**Abstract**— The biosorption of As (III) ions into dry, heat and NaOH treated Aspergillus fumigatus in aqueous solutions were studied in a batch mode. The effect of pH (2 - 6), temperature (25, 30, 35°C), and initial concentration (100 – 280 mg/l) of As (III) ions were investigated. The maximum biosorption rate of As (III) ions on the tested biosorbent were obtained at pH – 5 and 35°C in about 240 min. The maximum biosorption capacities of dry, heat and NaOH – treated fungal biomass were 134, 166 and 152 mg/g of dry biomass, respectively. The experimental results suggest that the second – order equation is the most appropriate equation to predict the biosorption capacity by dry, heat and NaOH – treated Aspergillus fumigatus. Biosorption equilibrium data were best described by Langmuir isotherm model followed by Freundlich model. The thermodynamics of As (III) ions in dry, heat and NaOH treated Aspergillus fumigatus biomass system indicates spontaneous and exothermic nature of the process.

**Keywords** - Aspergillus fumigatus, As (III) ions, kinetic characterization, chemical and physical treatment, thermodynamics

I. INTRODUCTION

Heavy metals, released by a number of industrial processes such as production of various chemicals and smelting of heavy metals, are major pollutants in soil, marine and industrial wastewaters [1]. Arsenic is an extremely toxic metalloid that adversely affects human health which can cause a variety of diseases including arsenical dermatitis, heart disease and skin cancer [2]. The toxicity of arsenite is due to the formation of strong bonds with functional groups, such as the thiols of cysteine residues and the imidazolium nitrogens of histidine residues from cellular proteins. In the case of arsenate its toxicity is the result of the mimetic effect of arsenate (AsO$_3^{3-}$) and phosphate (PO$_4^{3-}$) which affects the global cell metabolism [3]. Because of the toxicity the acceptable concentration of arsenic in drinking water is limited to 10 µg/l (Min and Hering, 1998). Conventional methods such as chemical precipitation, oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies are generally used for removing metals from aqueous solutions [4]. These processes may be ineffective or expensive, especially when the solutions containing in the order of 1-100 mg dissolved heavy metal ions / L [5]. Therefore removal of toxic heavy metals by an environmentally friendly manner is of great importance. Of the different biological methods, bioaccumulation and biosorption have been demonstrated to possess good potential to replace conventional methods for the removal of metals [6]. Fungal 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 [7]. Living fungal cells can be pretreated using physical or chemical treatment process to increase the metal biosorption capacity. Physical pretreatment methods include heat treatment, autoclaving, freeze-drying and boiling. Chemical pretreatment methods, such as contacting microbial cells with acids, alkali and organic chemicals, have showed enhancement or reduction in metal biosorption, depending on the fungal strains and treatment procedures used [8]. Many fungal species such as Aspergillus nidulans [9], Aspergillus fumigatus [10], Phanerochaete chrysosporium [11], Macor miehei [12] and Aspergillus niger [13] have been extensively studied for heavy metal biosorption and the process mechanisms seem to be dependent upon species. The aim of the study was to investigate the effect of biosorption of As (III) ions by dry, heat and NaOH treated fungal biomass of Aspergillus fumigatus. The biosorption efficiencies of As (III) ions from aqueous solution were studied using batch systems

II. MATERIALS AND METHODS

A. Preparation of fungal strain

The isolated As (III) ions resistant Aspergillus fumigatus was cultured in Sabourd Dextrose medium (Hi-media, Mumbai, India) and maintained in room temperature. The fungal biomass was harvested from the medium after 5 days by filtration through whatman No.1 filtration paper. The filtered fungal biomass was resuspended in purified water for washing and again filtered to make sure that no media remain on the cell surface. The fungal biomass was dried in a hot air oven at 40°C for 24 h to remove the water content from the fungal strain which was used for biosorption studies.

B. Pretreatment of fungal biomass

The dry mycelium of Aspergillus fumigatus were pretreated in two different ways to enhance the biosorption of arsenic from the aqueous solution

- Autoclaved for 30 min at 121°C and 15 psi
- 1 g of fungal biomass boiled for 15 min in 50 ml of 0.5 N sodium hydroxide solution

The fungal biomass those exposed to pretreatment were washed with generous amounts of deionized water and ground after dried at 60°C for overnight. The biomass pretreated with NaOH was washed with de-ionized water
The ph of the solution was in near neutral range (pH 6.8-7.2) before drying.

