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Biosorption of arsenic(III) ion from aqueous solution using *Aspergillus fumigatus* isolated from arsenic contaminated site

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ABSTRACT

The biosorption of As(III) ion onto the dry biomass of *Aspergillus fumigatus* isolated from the arsenic contaminated soil. The effect of initial As(III) ion concentration (100–260 ppm), pH (3, 4, 5) and temperature (25, 30, 35°C) on arsenic removal has been investigated. In addition the polarity and surface energy of the fungal biomass was determined by FTIR spectroscopy. The biosorbents varied with the pH of the medium and the maximum biosorption at initial concentration of 180 ppm of As(III) ion was obtained at pH-5. The effect of temperature on the biosorbents was varied with different As(III) ion concentration and the maximum adsorption occurred at 35°C. The maximum biosorption capacity (q_m) of fungal biomass were 106, 101 and 134 ppm at pH-3, 4 and 5 respectively. Similarly at 25, 30 and 35°C the maximum biosorption capacity (q_m) were 144, 125 and 175 ppm respectively for As(III) ion per gram of dry biosorbent. The experimental biosorption equilibrium data for As(III) ion were in good agreement with those calculate by Langmuir and Freundlich model

Key words: *Aspergillus fumigatus*; As(III) ion; biosorption; pH; sorption performance; temperature

1. Introduction

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 (As) 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 [1]. The toxicity of arsenite is due to the formation of strong bonds with functional groups, such as the thiols of cystein 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_4^{3-}) and phosphate (PO_4^{3-}) which affects the global cell metabolism [2]. Because of the toxicity the acceptable concentration of arsenic in drinking water is limited to 10 μg/L according to the Environmental Protection Agency [3]. Conventional technologies such as coagulation do not discriminate between arsenic and other elements and involve alteration of the water chemistry and addition of other chemicals [4]. Biological methods are gaining momentum because of their potential in heavy metal remediation as cost effective technology. During recent years, the study of microorganisms has contributed important insights into the basic problems an emerging technology that has received more attention in the development of biosorbents with high affinity and specificity [5,6]. Fungi cell wall contains large

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quantity of polysaccharides and proteins which offer many functional groups (such as carboxyl, hydroxyl, sulphate, phosphate and amino groups) for binding metal ions [7–9]. Many fungal species such as *Rhizopus arrhizus* [10], *Phenerochaecte chrysosporium* [11,12], *Mucor miehei* [13] and *Aspergillus niger* [14] have been extensively studied for heavy metal biosorption and the process mechanisms seem to be dependent upon species. However, the efficiency of fungal cell as sorbents varies greatly and depends on the physiological states, surface properties of cells, pH and other physicochemical parameters of the metal solution [15]. The ability of microbial cells to bind metals from aqueous solutions has been demonstrated to be a function of solution pH [16]. In addition, metal adsorption on both non-specific and specific sorbents is pH dependent, as the pH affects the availability of metal ions in solution as well as the metal binding sites onto cell surface [17]. In this investigation the fungal biomass of *Aspergillus fumigatus* was isolated from the arsenic contaminated sites were evaluated for their sorption efficiencies of As(III) ions from aqueous solution using batch systems. The effect of initial concentration, pH and temperature are the factors that influences sorption efficiency of metal ions in solution were also studied since it is a key parameter in most biological processed and controls the growth and the adsorption capacity of substances.

