Biotechnology: A Powerful Tool for the Removal of Cadmium from Aquatic Systems

(Bioteknologi: Alat yang Ampuh untuk Penyingkiran Kadmium daripada Sistem Akuatik)

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ABSTRACT

The prime objective of the present research work was to evaluate the efficiency of bio-machine for the removal of Cadmium (Cd) from aquatic systems. Aspergillus niger fungus was used as bio-machine to remove Cd from aquatic systems. Twenty three different strains (IIB-1 to IIB-23) were isolated from industrial effluents and the Langmuir and Freundlich models were applied to the best Cadmium removal strain IIB-23 in order to obtain the adsorption parameters. Different parameters such as pH, temperatur e, contact time, initial metal concentratio, and biomass dosage on the biosorption of Cd were studied. The percent removal of Cd initially increased with an increase in pH ranging from 5.5-6.5 and then decreased by increasing pH from 7.0-7.5. An optimized pH used for Cd removal from aquatic systems was found to be 6.5. Additionally, an optimum amount of biomass was 1.33 g for the maximum removal of Cd from the aqueous solutions with initial metal concentration of 75 mg/L. The results obtained thus indicated that Langmuir model is the best suited for the removal of Cd from aquatic systems.

Keywords: Adsorption; Aspergillus niger; Bio-machine; Biosorption; Biotechnology; Cadmium

ABSTRAK

Objektif utama penyelidikan ini adalah untuk menilai keberkesanan mesin biologi dalam menyingkirkan kadmium (Cd) daripada sistem akuatik. Kulat Aspergillus niger digunakan sebagai mesin biologi untuk penyingkiran Cd daripada sistem akuatik. Dua puluh tiga strain berbeza (IIB-1 IIB-23) telah dipencilkan daripada efluen industri dan model Langmuir dan Freundlich digunakan untuk penyingkiran kadmium terbaik strain IIB-23 untuk mendapatkan parameter penjerapan. Parameter berbeza seperti pH, suhu, masa hubungan, kepekatan logam pemula dan dos biojisim pada bioserapan CD telah dikaji. Peratus penyingkiran Cd pada mulanya meningkat dengan peningkatan dalam pH antara 5.5-6.5 dan kemudian menurun dengan peningkatan pH daripada 7.0-7.5. PH optimum yang digunakan untuk penyingkiran Cd daripada sistem akuatik adalah 6.5. Di samping itu, sejumlah biojisim optimum adalah 1.33 g untuk penyingkiran maksimum Cd daripada larutan berair dengan kepekatan logam pemula 75 mg/L. Keputusan yang diperoleh menunjukkan bahawa model Langmuir adalah yang terbaik untuk penyingkiran Cd daripada sistem akuatik.

Kata kunci: Aspergillus niger; bioserapan; bioteknologi; kadmium; mesin biologi; penjerapan

INTRODUCTION

Heavy metal pollution load in an aquatic system is of great environmental concern as these metals are toxic and non-biodegradable. Due to the increasing anthropogenic activities and use of improper drainage systems of industries and agriculture, these metals are accountable for dangerous chemical pollution of aquatic systems (Chopra & Pathak 2010). The toxic heavy metals responsible for water pollution are Cadmium (Cd), Zinc (Zn) Chromium (Cr), Nickel (Ni), Lead (Pb) and Mercury (Hg). At higher concentrations (above LD_{50} values), these metals become toxic but some of these metals such as Cr, Ni and Zn are essential at trace levels for human beings (Newman et al. 2010).

Cadmium is the most toxic metal which causes harmful effects to the humans as well as to environment. It is harmful pollutant originating from mining, ceramics and metal plating, and also causes cancer, kidney and bone diseases in humans. Long-lasting exposure causes liver damage, renal dysfunction and bone degradation (Ng et al. 2008). Conventional methods used for the removal of heavy metals from waste water include adsorption and evaporation (Ahluwalia & Goyal 2007; Aksu et al. 1999), ion exchange (Finley et al. 2013), chemical precipitation (Göksungur et al. 2005), chemical reduction (Moses et al. 2003) and different membrane processes (Colditz et al. 1995; Nourbakhsh et al. 1994). The disadvantages regarding conventional techniques are monitoring systems,

incomplete metal removal (Xiangliang et al. 2005), requirement for expensive equipment and generation of toxic sludge (Kobya et al. 2005).

