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REMOVAL OF FE(II) USING *Aspergillus flavus* FROM AQUEOUS SOLUTION

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ABSTRACT

Biosorption is receiving more attention for the removal of metal ions from waste water in comparison with the conventional technologies. In present investigation, *Aspergillus flavus* is tested for its abilities to absorb Fe(II) ions from aqueous solution. The biosorption capacity of Fe(II) was found to 5.17 mg/g of live biomass. The optimization of some important parameters, such as incubation time, pH, temperature and initial metal ion concentration on biosorption capacity were examined in batch experiments. Maximum biosorption rate was obtained at pH range from 7.0-8.0, after 5 days of incubation time and between 30-35°C of temperature range. It was also observed that increasing Fe(II) concentration decreased the biosorption capacity. Langmuir and Freundlich isotherm model were used for mathematical description of adsorption of Fe(II). The value of correlation coefficients (R^2) of Langmuir and Freundlich isotherm model were found to 0.974 and 0.872, respectively, therefore, the Langmuir model fitted the equilibrium data better than Freundlich isotherm model.

KEYWORDS: Biosorption; *Aspergillus flavus*; iron; Isotherms

The unceasing growth of industrialization and urbanization has increased the demand of natural resources. These demands of human development have also increased the level of pollution in the present environment. Water pollution by the heavy metals is one of the most significant problems, which is mainly due to the discarding industrial effluent, domestic wastewater or dumping solid waste nearby water resources. The heavy metals such as Fe, Zn, Cu, Pb, Mn, Hg, Cr and Cd etc. are widely distributed into earth's surface. Iron is commonly used in electroplating industries, steel and ferroalloy units etc. Almost all organisms and living cell require iron for the basic cellular process. However, excessive iron causes iron toxicity. In humans, vomiting, diarrhea and damage in the intestine are the symptoms of iron toxicity and it is also affecting the aquatic life through getting precipitated in the gills of fishes (Binupriya et al., 2006). The industrial effluents have containing heavy metals and other toxic substances and its disposal into natural water systems is a cause of serious environmental concern.

Conventional methods such as chemical precipitation, electrowinning, membrane separations, evaporation and ion exchange are very expensive as an economic point of view to remove heavy metal from wastewaters (Dursun, 2006). Biosorption is one of the methods to solve water pollution in an inexpensive manner. Biosorption is a physicochemical process that can be simply defined as the removal of substances from solution by biological material (Fomina and Gadd, 2014). A variety of biological materials is available for metal removal processes. Bacteria, yeast, algae and fungi have received more attention for metal removal and their recovery due to their good performance, low cost and large availabilities (Wang and Chen, 2009).

The main objective of this investigation is to reduce the higher concentration of iron from the industrial effluents using biosorption method. For this purpose, an artificial aqueous solution of iron (II) was tested in the laboratory. In the present study, the biosorption of Fe(II) by live biomass of *Aspergillus flavus* was investigated and effect of parameters such as incubation time, pH, temperature and initial metal ion concentration were studied in a batch system. For characterization of uptake capacity of Fe(II) isotherm model were also studied.

MATERIALS AND METHODS

Microorganism

Fungal strain *Aspergillus flavus* was isolated from the steel industrial effluent of Urla industrial area, Raipur (Chhattisgarh), India by serial dilution technique on Potato Dextrose Agar (PDA) and identified based on morphological and microscopic characteristics (Nagamani et al., 2006). The fungal culture was routinely maintained in PDA medium.

Preparation of Biosorbent

The fungal culture was inoculated in a Potato Dextrose Broth (PDB), pH 5.5-5.6 and after 3-4 days of inoculation, 100 µL of 10^{-1} fold diluted fungal spores suspension was transferred into the experimental batch setup. The batch biosorption experiment was carried out in Erlenmeyer flask (150 mL) containing 50 mL of PDB with Fe(II) approximately 5 mg/L of concentration. In the medium, ferrous ammonium sulfate (FAS) compound was used as Fe(II) source. The

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culture was incubated at 26±1ºC in 100 rpm on a rotary shaker.