2. Material and methods

2.1. Sampling and analytical method

Thoothukudi is situated in the extreme south-eastern corner of Tamilnadu state on the east and south east by Gulf of Mannar. The total area of the district is 4621 Sq.kms and has a coastal line of 135 kms. The contaminated site is located at 8°49′09.27″N and 78°04′54.81″E at an elevation of 18 m. The climatically conditions is usually hot and dry. There are over 2,200 and above small scale industries registered in the area and 12 major industries. They are engaged in the production of various chemicals, smelting of heavy metals, cotton and staple yarn, caustic soda, PVC resin, fertilizers, soda-ash, carbon dioxide gas in liquid form etc. Soil samples were collected from the arsenic contaminated sites in Thoothukudi district. Soil samples were collected at different sites of the field by using sterile scalpel and transferred to sterile polythene bags for further analysis. The soil samples were acidified with 1:1 HCl and the arsenic was analyzed using a PG–990 atomic absorption spectrophotometer (AAS) equipped with flame and graphite furnace. Argon gas of ultra-high purity (99.99%) was used to sheath the atomizer and to purge it internally. An arsenic hollow cathode lamp was used with emitting wavelength of 193.7 nm with a slit width of 0.5 nm. Palladium solution and magnesium nitrate solution were used as the matrix modifier for calibration. One gram of soil was added to 60 mL of 1:1 HCl in a conical flask and the solution was heated on a hot plate to boiling point. After eight hours the iron oxide in the medium was completely dissolved and the acid solution turned yellow. At this point, digestion was discontinued; the solution was made up to 250 mL with distilled deionized water, filtered through a 0.45 μm filter and the iron content determined by AAS. The arsenic content in the contaminated soil was found to be145 ppm.

2.2. Isolation and identification of As(III) ion resistant fungi

Ten grams of soil sample were added into 90 mL sterile distilled water and agitated for uniform microbial suspension. Serial dilutions were performed by decimal dilutions and were made up to 10^{-7}. From these dilutions 1 mL aliquots were poured into sterile pertriplates and 15–20 mL sterile Sabourd Dextrose Agar medium (Hi–media, Mumbai, India) supplemented with chlorotetracycline (10 mg/mL). 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. Slide culture techniques [19] were used to observe morphological characteristics of fungi [20].

2.3. FT-IR spectroscopy

FT-IR spectra of *A. fumigatus* was obtained by using a FT-IR spectrophotometer (Mattson 1000 FT-IR, England). The dry sample (about 0.1 g) mixed with KBr (0.1 g) and pressed into a tablet form. The FT-IR spectrum was then recorded.

2.4. Biosorption studies

The biosorption of As(III) ions on the isolated fungi from aqueous solution containing metal ions was 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 of the arsenic resistant fungi with As(III) ion was investigated in the pH range (3, 4, 5) (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, 35°C) respectively. The pH was maintained at 5 (optimum). The effect of the initial As(III) ion concentration on the

biosorption was studied at different pH and temperatures described above except that the concentration of heavy metal ion in the adsorption medium was varied between 100 to 260 ppm. After the desired incubation period the aqueous phases were separated from the materials and the concentrations of the metal ions in these phases were measured by using an Atomic Absorption Spectrophotometer.

2.5. 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 [21].

$$q = [(C_o - C_l)\ V]\ /\ M \quad (1)$$

where q is the amount of heavy metal adsorbed onto the unit amount of the adsorbents (mg/g) and C_o and C_l 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).

2.6. Adsorption isotherms

The adsorption isotherm model was used to characterize the interaction of As(III) ion with the fungal biomass. The Langmuir model is based on the assumption that maximum adsorption occurs when a saturated monolayer of solute molecules is present on the adsorbent surface, the energy of adsorption is constant and there is no migration of absorbate molecules in the surface plane. The Langmuir model is described by the following equation

$$q = q_m C/k_{d+C} \quad (2)$$

where C and q also show the residual metal concentration and the amount of metal adsorbed on the adsorbent at equilibrium respectively. The $k_d = k_2/k_1$ is the Langmuir constant of the system. The semi-reciprocal plot of C/q vs C was employed to generate the intercept k_d/q and the slope $1/q$.

