with the uptake of arsenic ions, because final pH of solutions was less...
than the initial pH. Therefore ion exchange was confirmed to be one of the biosorption mechanisms. The amount of biosorbed As (III) ion by Aspergillus fumigatus at pH 3, 4 and 5 was found to be 106, 101 and 134 mg/L respectively. The biosorption of Cd (II), Pb(II) and Cu (II) on inactivated P. chyrsosporium was pH dependent and maximum biosorption was obtained at pH – 6.

Effect of temperature on different As (III) ions concentration

The temperature of the adsorption 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 processes responsible for biosorption are largely physico-chemical in nature. The effect of temperature on the metal biosorption experiment was investigated at three different temperatures. Figure 4 shows the effect on temperature on the biosorption of As (III) ions at different concentrations. As can be seen from the figure the maximum biosorption of As (III) ions on fungal biomass was observed around 35°C. Mostly adsorption is an exothermic process whereas some examples of endothermic adsorption have also been reported. In the present investigation the biosorption of As (III) ions at different temperatures is an exothermic process. The arsenate and arsenite, the sorption capacity at 20°C was higher than that at 35°C when the same initial arsenic concentrations were used, indicating that a lower temperature was favourable for arsenic sorption. The arsenic (V) resistant GBFH strain grew optimally at 37°C with a maximum growth rate of 0.12/h. Although growth was not observed at 4 or 45, small amounts of As (V) respiration (2 mM) occurred at both temperatures.

Biosorption kinetic modeling

In order to investigate the mechanism of biosorption of As (III) ions and its potential rate controlling step such as mass transport and chemical reaction processes, kinetic models have been used to test experimental data. Figures 1 and 2 present the remaining concentration of As (III) ions in aqueous solution as a function of time at different pH and temperature were succeeded at 240 min, from that point on, the As (III) ions concentration declines at a much lower rate and gradually levels – off towards at the end of the experiment (240 min). The rapid kinetics has significant practical importance, as it will facilitate smaller reactor volumes ensuring high efficiency and economy (the first and second order equations) can be used in this case assuming the measured concentrations are equal to cell surface concentrations.

The comparison of experimental biosorption capacities and the theoretical values at different pH and temperatures were estimated from the first order rate equations and are given in Tables 1 and 2. The theoretical $q_{eq}$ values estimated from the first order kinetic equation are calculated and compared to the experimental $q_{eq}$ values.
kinetics model gave significantly different values compared to experimental values and the correlation coefficients for the linear plots of \( \log (q_e - q_t) \) against \( t \) were also found to be slightly lower than 0.995 for both pH and temperatures. The correlation coefficients for the linear plots of \( t/q_t \) against \( t \) for the second-order equation are greater than 0.995 for *Aspergillus flavus* for contact times of 270 min at different pH and temperatures (Figs 5 and 6). The theoretical \( q_{eq} \) values for different pH and temperatures were very close to the experimental \( q_{eq} \) values in the case of pseudo second order kinetics. These results suggest that the second-order mechanism is predominant and the chemisorptions may be the rate-limiting step that controls the biosorption process\(^{38-40}\). Therefore, the results of this investigation shows that the biosorption systems best described in pseudo second order kinetic model.

**Langmuir and Freundlich adsorption isotherms**

The two most commonly used adsorption isotherms for biosorption studies (the Langmuir and Freundlich isotherms) were investigated. Result of this study shows that Freundlich isotherm model fitted well when compared to Langmuir isotherm model. Figure 7 shows the Langmuir plot for As (III) ions by fungal biomass at different pH. The Langmuir constants \( (q_m \) and \( k_d) \) along with correlation coefficients \( (R^2) \) have been calculated from the plots for biosorption of As (III) ions on the biosorbents and the results are given in Tables 3 and 4. The maximum capacity \( q_m \) determined from the Langmuir isotherm defines the total capacity of the biosorbents for As (III) ions. The order of maximum capacity \( (q_m) \) for the biosorbents for heavy metal ions removal was found as pH 5 > pH 3 > pH 4 (Table 3). Similarly the Langmuir isotherm model and correlation coefficient have been calculated at different temperatures. The order of maximum capacity \( (q_m) \) for the biosorbents for heavy metal ions removal was found to be 35°C > 25°C > 30°C (Table 4). It is clear that this increase in the \( q_m \) value is due to an increase in the adsorptive sites on the biosorbents. The living organisms induce the production of metallothioneins which are protein that contain large amounts of cystein and bind heavy metal ions in order to respond to the effects of heavy metals\(^{41}\).