Biosorption Studies

The optimum time required to biosorption of Fe(II) by *A. flavus* was determined for the period of 1st to 9th day. The optimization of pH was carried out at different pH range from 3.0 to 11.0 at optimum incubation time. The pH of the solution was adjusted using 0.1N HCl and 0.1N NaOH. The optimization of temperature was studied at the different temperature range from 20 to 40°C at optimum conditions of pH and incubation time. The effect of initial metal ions concentration on biosorption of Fe(II) ions was investigated at the different concentration ranging from 5 to 50 mg/L at optimum conditions of aforesaid parameters. The fungal mat was removed by filtration using Whatman No.1 filter paper and filtrate were subjected to determination of residual iron concentration in the medium.

The iron concentration was measured using an iron test kit (Iron Test 0.005-5.00 mg/L, Merck, Germany) and its absorbance level was measured by Spectroquant NOVA 60 (Merck, Germany). Biosorption experiment was performed in duplicates and average values were used in the analysis.

The percent (%) removal of metal ions was calculated by following equation given by (Olusola and Aransiola, 2015):

$$\%\, of\ metal\ removal\ = \left(\frac{C_i - C_f}{C_i}\right) \times 100$$

Where, C_i = initial metal ion concentration, mg/L; C_f = final metal ion concentration, mg/L.

Adsorption isotherm studies

The biosorption data obtained for Fe(II) was analyzed using Langmuir isotherms. Langmuir's isotherm model assumes a monolayer adsorption on to a surface containing a finite number of identical sites (Langmuir, 1918), which is represented as:

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \left(\frac{1}{q_{max}K_L}\right)\frac{1}{C_e}$$

where, q_e is the amount adsorbed at equilibrium (mg/g); C_e is the equilibrium concentration (mg/L); q_{max} is the maximum amount adsorbed per unit weight of biosorbent to form a complete monolayer on the surface and K_L is a constant related to the energy of biosorpion (Lmg^{-1}).

The Freundlich equation (Freundlich, 1906) proposes an empirical model which is based on the sorption on heterogeneous surface and the Freundlich adsorption equation can be written as:

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e$$

where, K_F (mg/g) and n are Freundlich isotherm constants and indicator of the biosorption capacity and biosorption intensity, respectively; C_e is the equilibrium concentration (mg/L); q_e is the amount adsorbed (mg/g).

RESULTS AND DISCUSSION

Optimization of Incubation Time

Effect of incubation time on interaction between metal ions and living biomass of *A. flavus* on sorption is illustrated in Figure,1. It was observed that the percentage removal of Fe(II) was increased rapidly during the 1st to 5th day (29.09% to 92.31%) of incubation time and remained nearly constant after 5th day of incubation time. Similar results was reported by Shivakumar et al. (2014) for Cu, Zn, Pb, Cr and Ni. This incubation time was considered for further optimization of parameters.

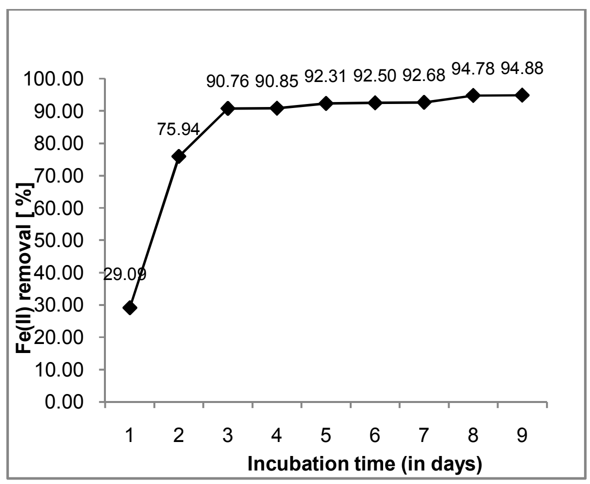

Figure 1: Effect of incubation time on biosorption capacity of living biomass of *A. flavus.*

Optimization of pH

The pH is described as the most important parameter in the quantity of metal ion itself to cation competition with hydrogen ions (Adeogun et al., 2012). The maximum percentage removal of Fe(II) 93.58%-94.13% was obtained in the pH ranging from 7.0-8.0 (Figure,2). Our result showed positive agreement with previously findings of Kareem et al. (2014) for Fe(II) removal by *T. viride* at pH 7.0 and Binupriya et al.