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. The Freundlich model is described by,

$$q_e = K_F\ C_e^{1/n} \quad (3)$$

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

3. Results and discussion

3.1. Identification and cultural characteristics of arsenic resistant fungi

The present work was taken up to investigate to remediate heavy metal contaminated soil were to immobilize the metal *in situ* by microbes to reduce metal bioavailability and mobility or to remove the metal from the soil. In this preliminary screening in total count of fungus in the soil sample was ranged from $123 \pm 7.31 \times 10^2$ to $3.7 \pm 0.23 \times 10^7$ 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 grown well in 35°C and they show green brown shade with velvety texture on sabourd dextrose agar plate. The conidial heads are smooth and may reach up to 300–500 µm (L) × 5–8 µm (W). The vesicle is club shape and measures up to 20–30 µm in diameter. The sterigmata are uniseriate and measures 5–10 µm × 2–3 µm. Based on these characteristics the resistant fungus was identified as *A. fumigatus* [20].

3.2. Fungal viability and survival with and without As (III) ions

The viability of *A. fumigatus* were checked with and without the presence of As(III) ions at different pH, temperature and initial concentration were investigated. The maximum growth profile of *A. fumigatus* at pH-5 were found to be 6.15 g/L with As(III) ions and 5.86 g/L without As(III) ions. At pH below 5 with and with out As(III) ions the growth profile were found to declined. Similarly the maximum growth profile was obtained for the resistant fungal isolate at 35ºC. The dry weight of the mycelium were found to be 5.56 g/L and 4.10 g/ L with and without the presence of As(III) ion respectively. The resistant fungal mycelium was cultivated in fungal broth media amended with various concentration of As(III) ion (100–260 ppm). It appears that the fungal mycelium was able to survive at metal concentrations as high as 180 ppm of As(III) ion and above this concentration *A. fumigatus* cannot survive and the dry weight were declined. *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 [22]. The arsenic tolerance *Aspergillus nidulans* grown in SD broth with different concentrations of As(III) ions ranging from 20 to 500 mg/L. *Aspergillus nidulans* showed tolerance to

the maximum concentration of 500 mg/L of As(III) ions [23]. Lower sorption of arsenic by biomass, which is in solution presented as negatively charged oxyanion may relate with repulse electrostatic interactions between negatively charged surface of biomass and AsO_4^{3-} [24]. Arsenate 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 [25]. The maximum uptake by *Spirulina platensis* were found to be 44.56 mg/g dry weight biomass was obtained at 40 mg/L of cadmium in aqueous solution [26]

3.3. Characterization of biosorbent

The functional groups responsible for heavy metal ion biosorption on *A. fumigatus* are confirmed by FT-IR spectra. The functional groups for heavy metal ions binding on the fungal cell walls are carboxyl (–COOH), phosphate (PO_4^{3-}), amide (–NH_2), thiol (–SH) and hydroxide (–OH). In fungal cell walls chitin and its associated proteins contain many carboxyl groups with pka values in the range of 4.0–5.0 [27]. Phosphate groups are mainly in glycoproteins and are believed to play an important role in biosorption because they can exhibit a negative charge above pH 3.0 [28]. The changes in the functional groups and the surface properties of the fungal pellets are confirmed by the FT-IR spectra of before and after metal loading (Fig. 1). The FT-IR spectra of before and after metal loading fungal biomass have intense peaks at the frequency level of 3400–3200 cm^{-1} representing –OH stretching of carboxylic groups and also representing stretching –NH groups. The strong peaks at around 1645 cm^{-1} are caused by the bending N–H groups of chitin on the cell wall structure of fungal pellets. The peaks at 2920, 1550 cm^{-1} representing C–H stretching vibrations, N H bending and C–OH stretching vibrations on the functional groups present on the fungal cell wall. The peaks at 1000 cm^{-1} represents N–H stretching vibrations on fungal cell wall.