Biomass is very useful for the accumulation of heavy metals. According to the environmental remediation, biomass must be a waste material and it comes from nature (Sawalha et al. 2006). Different types of biomasses have been obtained from fungi, bacteria, yeast, microbial, plants derived, molds and crab shells (Nagajyoti et al. 2010) which were used for the biosorption of different metals (Martín-González et al. 2006).

Biosorption is the binding and concentration of heavy metals from aqueous solutions (very dilute) by the use of dead, microbial and plant derived biomass. Biomass acts as an ion exchanger and chemical substance of biological origin. It was actually the cell wall of certain biomass which is responsible for the biosorption. Biosorption efficiency depends upon specificity and affinity of the biosorbent and their physical and chemical conditions in effluents. Biosorption is highly competitive because of cost effectiveness, regeneration of metals, no sludge formation, no additional nutrient required and heavy metal selectivity (Pittman et al. 2011). Due to the structural complexity of the microorganism there are many ways for the metal uptake by the microbial organism. According to the various criteria, the biosorption mechanisms are classified via metabolism dependence, biosorption mechanism as metabolism and non-metabolism dependent. Similarly, on the metal removal from the solution, biosorption may be classified as extra-cellular accumulation, cell surface sorption and intra-cellular accumulation. Depending upon metabolic biosorption, transport of the metal across the cell membrane yields intra-cellular accumulation which is highly dependent upon the cell's metabolism. It means that biosorption only takes place with viable cells. It is associated with the active defense system of the microorganism, which further reacts in the presence of toxic metal. According to non-metabolic dependent biosorption, metal uptake is the physiochemical interaction between the functional groups present on the microbial cell surface and metal, during non-metabolic dependent biosorption. It is based upon chemical precipitation, ion exchange and physical adsorption. The cell wall of microbial biomass is made up of proteins, polysaccharides and lipids. Metal binding takes place with functional groups such as sulphate, phosphate, carboxyl, hydroxyl and amino groups. This type of biosorption is reversible and fast (Borcherding 2014).

Many researchers have been focused upon the characteristics of metal uptake by using fungi, particularly when the heavy metals present at low and different concentrations rather than the current interest upon microbial detoxification of industrial effluents.

As for as the removal of toxic metals from polluted aquatic systems are concerned, there are numerous studies carried out with mechanistic approach of physico-chemical adsorption. In this context, we embarked a project aiming at the isolation of *Aspergillus niger* as fungal biomass from the industrial effluents of Pakistan. The isolated fungal biomass was investigated as efficient biosorbent for the removal of Cd from industrial waste water by employing batch biosorption studies.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PRESERVATION

Samples of different industrial effluents such as textile, pharmaceuticals, and leather were collected from Quid-e-Azam industrial estates Kot Lakh path, Lahore, Pakistan (Figure 1). Samples were collected (1.5 L of each) in sterilized plastic containers. After collection samples were preserved first into ice box and then shifted to research laboratory (Institute of Industrial and Biotechnology Department GCU Lahore) and kept in refrigerator at 4°C to stop any physico-chemical change and microbial activity.

DETERMINATION OF HEAVY METAL CONCENTRATION

Initial as well as post biosorption treatment metal (Cd) concentration in the industrial effluent was determined using Atomic Absorption Spectrophotometer ModelAA-7000F, Shimadzu Japan and standard of Merck pharmacy was used having concentration range of 1000 ppm.

ISOLATION OF BIOSORBENTS

Potato dextrose agar (PDA) medium was prepared, (100 mg/L) of Cd solution was added, Levofloxacin 500 mg was also added into the medium to inhibit the bacterial growth and make the volume up to the mark. Nutrient agar medium was prepared for bacterial growth followed by pouring of medium into the plates under the laminar flow. After solidifying media, industrial effluents were spread onto the plates by using micropipette. Plates were incubated at 30°C for 6-7 days. When colonies were seen, re-inoculation of hyphen tips was done to purify the isolates. To get pure cultures of fungal species, they were re-inoculated on the new plates for three times. From the established culture of 6-7 days, spores were inoculated onto the PDA slants at 30°C, pure culture were obtained and used for inocula preparation. These slants were stored at 4°C for further use.

