ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Removal of Copper from Aqueous Solution by *Aspergillus Niger,* NCIM 616 Using AAS and ICPMS

M Sravani[1], V Sridevi[2*], K Vijay Kumar[3], N Harsha[4] and T Kanthi Kiran

[1]M.Tech, Center for Biotechnology, Dept of Chemical Engineering, Andhra University, AP, INDIA.
[2*]Associate professor, Dept of Chemical Engineering, Andhra University, AP, INDIA.
[3]M.Tech, Center for Biotechnology, Dept of Chemical Engineering, Andhra University, AP, INDIA.
[4]M.Tech, Center for Biotechnology, Dept of Chemical Engineering, Andhra University, AP, INDIA.

ABSTRACT

Heavy metal pollution represents an important environmental problem due to the toxic effects of metals, and their accumulation throughout the food chain leads to serious ecological and health problems. Microorganisms can be important biosorbents for heavy metal remediation of contaminated soils and wastewaters. The biosorption from synthetic waste water of Cu(II) on to the dry fungal biomass of Aspergillus niger, NCIM 616 was studied. The maximum absorption of Cu(II) on the fungal biomass was obtained at pH 5, temperature-35oC, and the biosorption equilibrium was established after about 6 hrs using AAS and ICPMS which were influenced more at the removal of Cu(II) and the comparision between the two instruments was also studied. The studies indicate that A.niger NCIM 616 is an effective biosorbent for Cu(II) removal. The maximum Cu(II) biosorption capacity has been found to be 48.37% Cu (II) of dry weight of biomass at an fungal dose of 20 mg/dm3 in of contact time 8 days. Accurate results of biosorption of metals were obtained in ICPMS compared to AAS.

Key words: A. niger, NCIM 616, biosorption, copper removal, metal concentration, pH, temperature, time.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Environmental Pollution

The pollution of the environment with toxic heavy metals is spreading through the world along with industrial progress [1,2] Heavy metals are major pollutants in marine, ground industrial and even treated wastewaters [3,4]. The specific problem associated with heavy metals in the environment is their accumulation in the food chain and their persistence in nature. Aqueous effluents emanating from the mining industry and metal plating factories contain dissolved heavy metals. If these discharges are emitted without treatment, they may have on adverse impact on the environment [5].

Heavy metal ions are used in various industries due to their technological importance. Waste waters from these industries include metal ions having permanent toxic effect [6] Algae, fungi, yeast and bacteria remove heavy metals from waste waters through functional groups such as ketones, aldehydes, carboxyls on their cell walls. Feasible and useful treatment methods have been developed to purify industrial waste waters. [7, 8] Removal and recovery of heavy metals are very important with respect to environmental and economical considerations. Conventional methods for removing heavy metals include chemical precipitation and ion exchange. These become inefficient or expensive especially when the concentration of the heavy metal ion is low, the order of 1 to 100 mg^{-1}. In order to qualify for industrial applications, biosorbents have to be produced at a low cost.

Many studies showed that soluble metal ions in the environment could be captured by cell wall because of negative charged groups attach within its fabric. In last ten decades, bacteria, algae and fungi or their separated components have been used successfully as biosorbent for heavy metal removal. The metal uptake process, however, is complex and dependent on the chemistry of the metal ions, specific surface properties of the organisms, cell physiology and the physicochemical influence of the environment like pH, temperature and metal concentration [9-12]

In this study, the use of *A. niger,* NCIM 616 as a biosorbent for heavy metals biosorption from synthetic wastewaters was studied. The maximum biosorption capacity of the fungal biomass, based on dry weight, was determined under pH controlled conditions by varying the concentration of the heavy metals ions in the artificial wastewaters.

MATERIALS AND METHODS

Microorganisms

A.niger NCIM 616, procured from National Collection of Industrial Microorganisms (NCIM), NCL, Pune. The organism was sub-cultured at regular intervals of 4 weeks. The culture was grown at 28^{o} C for 5 days and stored at 4^{o} C for further use.

ISSN: 0975-8585

Maintenance of Culture

The experiments described in this study were carried out using *A.niger* NCIM 616 which was maintained on potato dextrose agar (PDA) at 4^{o} C and subculture was done frequently in the laboratory. Fresh slants were prepared for running experiment.

Spore Suspension /Inoculum

10 ml of sterilized 0.1 % Tween 80 solution is added to the 5 days old slant and the culture was scraped with the help of inoculating loop. The required volume was pipetted into each flask containing autoclaved (at 121 ^{0}C, 15 lbs for 15 min) basal medium.

Cultivation of Fungi Species for Biomass Production

The basal medium contained (g/dm^3): ammonium nitrate, 2.06; mono potassium phosphate, 0.55; $MgSO_4.7H_2O$, 0.25; sucrose, 50. The basal medium was made up with distilled water. The medium was swirled while still hot and then allowed to stand overnight .This medium was then adjusted to pH 5 using either NaOH or HCL and distributed into 250 ml conical flasks. The media in all the flasks were autoclaved at 121^{o} C, 15 lbs, for 20 min. For the transfer of fungal cultures, spore inoculum was prepared by taking a loops full of spores from the slants of 5 day mother culture using inoculating loop in sterilized conditions. Tween80 acts as surfactant. In each conical flask, 10 ml of spore inoculums was added .Then these flasks were incubated in orbital shaker (100 rpm) at 28^{o} C for 4 days.