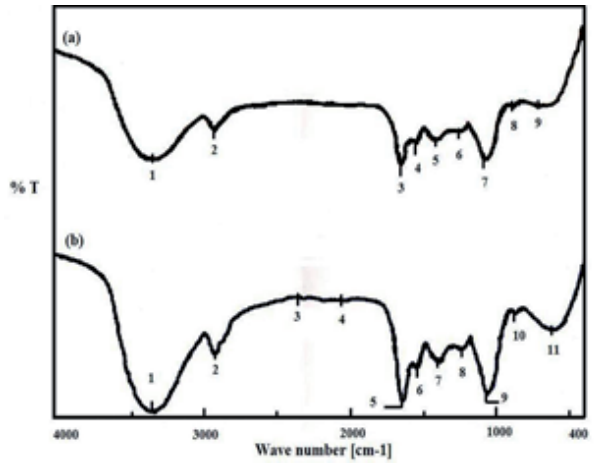

Fig. 1. FTIR Spectra of *Aspergillus fumigatus* on As(III) ion before (a) and after (b) metal loading.

3.4. Effect of pH on different As(III) ion concentration

It is well known that metal ion adsorption on non-specific and specific sorbents is pH dependent [14]. 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 [29]. In this study batch experimental system were used in the optimization of metal concentration by fungal biomass with respect to pH. Fig. 2 shows the effect of pH on different As(III) ion concentration. Biosorption capacity of the fungal biomass increased with increasing initial concentration of As(III) ion in the medium and reached a saturated value at 180 ppm of As(III) ion at different pH (3, 4, 5). As seen from Fig. 2 the amount of biosorbed As(III) ion on pH 3, 4 and 5 were 106, 101 and 134 ppm respectively. The metal biosorption hence depends on the protonation or deprotonation of these carboxyl groups, which have pKa between 3 and 4 [29] Arsenic removal by pretreated waste tea fungal biomass was obtained at pH-3 and 5 [30]. 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 biosorption of Cd(II), Pb(II) and Cu(II) on inactivated *P. chyrsosporium* was pH dependent and maximum biosorption was obtained at pH-6 [12].

3.5. Effect of temperature on different As(III) ion concentration

The temperature of the adsorption medium could be important for energy dependent mechanisms in metal

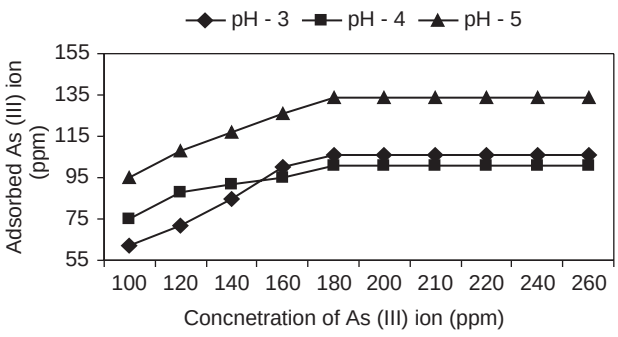

Fig. 2. Effect of different pH on biosorption capacities of *Aspergillus fumigatus* for As(III) ions.

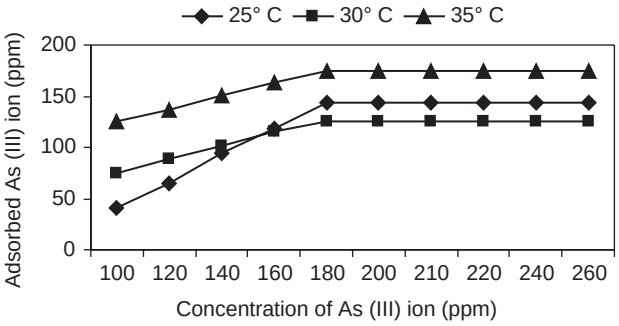

Fig. 3. Effect of different temperature on biosorption capacities of *Aspergillus fumigatus* for As(III) ions.