EVALUATION OF MICROBIAL STRAINS BY SCREENING

Nutrient broth was prepared in a conical flask and cotton plugged. The prepared medium was sterilized in autoclave for 20 min at 15l bs. and 121°C. After sterilization, the medium was cooled to room temperature. Under laminar flow cabinet, the inoculum was added in it. For three days, those cultures were incubated on rotary shaker at 30°C and 160 rpm. After 72 h, fungal growth was observed and biomass was centrifuged at 6000 rpm for 10 min. Pallet was taken and makes Cd solution of known concentration of 100 ppm and added into it. Treat for 1 h at rotary shaker and again centrifuged at 6000 rpm for 10 min. Supernatant was collected and pallets were separated for determination

FIGURE 1. Map and view of Quid-e-Azam Industrial Estates Kot Lakh Path, Lahore, Pakistan

of strain shows best Cd removal. Supernatant was run onto the atomic absorption spectroscopy. The strain having least amount of Cd shows best cadmium removal. The best results showed by a fungal strain which was used for further biosorption studies.

INOCULUM PREPARATION

The inoculum was prepared by taking 0.2 g nutrient broth in a 250 mL conical flask and 20 mL distilled water was added to it. After dissolving and diluting volume up to 25 mL with distilled water. The flask was Cotton plugged and autoclaved at 121°C at 15 lbs pressure for 15 min. Czepekdox medium was used for fungal growth, consisting of (g/L): KH_2PO_4 7.0; $(NH_4)_2SO_4$: 1.0; K_2HPO_4: 2.0; glucose 10, yeast extracts, 0.6 mixed in distilled water and volume raised to 1.0 liter. From which 25 mL was transferred to separate conical flask of 250 mL. A slant having *A. niger* was taken and 10 mL of the sterilized distilled water added. By using inoculating needle, the slant was then scratched and spore suspension was made. For the inoculation of cultural media, one milliliter of this spore suspension was used. Samples were filtered by using syringe filter paper nylon membrane having porosity 0.22 μm (AISIMO).

SUBMERGED FERMENTATION

The fungal strains were inoculated in liquid media in 250 mL Erlenmeyer flasks and then these flasks were cotton plugged and kept in autoclave for 15 min at 15 lbs. and 121°C. After sterilization, the media was cooled down at room temperature and inoculum was added in it in laminar air flow. Those cultures were incubated on rotary shaker at 160 rpm and 30°C for 72 h. After 3 days, fungal biomass was centrifuged. All the experiments were performed in triplicate. Culture broth was centrifuged at 6000 rpm for 10 min after three days of incubation period. Supernatant was collected and pallets were separated for the determination of dry cell mass. The supernatant was used for further biosorption studies.

IDENTIFICATION OF FUNGAL SPECIE

The fungal specie *Aspergillus niger* was identified with the help of expert of Department of Botany, University of the Punjab, Lahore, Pakistan and Government College University (GCU) Lahore, Pakistan, and thus was used as biosorbent for the removal of heavy metals like Cd from the waste water.

MORPHOLOGICAL CHARACTERISTICS AND TAXONOMIC POSITION OF *ASPERGILLUS NIGER*

Colonies of *Aspergillus niger* were grew fast upon plates and gives appearance like cotton. A little bit contamination was appeared; hence, pure colonies of black color were picked up with the help of inoculating loop and transferred to slants. These were initially black in colorbut with the passage of time; the colonies became blackish to brownish black. Conidiophores were narrowly placed with hyaline erect stem. Vesicles were globular to club shaped over the surface of which rod-shaped sterigmata are born. Conidiophores were 949-1060 μm in length, hyaline and finely roughened, smooth-walled and had conical terminal vesicles (42 μm in diameter), which supported a single row of phialides with 14-16 μm in length (Figure 2).

METAL UPTAKE AND BIOSORPTION EFFICIENCY

The uptake of metal ions was calculated by the given formula:

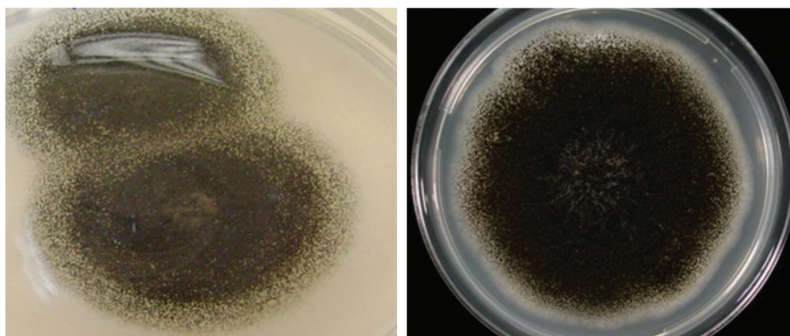FIGURE 2. Images showing colonies of microbes with best Cd removal

$$q = V (Ci-Cf)/m \qquad (1)$$

where Q is the mg of metal ions per g of biosorbent; V is the volume of reaction; Ci is the initial metal concentration; Cf is the final metal concentration; and M is the amount of biosorbent.