C. Biosorption Studies

The biosorption of As (III) ions on the dry, heat – treated and NaOH - treated Aspergillus fumigatus from aqueous solution containing metal ions was investigated in batch experiments. The As (III) ion concentration of 100–280 mg/l was prepared by diluting stock solution (1000 ppm) in deionized water. The diluted solution was sterilized by filtration through a flow pore filter with a 0.45 μm pore size and was used for further preparation of metal concentration. The biosorption of As (III) ions experiment were conducted in Erlenmeyer flask containing 100 ml of As (III) ionic solution and 0.1 g of dry, heat and NaOH – treated Aspergillus fumigatus were added in batch experiments. The biosorption rate and capacity was studied in the heavy metal solution at different pH, temperature and initial As (III) ions concentration.

The effect of pH was investigated in the pH range (2 – 6) (which was adjusted with HCl or NaOH at the beginning of the experiment). The general experimental procedure was repeated for various values of temperature such as (25, 30, and 35º C) respectively. The effect of the initial As (III) ions concentrations (100 ml) was studied at the concentration ranging 100 to 280 mg/l at pH 5 (optimum). After the desired incubation period the aqueous phases were separated from the fungal biomass by centrifuged at 300 rpm (KEMI, R8 – C). The concentrations of remaining As (II) ions in the heavy metal solution were determined by Atomic Absorption Spectrophotometer (PG – 990, Germany) as per the standard methods [14].

D. Data Analysis

The amount of adsorbed As (III) ions by dry, heat and NaOH – treated Aspergillus fumigatus (mg metal ions/g dry biosorbent) was obtained by using the following expression [15].

\[ q = \frac{(C_0 - C_e)V}{M} \] (1)

where q is the amount of As (III) ions adsorbed onto the unit amount of the adsorbents (mg/g) and C₀ and Cₑ are the concentrations of the As (III) ions in the aqueous 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)

E. Pseudo – first and second order equation

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 the pseudo first order [16] and pseudo second order.

The Pseudo first order equation [16] is generally expressed as follows,

\[ \log (q_{eq} - q_t) = \log q_{eq} - \frac{K_1 q_{eq}}{2.303} t \] (2)

The applicability of the kinetic first order model is confirm by the straight line against Log (qe – q) against t. The first - order process qe should be equal to the intercept of a plot of Log (qe – q) against t

The second order mechanism for the biosorption rate of As (III) ions by dry, heat and NaOH – treated Aspergillus fumigatus [17] is expressed as Eq. 4

\[ \frac{1}{q_t} = \frac{1}{k_2 q_{eq}^2} + \frac{1}{q_{eq}} t \] (3)

The applicability of the kinetic second order model is confirmed by plot of t / qₑ vs t give a linear relationship between As (III) ions and biosorbents. From the intercept and slope the rate constant (k₂) and adsorption at equilibrium (qe) are calculated respectively.

F. Adsorption isotherms

The Langmuir isotherm relates the sorption density qₑ (metal uptake per unit weight of sorbent) to equilibrium sorbate concentration in the bulk fluid phase, Ce. The langmuir isotherm is described by the following equation

\[ C_e = \frac{1}{q_m K_a} + \frac{C_e}{q_m} \] (4)

Where qₑ is the amount adsorbed per unit mass of adsorbent (mg/g), Ce the equilibrium concentration of the adsorbate (mg/l), qₑm the equilibrium sorption capacity for complete monolayer (mg/g) and Ka the sorption equilibrium constant (l/mg). When Ce/Åₑ was plotted against Ce, a straight line with slope 1/Ka qₑm and an intercept of 1/qₑm were obtained.

The Freundlich equation is described by the following equation

\[ q_{eq} = K_F C_e^{1/n} \] (5)

where qₑ – Metal uptake at equilibrium concentration (mg / g) ; Cₑ – Equilibrium metal ion concentration, (mg / g) ; K_F – Freundlich’s constant of adsorption capacity ; n – Freundlich’s constant of adsorption intensity. The Kᵢ was estimated from the y – intercept and n was calculated from the slope.