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 [31]. The effect of temperature on the metal biosorption experiments was investigated at three different temperatures. Fig. 3 shows the effect on temperature on the biosorption of As(III) ion at different concentrations. As can be seen from the Figure the maximum biosorption of As(III) ion on fungal biomass were observed around 35°C. Mostly adsorption is an exothermic process [32] whereas some examples of endothermic adsorption have also been reported [33]. The sorption capacity both arsenate and arsenite 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 [34]. The arsenic (V) resistant *Desulfitobacterium* 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 [35].

3.6. Langmuir and Freundlich isotherms

The two most commonly used adsorption isotherms for biosorption studies (the Langmuir and Freundlich isotherms) were investigated. Figs. 4–7 shows the Langmuir plot for As(III) ion 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) ion on the biosorbents and the results are represented in Tables 1 and 2. The maximum capacity q_m determined from the Langmuir

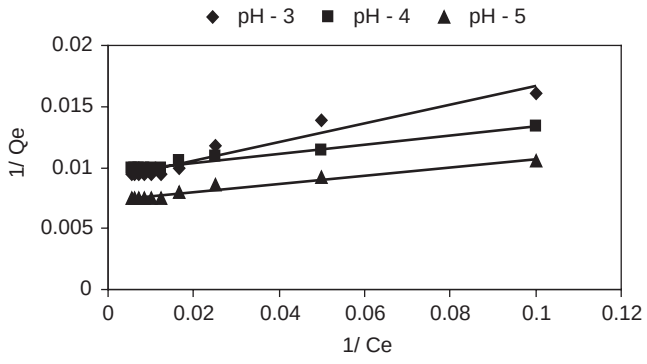

Fig. 4. Langmuir plot of As(III) ions on *Aspergillus fumigatus* at different pH.

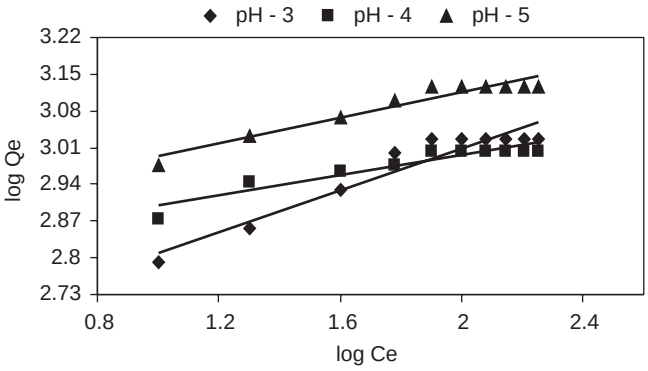

Fig. 5. Freundlich isotherm plot of As(III) ions on *Aspergillus fumigatus* at different pH.

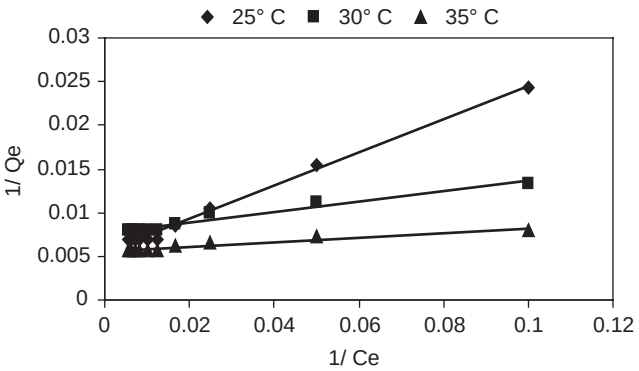

Fig. 6. Langmuir isotherm plot of As(III) ions on *Aspergillus fumigatus* at different temperature.

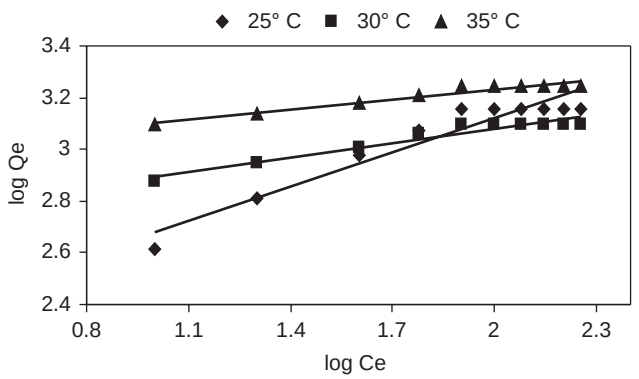

Fig. 7. Freundlich isotherm plot of As(III) ions on *Aspergillus fumigatus* at different temperature.