The metal ion concentration in the filtrate was determined by using the Flame Atomic Absorption Spectrometry, AA-7000F Shimadzu Japan.

The efficiency of heavy metal ions was calculated by the following formula:

$$Y = Ci-Cf/Ci \times 100 \qquad (2)$$

where Y is the percentage Cd removal; Ci is the initial Cd concentration; and Cf is the final Cd concentration.

REPRODUCIBILITY

All the experiments were performed in triplicates. Controls were also run without addition of biosorbent using the same protocol. All the parameters were identified after the screening of best Cd removal strain that is *Aspergillus niger* was used for the optimization of biomass amount by using different batch biosorption experiments as well as for kinetic isotherms.

RESULTS AND DISCUSSION

ISOLATION AND SCREENING OF BEST CADMIUM REMOVAL STRAIN

The results indicated that best Cd removal strain is IIB-23 specie because percentage removal of Cd is higher in that specie (Figure 3). Furthermore, this species is identified and used for the analysis of biosorption parameters. Graph depicted that IIB-23 specie have least amount of Cd left i.e. 0.332 (mg/L) so it is a best Cd removal strain used for further biosorption studies. Cadmium ions from industrial waste water were absorbed more rapidly within 15-20 min than other ions such as Iron (Fe), Copper (Cu), and Lead (Pb) by *A. niger* biomass. Experiments indicated that equilibrium reached within 20 min while using industrial waste water samples in comparison to the single ions

solution equilibrium reached within 30 min. The removal percentage order at equilibrium was: Cd (95%), Pb (88%), Fe (70%), Cu (60%), Ni (48.9%) and Zn (15.4%) (Goyal et al. 2003).

The results indicated that best Cd removal strain is fungal specie, *Aspergillus niger*, used for further biosorption parameters. It is an efficient fungus used for the biosorption of heavy metals. It is selected on the basis of its resistance to multi-metals and major occurrence.
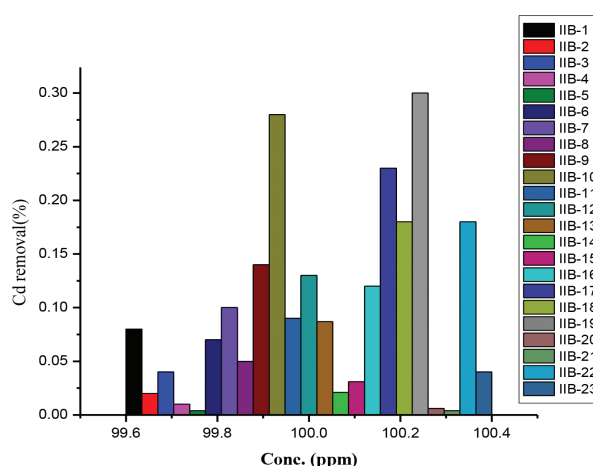

FIGURE 3. Results of isolated microbial strains showing Cd removal

OPTIMIZATION OF PARAMETERS

Effect of pH The results indicated that optimized pH for the biosorption of Cd by using *Aspergillus niger* as adsorbent is 6.5 (Figure 4). It starts increasing from pH 5.5-6.5 which is an acidic media further it starts decreasing from pH 7-7.5 and conditions for this parameter are pH 5.5-7.5 (Wilhelmi & Duncan 1995), temperature 30°C, adsorbent amount 1 mL in the form of spore suspension and initial Cd concentration is 100 (mg/L) (Joo et al. 2010). The biosorption process for heavy metals optimized at pH (5.0-5.2) showed the ionic strength affects the adsorption between the solute and fungal biomass. Biomass connection with water molecules bounded to each other with an electrical double layer and showed the

electrostatic interaction (Volesky & May-Phillips 1995). So, the adsorption process decreased by the increasing the electrostatic interaction. The metal uptake dependence upon pH is specifically related to the chemistry of metal in solution and functional groups present on the biomass (Park et al. 1999). The approach of the metal cation is restricted because the surface ligands are closely associated with the hydronium ion at lower pH of 2-3 and due to the presence of repulsive forces, sorption of the metals at lower pH become decreased and dissociation of carboxyl group is not occur and cannot bind to the fungal cell wall. The cell surface sites are closely associated to the H^+ ions and making them unavailable for metal cation at lower pH (Yin et al. 1999).
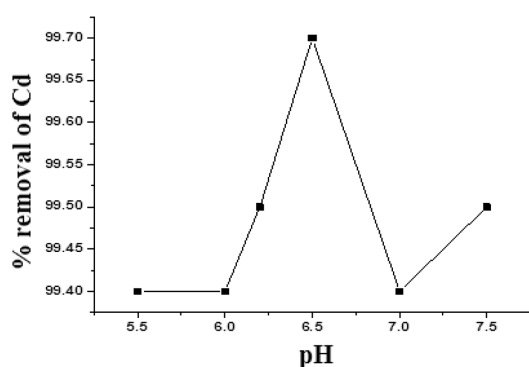