The Langmuir constant \( (k_d) \) estimated from the intercept is a measure of the stability of the complex formed between metal ions and adsorptive surface layer of the biosorbents under specified experimental conditions. The presence of small \( k_d \) value indicates that the metal ions has a high binding affinity for the biosorbent and the \( k_d \) values are presented in Tables 3 and 4. The \( k_d \) values for the adsorption of As (III) ions was 3.86, 3.35 and 2.25 at pH – 3, pH – 4 and pH – 5, respectively. The \( k_d \) value was low at pH- 5 and it indicates that the high binding affinity between the biosorbents and heavy metal ions. Similarly the \( k_d \) values was found to be 1.62, 1.58 and 1.57 at 25°C, 30°C and 35°C, respectively. Here, the \( k_d \) value was

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Fig. 5—The Pseudo first order kinetics constant \((\log q_m - q_t)\) plot at different temperatures on biosorption of arsenic on *Aspergillus flavus* at pH - 5 from aqueous solution (Initial concentration 200 mg/L; biomass concentration - 0.1 g)

Fig. 6—The Pseudo second-order kinetics constant \(t/q_t\) at different pH on biosorption of arsenic on *Aspergillus flavus* from aqueous solution (Initial concentration 200 mg/L; biomass concentration - 0.1 g; temperature 35°C)

Fig. 7—Langmuir isotherm plot of As (III) ions on *Aspergillus flavus* different pH (Biomass concentration - 0.1 g; temperature 35°C)
Table 3—Langmuir and Freundlich isotherm model constant and correlation co efficient for biosorption of As (III) ion from aqueous solution by Aspergillus flavus at different pH

<table>
<thead>
<tr>
<th>Biosorbent at different pH</th>
<th>Experimental $q_{eq}$ (mg/g)</th>
<th>Langmuir Constant</th>
<th>Freundlich Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$q_{eq}$ (mg/g)</td>
<td>$q_m$ (mg/g)</td>
<td>$k_d \times 10^4$ (M)</td>
</tr>
<tr>
<td>3</td>
<td>185</td>
<td>188</td>
<td>3.86</td>
</tr>
<tr>
<td>4</td>
<td>182</td>
<td>185</td>
<td>3.35</td>
</tr>
<tr>
<td>5</td>
<td>191</td>
<td>192</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Table 4—Langmuir and Freundlich isotherm model constant and correlation co efficient for biosorption of As (III) ion from aqueous solution by Aspergillus flavus at different temperatures

<table>
<thead>
<tr>
<th>Biosorbent at different temperature (°C)</th>
<th>Experimental $q_{eq}$ (mg/g)</th>
<th>Langmuir Constant</th>
<th>Freundlich Constant</th>
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<tbody>
<tr>
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<td>$q_{eq}$ (mg/g)</td>
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<td>$k_d \times 10^4$ (M)</td>
</tr>
<tr>
<td>25</td>
<td>187</td>
<td>188</td>
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<td>30</td>
<td>185</td>
<td>185</td>
<td>1.58</td>
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<tr>
<td>35</td>
<td>190</td>
<td>188</td>
<td>1.57</td>
</tr>
</tbody>
</table>

Fig. 8—Freundlich isotherm plot of As (III) ions on Aspergillus flavus at different temperatures (pH – 5; biomass concentration - 0.1 g)

Conclusions

Aspergillus flavus have been successfully used as the biosorbing agent for removal of As (III) ions from aqueous solution. The biosorption of As (III) ions depend on the experimental conditions particularly medium pH, temperature and concentration of metal ion in the medium. The results from this study show that pH, temperature and As (III) ions concentration highly affect the overall metal uptake capacity of biosorbent. The biosorption of As (III) ions on the biosorbent seems to follow the second-order kinetics than first-order kinetics. The Freundlich and Langmuir adsorption models were employed for the mathematical description of biosorption equilibrium data regarding As (III) ions to Aspergillus flavus for varying pH, temperature and heavy metal concentration. The calculated isotherm constants were used to compare the biosorptive capacity at different experimental for the removal of As (III) ions. The result of this investigation demonstrate that the Freundlich model fits a little better than the Langmuir model the adsorption equilibrium data in the examined concentration range.

References