(2006) for maximum removal of Fe(II) was in neutral pH and Ni(II) was in alkaline pH. They also suggested that at high pH, negatively charged ions of the surface of biomass attracts more positively charged metal ions for binding.

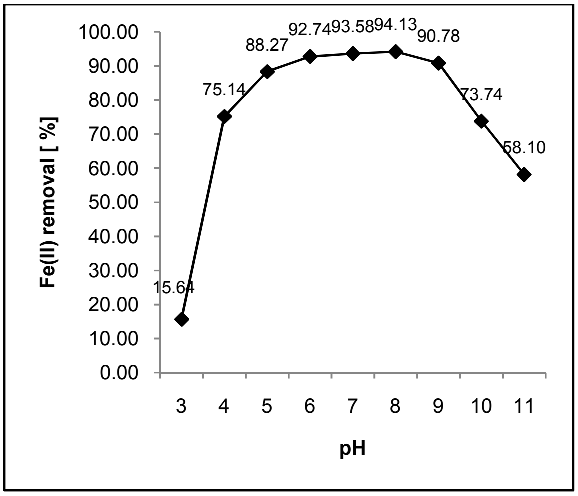

Figure 2: Effect of pH on biosorption capacity of living biomass of *A. flavus.*

Optimization of Temperature

Temperature has also one of the important factors which influence the biosorption capacity of metal ions. The maximum removal of Fe(II) 93.71%-94.36% was obtained at 30-35°C of the temperature range (Figure,3). Several researchers also suggested that the biosorption capacity remains unaffected within 20-35°C temperature range (Ahemad and Kibret, 2013; Kareem *et al*., 2014). Biosorption process is usually operated at low temperature range because high temperature will increase the operational cost (Wang and Chen, 2006).

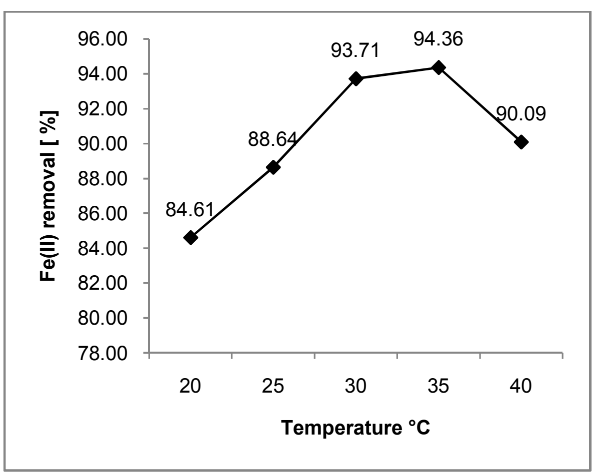

Figure 3: Effect of temperature on biosorption capacity of living biomass of *A. flavus.*

Optimization of Initial Metal Ion Concentration

In the current study, maximum Fe(II) removal (96 %) was observed at an initial metal ion concentration of 5 mg/L. As initial concentration of Fe(II) increased from 5-50 mg/L, percentage removal decreased from 96% to 59.71%, respectively (Figure,4). Several authors also suggested that at lower concentration of metal ions, the number of available binding sites is high; therefore higher biosorption yields were obtained but as initial concentration of metal ion increased, availability of binding sites of biosorbent relatively decreased, which resulted in reduction of biosorption capacity (Dursun, 2006; Wang and Chen, 2006 and Fomina and Gadd, 2014).