FIGURE 4. Percent (%) removal of Cd at different values of pH

Metal Uptake at Different Metal Concentrations Figure 5 illustrates that due to the increase of metal uptake initially increase removal of metal and then goes on decreasing. The maximum metal uptake was obtained at 75 ppm initial metal concentration. The percent (%) Cd removal efficiency was observed in direct relation to the biomass size until equilibrium was attained and biomass dosage was optimized by using batch biosorption studies in which the optimized amount of biomass was 1.33 g having concentration of solution 75 mg/L.
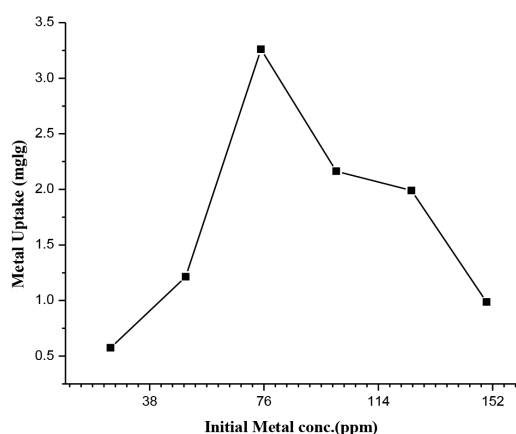

FIGURE 5. Metal uptake at different metal concentrations

Percent (%) Removal of Cd at Different Biomass Amount Figure 6 shows that initial biomass size increases the removal of Cd and then it gradually goes on equilibrium. The optimized biomass value is 1.33 in which concentration of solution is 75 (mg/L), volume of the solution is 100 mL, adsorbent size varying from 0.19-0.33, pH 6.5 and temperature is 30°C. It has been observed that by using *Aspergillus niger* as adsorbent, percentage removal of Cd metal increased due to the increasing concentration of biomass. The maximum removal of Cd was achieved at biomass dose of 1.33 g with percentage removal of 97% was achieved. Therefore, it can be concluded that optimum amount of biomass was 1.33 g for the maximum removal of Cd from the aqueous solutions with initial metal concentration of 75 mg/L. Adsorption experiments were carried out by varying amount of biomass dosage 0.19-0.33 g in mixed ions solution. The maximum biosorption efficiency was achieved at biomass dose of 1.33 g/L with slight significant increase in percentage removal at lower biosorbent amount.
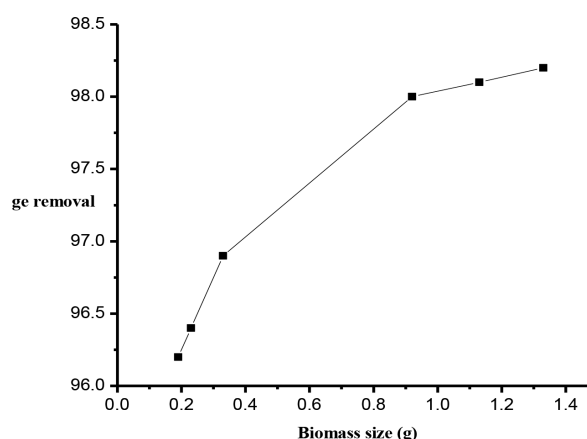

FIGURE 6. Percent (%) removal of Cd at different biomass amount

Biosorption Isotherms These are graphical curves which show relationship between equilibrium concentration at uniform temperature and sorbatesorbed by the adsorbent. The biosorption isotherms for Cd onto the fungal strain were studied in batch experiments by varying concentrations. Therefore, Langmuir and Freundlich isotherms were successfully applied for the verification of analysis.

Langmuir Biosorption Isotherm It tells about monolayer adsorption which means that no adsorbate deposits on it. The number of active sites is static and homogenous distribution on the sorbent surface occurs (Robbins 2012).