G. Thermodynamics of biosorption of As (III) ions

In the present study, the biosorption experiments were carried out in the temperature (25, 30, 35ºC). The values of the thermodynamic parameters such as ΔG°, ΔH° and ΔS°, describing As (III) ions uptake by dry, heat and NaOH – treated Aspergillus fumigatus were calculated using the thermodynamic equations. The apparent equilibrium constant for the process has been shown to be

\[ K_c = \frac{r_{ads}}{r_{des}} \] (10)

The change in Gibbs free energy of the biosorption process is thus given as

\[ ΔG° = -R T \ln K_c \] (11)
Where $\Delta G^o$ is the standard Gibbs free energy change for the biosorption (J / mol), R the universal gas constant (8.314 J/mol/K) while T is the temperature (K). From thermodynamics,

$$\Delta G^o = \Delta H^o - T\Delta S^o$$

or

$$\Delta G^o = -\Delta S^o (T) + \Delta H^o$$

A plot of T against $\Delta G^o$ gives a straight line with slope $-\Delta S^o$ and an intercept of $\Delta H^o$ was obtained.

**F. Statistical analysis**

All data represent the mean of three independent experiments. Standard deviation and error bars are indicated whenever necessary. All statistical analysis and plots were performed using Sigma Plot for Windows version 10 software (Systat Software, Inc).

**III. RESULTS AND DISCUSSION**

**A. Biosorption rate of As (III) ions**

The biosorption rate of As (III) ions by dry, heat and NaOH – treated fungal biomass from solution containing 200 mg/l of As (III) ions are present in Fig. 1. The biosorption rate for dry, heat and NaOH – treated fungal biomass was found to be 150, 182 and 160 mg/g of As (III) ions respectively. The saturation level occurred after 240 min at pH = 5 in dry, heat and NaOH – treated fungal biomass. There was no significant change after 240 min in the biosorption of As (III) ions. The biosorption of As (III) and As (V) by the macro fungus *Inonotus hispidus* and the biosorption equilibrium occurred at 30 min [18]. The biosorption rate of As (V) by *Lessonia nigrescens* occurred during the first 120 min of adsorption [19]. The equilibrium time for removal of arsenic by iron – oxide coated *A. niger* was found to be approximately 7 h [20].

As seen from the figure the amount of biosorbed As (III) ions on dry, heat and NaOH – treated fungal biomass were 150 mg/g, 182 mg/g and 170 mg/g of As (III) ions respectively. In this study the equilibrium biosorption of As (III) ions for all the fungal biomass preparations were found to be similar in pH = 5. The metal biosorption hence depends on the protonation or deprotonation of these carboxyl groups, which have pKa between 3 and 4 [21]. The arsenic removal by pretreated fungal biomass isolated from tea waste was obtained at pH - 4. 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 [22]. It was suggested that at lower pH value the surface of the adsorbent is surrounded by proton (H⁺) thereby preventing the metal ions from approaching the binding sites of the sorbent [23]. This means that at higher H⁺ concentration, the biosorbent surface become positively charged such that the attraction between the biomass and the metal cations reduced [24]. In contrast, as the pH increases, more negatively charged surface becomes available thus facilitating the arsenic removal from aqueous solution. *A. niger* biomass coated with iron oxide showed maximum removal (approximately 95% of As (V) and 75% of As (III)) at a pH of 6 [25].

The temperature of the heavy metal solution could be important for energy dependent mechanisms in metal biosorption by microorganisms. The biosorption of As (III) ions by all the fungal biomass preparations appears to be temperature – dependent over the temperature range such as 25, 30 and 35°C. The biosorption capacities of the dry, heat and NaOH – treated fungal preparations for As (III) ions increased at 35 °C. 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 favorable for arsenic sorption [26]. The arsenic (V) resistant Desulfitobacterium GBFH strain grew optimally at 37°C with a maximum growth rate of 0.12 g / h. Although growth was not observed at 4 or 45, small amounts of As (V) respiration (2 mM) occurred at both temperatures [27].

C. Effect of initial As (III) ions concentration on biosorption

The biosorption capacities of dry, heat and NaOH – treated fungal biomass at different equilibrium concentration of As (III) ions (100 – 280 mg/l) are shown in Fig. 3.