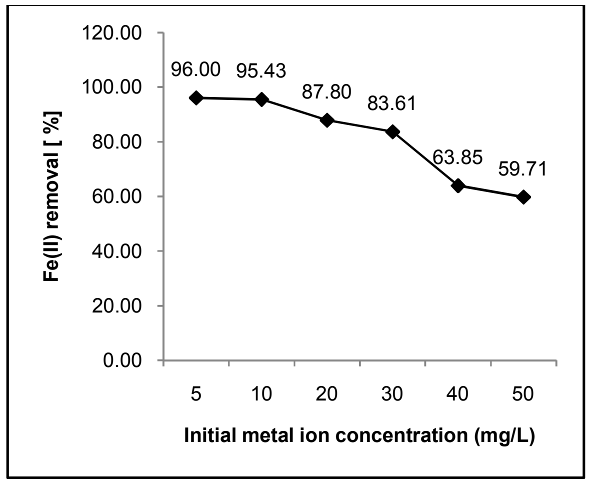

Figure 4: Effect of initial metal ion concentration on biosorption capacity of living biomass of *A. flavus.*

Biosorption Isotherms

The data obtained for Fe(II) biosorption at different initial metal ion concentrations from 5 to 50 mg/L with incubation time, optimum pH and temperature were used to fit the Langmuir and Freundlich adsorption model. Isotherm constant was determined to find out the adsorption capacity of living biomass of *A.flavus* for Fe(II). The value of correlation coefficients $R^2 \geq 0.96$ was found to be 0.974 for Langmuir and 0.872 for Freundlich model. This result indicated that the biosorption data was best fitted in Langmuir as compare to Freundlich model (Figure,5). The Langmuir constant (K_L) and Freundlich constants (K_F and n) values were determined from slope and intercept of the plot and shown in Table 1.

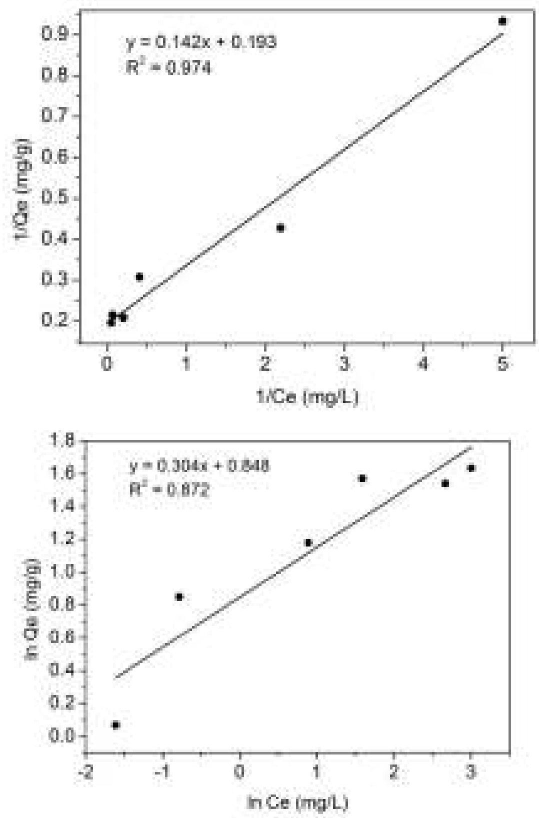

Figure 5: Biosorption isotherms (Langmuir and Freundlich) for Fe(II) biosorption on living biomass of *A. flavus.*

Table 1: Isotherm parameter for the biosorption of Fe(II) by living biomass of *A. flavus.*

Langmuir isotherm model			Freundlich isotherm model		
q_{max} (mg/g)	K_L (L/mg)	R^2	K_F (mg/g)	n	R^2
5.17	1.361	0.974	2.334	3.284	0.872

CONCLUSION

In the current study, the living biomass of *A. flavus* showed a higher affinity towards Fe(II) at pH range 7.0-8.0 and temperature range 30-35°C. The maximum biosorption rate was found in the 5th day of incubation time. An increase in initial Fe(II) concentration had resulted in decreased biosorption capacity. The equilibrium data fitted well with Langmuir isotherm model to evaluate the performance of the living biomass of *A. flavus*. The maximum biosorption capacity of *A. flavus* was found to be 5.17 mg/g for Fe(II). Finally, results indicated that the fungal strain isolated from iron polluted sites can be use in order to remove iron from industrial effluents because of its high biosorption capacity and cost effectiveness.

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