Table 1
Langmuir and Freundlich Isotherm model constant and correlation co efficient for biosorption of As(III) ion from aqueous solution by *Aspergillus fumigatus* at different pH.

Biosorbent at different pH	Experimental q_{eqex} (mg/g)	Langmuir Constant			Freundlich Constant		
		q_m (mg/g)	$k_d \times 10^{-4}$ (M)	R^2	k_F	N	R^2
pH-3	106	105	7.35	0.948	0.42	5.07	0.920
pH-4	103	103	3.56	0.978	0.45	10.35	0.893
pH-5	136	136	2.51	0.962	0.46	8.22	0.931

Table 2
Langmuir and Freundlich Isotherm model constant and correlation co efficient for biosorption of As(III) ion from aqueous solution by *Aspergillus fumigatus* at different temperature.

Biosorbent at different temperature	Experimental q_{eqex} (mg/g)	Langmuir Constant			Freundlich Constant		
		q_m (mg/g)	$k_d \times 10^{-4}$ (M)	R^2	k_F	N	R^2
25°C	144	185	10.32	0.997	0.35	2.27	0.923
30°C	125	129	4.65	0.959	0.43	5.43	0.932
35°C	175	178	1.48	0.929	0.47	7.99	0.939

isotherm defines the total capacity of the biosorbents for As(III) ion. 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 1). Similarly the Langmuir isotherm model and correlation coefficient have been calculated at different temperature. The order of maximum capacity (q_m) at different temperature for the heavy metal removal ions was found to be 35°C > 25°C > 30°C (Table 2). 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 [36].

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 1 and 2. The k_d values for the adsorption of As(III) ion were 7.35, 3.56 and 2.51 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 10.32, 4.65 and 1.48 at 25, 30 and 35°C respectively. Here the kd value was low at 35°C and leading to better affinity than that of 25°C and 30°C. *Aspergillus fumigatus* removed uranium (VI) very rapidly and reached equilibrium within 1 h of contact of biomass with the aqueous metal solution. Biosorption data fitted to Langmuir model of isotherm and a maximum loading capacity of 423 mg U/g dry wt was obtained. Distribution coefficient as high as 10,000 (mg U/g) / (mg U/mL) at a residual metal ion concentration of 19 mg/L indicates its usefulness in removal of uranium (VI) from dilute waste streams [37].

The Freundlich constants k_F and n shows easy separation of metal ions from aqueous medium and indicate favorable adsorption [38]. The intercept k_F value is an indication of the adsorption capacity of the adsorbents; the slope 1/n indicates the effect of concentration on the adsorption capacity at different pH was found to be increased with increasing the pH in the aqueous solution. The n values showed easy uptake of As(III) ion from aqueous medium with a high adsorption capacity as seen from Table 1. Table 2 shows the adsorption capacity at different temperature was increases at 35°C and shows the high adsorption capacity by the fungal biomass and n values were found high enough for separation. In these systems, the metal removal process is based on solid–liquid contacting and separation process. Such preparations offer advantages in terms of mechanical strength and durability, handling and ease scale up.

4. Conclusions

Aspergillus fumigatus have been successfully used as the biosorbing agent for removal of As(III) ion from aqueous solution. The biosorption of As(III) ion 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) ion concentration highly affect the over all metal uptake capacity of biosorbent. The Freundlich and Langmuir adsorption models were employed for the mathematical description of biosorption equilibrium data regarding As(III) ion to *A. fumigatus* 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) ion. 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.

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