**Table 1(a)** The Pseudo first order kinetics constants for biosorption of As (III) ions on the fungal biomass

<table>
<thead>
<tr>
<th>Biosorbents</th>
<th>Experimental qe (mg/g)</th>
<th>Pseudo first order reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Biomass</td>
<td>150</td>
<td>212</td>
</tr>
<tr>
<td>Heat – treated biomass</td>
<td>160</td>
<td>196</td>
</tr>
<tr>
<td>NaOH – treated biomass</td>
<td>161</td>
<td>162</td>
</tr>
</tbody>
</table>

As seen from the Fig. 3 the amount of biosorbed As (III) ions on the dry, heat and NaOH – treated fungal biomass were found to be 134, 166 and 152 mg/ g respectively. The higher As (III) ions biosorption capacity were obtained in heat – treated biomass may be increases the availability of the binding sites by fixing the soluble protein in the cell wall after denaturation with heat. From these results it was showed that the surface properties of the fungal cell could be improved upon application of heat. The heat treatment could erode microbial cell surface integrity causing the walls to become leaky with a marked increase in the passive diffusion of metal ion to the interior part of the cell wall [28]. The biosorption of Cr (VI) is 44%, 49.6% and 66.6% is maximum at 240 min by live, dead and immobilized cells of Pseudomonas sp. [29]. The biosorption capacity of dead Fusarium flocciferum was 19.2 mg Cd (II) / g dry biomass [30]. The biosorption capacity of the NaOH pretreated A. niger was 7.24 mg of lead, 3.43 mg for cadmium and 66 mg for copper per gram dry biomass [13]. The uptake of As(III) by Paecilomyces Sp., was observed 64.5% and 58% at lower concentrations (1-2mg/L) and 49% and 42% at higher concentrations (4-5mg/L) [31]. The maximum absorption of arsenic by R. rosa, H. rosasinensis, T. erecta, and C. indica, initial concentration of 500 ppb was found to be 98, 96,92 and 85% respectively [32].

**D. Biosorption kinetic modeling**

The pseudo first and second order rate constant for dry, heat and NaOH – treated fungal biomass are shown in Table 1(a&b). The experimental values and correlation coefficients were lower than theoretical values for dry, heat and NaOH treated fungal biomass gave different values. The correlation coefficients for dry, heat and NaOH – treated fungal biomass for the second order equation were greater than 0.990. The experimental and theoretical values were very close in pseudo second order kinetics. Thus the second – order mechanism were predominant in the biosorption process for dry, heat and NaOH – treated Aspergillus fumigatus.

**Table 1(b)** The Pseudo first order kinetics constants for biosorption of As (III) ions on the fungal biomass

<table>
<thead>
<tr>
<th>Biosorbents</th>
<th>Experimental qe (mg/g)</th>
<th>Pseudo first order reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Biomass</td>
<td>150</td>
<td>151</td>
</tr>
<tr>
<td>Heat – treated biomass</td>
<td>182</td>
<td>185</td>
</tr>
<tr>
<td>NaOH – treated biomass</td>
<td>160</td>
<td>161</td>
</tr>
</tbody>
</table>

The pseudo first and second order rate constant for dry, heat and NaOH – treated fungal biomass are shown in Table 1(a&b). The experimental values and correlation coefficients were lower than theoretical values for dry, heat and NaOH treated fungal biomass gave different values. The correlation coefficients for dry, heat and NaOH – treated fungal biomass for the second order equation were greater than 0.990. The experimental and theoretical values were very close in pseudo second order kinetics. Thus the second – order mechanism were predominant in the biosorption process for dry, heat and NaOH – treated Aspergillus fumigatus. **Table 1(a)** The Pseudo first order kinetics constants for biosorption of As (III) ions on the fungal biomass **Table 1(b)** The Pseudo first order kinetics constants for biosorption of As (III) ions on the fungal biomass