The Langmuir isotherm equation is linear and which is represented as (Doyle 1963).

$$Ce/qe = Ce/qm + 1/R_L qm$$

where RL is the Langmuir constant; Qe is the adsorption capacity; Ce is the equilibrium concentration; and Qm is the maximum adsorption capacity.

The Langmuir isotherm can be clearly identified by the following equation (Bardy et al. 2005; Villaescusa et al. 2011),

$$RL = 1/1 + KaC_0$$

Freundlich Biosorption Isotherm The Freundlich isotherm based upon multilayer adsorption and it tells about heterogeneous surface. It is plotted between concentration of solute in a liquid and concentration of solute on the surface of adsorbent.

The Linear equation for Freundlich isotherm is given as (Zhou & Kiff 1991),

$$lnqe = lnk_f + 1/n \, lnCe$$

where Ce is the equilibrium concentration (mg/L); K_f is the distribution coefficient; N is the intensity of adsorbtion; and Qe is the adsorption capacity (mg/g).

Comparison of Langmuir and Freundlich Isotherms Figure 7 concludes that slope for Langmuir model is 0.0936, intercept is -0.0084 and R^2 value is 99%. According to Freundlich isotherm (Figure 8), the value of slope is 0.1085, intercept is 1.260 and R^2 is 21%. Larger the value of R^2, the better is the isotherm model. So the Langmuir model is best fitted to the biosorption of Cd from *Aspergillus niger* biomass.
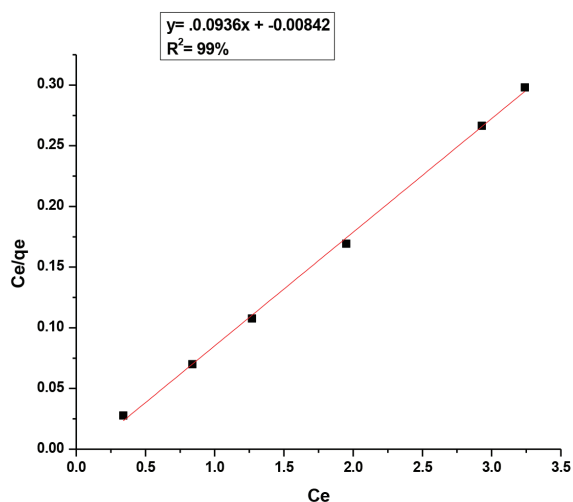

FIGURE 7. Langmuir isotherm for Cd biosorption on
Aspergillus niger

CONCLUSION

In the present study, a fungal strain *Aspergillus niger* was used as adsorbent for the removal of Cd from end of pipe waste water (effluents). For this purpose batch biosorption experiments were done. The study for the optimum pH
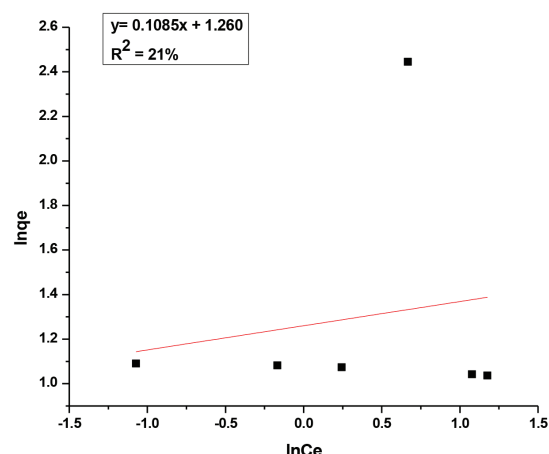

FIGURE 8. Freundlich isotherm for Cd biosorption on
Aspergillus niger

showed that fungal adsorbent worked maximum at pH6.5 and optimized amount of biomass was 1.33 g which was achieved at 75 ppm Cd concentration initially and 98.97% of Cd was removed from the solution. Applicability of Langmuir and Freundlich methods were assessed using experimental data. The experimental results showed Langmuir model was best fitted to our experimental data. Furthermore, it could efficiently be used as potential and cost-effective substituent regarding to imported and costly sorbents currently being applied. Biosorption, by using microbial biomass i.e. algae, fungi, bacteria has been considered as best alternative biological technique to the existing technologies used for the removal of heavy metals from the aqueous solutions. Fungal adsorbents are the economic because these microbes were grown upon PDA and Czepekdox media which were easily available and commercially economical. By using un-sophisticated fermentation techniques, a huge amount of fungi can grow on an inexpensive growth media. So it is recommended that fungal biomass can be used for the removal of metal ions from waste water because biomass can regenerate as well.

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