**E. Langmuir and Freundlich Adsorption Isotherms**

Table 2 (a&b) shows that the langmuir constant and correlation coefficients calculated from the plots for biosorption of As (III) ions on the dry, heat and NaOH – treated Aspergillus fumigatus. The maximum biosorption capacity for the biosorbent of As (III) ions determined from the langmuir isotherm constant. The maximum capacity (q_m) for the biosorption of As (III) ions was found to be heat – treated > dry > NaOH treated fungal biomass. The increase in q_n value was increase due to the adsorptive sites on the fungal biomass. The langmuir constant measure the stability of the intricate between the As (III) ions and the adsorptive layer of the fungal biomass in experimental conditions. The high binding affinity of the fungal biosorbents due to the presence of small k_d values which present in Table 2 (a&b). The k_d values for the adsorption of As (III) ions were found to be 2.51, 0.60 and 2.22 at dry, heat and NaOH – treated fungal biomass, respectively. The biosorption of As (III) ions by heat –
treated fungal biomass is high due to the presence of low \( k_d \) value.

**Table – 2(a).** Langmuir Isotherm model constant and correlation co efficient for biosorption of As (III) ions on the fungal biomass

<table>
<thead>
<tr>
<th>Biosorbents</th>
<th>Experimental ( q_e ) (mg/g)</th>
<th>Langmuir Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( q_m ) (mg/g)</td>
<td>( k_d \times 10^3 ) (M(^{-1}))</td>
</tr>
<tr>
<td>Dry Biomass</td>
<td>134</td>
<td>188</td>
</tr>
<tr>
<td>Heat – treated biomass</td>
<td>166</td>
<td>178</td>
</tr>
<tr>
<td>NaOH – treated biomass</td>
<td>152</td>
<td>238</td>
</tr>
</tbody>
</table>

**Table – 2(b).** Freundlich Isotherm model constant and correlation co efficient for biosorption of As (III) ions on the fungal biomass

<table>
<thead>
<tr>
<th>Biosorbents</th>
<th>Experimental ( q_e ) (mg/g)</th>
<th>Freundlich Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( k_f )</td>
</tr>
<tr>
<td>Dry Biomass</td>
<td>134</td>
<td>0.38</td>
</tr>
<tr>
<td>Heat – treated biomass</td>
<td>166</td>
<td>0.47</td>
</tr>
<tr>
<td>NaOH – treated biomass</td>
<td>152</td>
<td>0.35</td>
</tr>
</tbody>
</table>

The favorable adsorption of As (III) ions from aqueous solution by fungal biomass showed by the freundlich constant \( k_f \) and \( n \). Table – 2 (a&b) shows the effect of concentration on the adsorption capacity of dry, heat and NaOH – treated fungal biomass in the heavy metal solution. The high adsorption capacity of As (III) ions by the fungal biomass showed by the \( n \) values calculated from the slope. In the present investigation it is clear that Langmuir model fits well than the freundlich isotherm model.

**F. Thermodynamics of biosorption of As(III) ions**

The thermodynamic parameters for the adsorption system by the fungal biomass at different temperatures are present in Table 3. The spontaneous adsorption of As (III) ions by the fungal biomass is shown by the negative values of \( \Delta G^\circ \). The Gibbs energy of the interactions demonstrated that the processes are favorable for the formation of electrostatic interaction and/or arsenic – adsorbent complexes. However the negative value of \( \Delta G^\circ \) decreased with an increase in temperature, indicating that the spontaneous nature of sorption of As(III) ions by the fungal biomass is inversely proportional to the temperature. The negative value of \( \Delta G^\circ \) shows that the As (III) adsorption by the fungal biomass is an exothermic process.

**Table – 3 Free energy values obtained from the biosorption of As(III) ions using fungal biomass at different temperatures**

<table>
<thead>
<tr>
<th>Kelvin (K)</th>
<th>Dry biomass ( \Delta G^\circ ) (J/mol/K)</th>
<th>Heat – treated biomass ( \Delta G^\circ ) (J/mol/K)</th>
<th>NaOH treated biomass ( \Delta G^\circ ) (J/mol/K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>-1.404</td>
<td>-0.851</td>
<td>-6.365</td>
</tr>
<tr>
<td>303</td>
<td>-1.427</td>
<td>-0.865</td>
<td>-6.471</td>
</tr>
<tr>
<td>308</td>
<td>-1.451</td>
<td>-0.879</td>
<td>-6.578</td>
</tr>
</tbody>
</table>

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