Preparation of the Microorganism for Biosorption

After 4–5 days of growth, the harvested cells were washed with generous amounts of deionised distilled water till the pH of the wash solution was in the near neutral range. Then, it was dried at 60^{0}C for 24 h before use. Ten grams of dried microorganism was suspended in 100 dm−3 deionised distilled water and homogenized for 20 min in a homogenizer at 8000 rpm for 20 min and then stored in the refrigerator at 4^{0}C.

Incubation of Fungal Biomass with Metal Ion

A microorganism suspension of 10 cm^3 was mixed with 90 cm^3 of solution containing a known concentration of metal ions in 250 cm^3. Erlenmeyer flasks at desired temperature and pH for evaluating their influence on metal adsorption. All the final solutions contained a fixed concentration of bio sorbent (1.0 g dm^{-3}) the pH was adjusted to the desired value. The flasks were agitated on a shaker at a 100 rpm constant shaking rate for 7 days.

Metal Determination

Samples of 5 cm^3 were taken at predetermined time intervals for the residual metal ion concentrations in the solution. Before analysis the samples were centrifuged at 4000 rpm for 3

min and the supernatant fraction was analyzed for the remaining metal ions. All experiments were carried out at least twice. Values used in the calculations were the arithmetic averages of the experimental data. The residual Cu(II) ions in the biosorption media were determined by using an atomic adsorption spectrophotometer (UNICAM 929) and Inductively coupled plasma mass spectrometry (AGILENT). Thus the sample is collected and digested using Conc. HNO_3 for metal concentrations in AAS and ICPMS. The amount of metal taken up by the biomass was calculated as the difference between the initial and final concentration of the metal in the aqueous solution.

RESULTS AND DISCUSSION

Optimization of Fermentation Process

Experiments were carried out by *A.niger* NCIM 616 with heavy metal such as copper sulphate for maximizing the reduction of metal concentration by optimizing the process parameters under submerged state fermentation. Optimization was done on "one parameter a time basis" i.e., by changing one independent variable while fixing the others at a certain constant level. The optimum conditions obtained in each parameter was applied to the subsequent experiments. All the experiments were conducted in triplicate and the mean values are reported.

Effect of pH on Biosorption

The most important parameter influencing the sorption capacity is the pH of adsorption medium. The initial pH of adsorption medium is related to the adsorption mechanisms onto the adsorbent surface and reflects the nature of the physicochemical interaction of the metal in solution and the adsorptive sites of adsorbent. The impact of the solution pH on the metal biosorption was investigated in the biomass *A.niger* NCIM 616. Since pH is one of the main variables affecting the biosorption process [13], the optimum pH value for the uptake of metals was determined. Six different pH tests, chosen within the solubility range of the metals used, were carried out. Growth conditions of this species were optimized at various pH (3-7).The pH of the culture broth dropped significantly as compared to the control, where it remained constant at 7. The Copper ions maximum adsorption of 8.67 mg/g (AAS) & 9.18 mg/g (ICPMS) was seen at pH 5.

Adsorption of copper ion is over the pH range 5, pH-related effects were significant (Fig.1). Meanwhile, at the pH values of 3, the adsorption values started to decrease. At low pH, protons would compete for active binding sites with metal ions. The protonation of active sites thus tends to decrease the metal sorption. At a low pH, of almost 2 & 3, all the binding sites may be protonated, thereby desorbing all originally bound metals from the biomass [14 & 15]. An additional possible explanation why sorption increases with increasing pH is that the solubility of many metals in solution decreases with increasing pH. A further possible explanation of increasing sorption with increasing pH is that hydrolyzed species have a lower degree of hydration, i.e. less energy is necessary for removal or re-orientation of the hydrated

ISSN: 0975-8585

water molecules upon binding [16]. At a further increase of pH (6–7) the solubility of metals decreases enough for precipitation to occur. This should be avoided during sorption experiments as distinguishing between sorption and precipitation metal removal becomes difficult [16].

Kapoor and Viraraghavan, (1997) [17], reported that amine and carboxyl groups are important functional groups involved in biosorption of heavy metals by *A. Niger* NCIM 616 and biosorption of heavy metals was severely inhibited when these groups were modified. At highly acidic pH, the overall surface charge on the cells became positive and metal cations and protons compete for binding sites on cell wall, which results in lower uptake of metal. It has been suggested that at low pH values, cell wall ligands would be closely associated with H_3O^+ that restrict access to ligands by metal ions as a result of repulsive forces. At pH values above the isoelectric point, there is a net negative charge on the cell surface and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such that so as to promote reaction with metal ions, hence the rapid binding efficiency was obtained. At various pH, the following adsorbance of copper was observed under AAS & ICPMS.

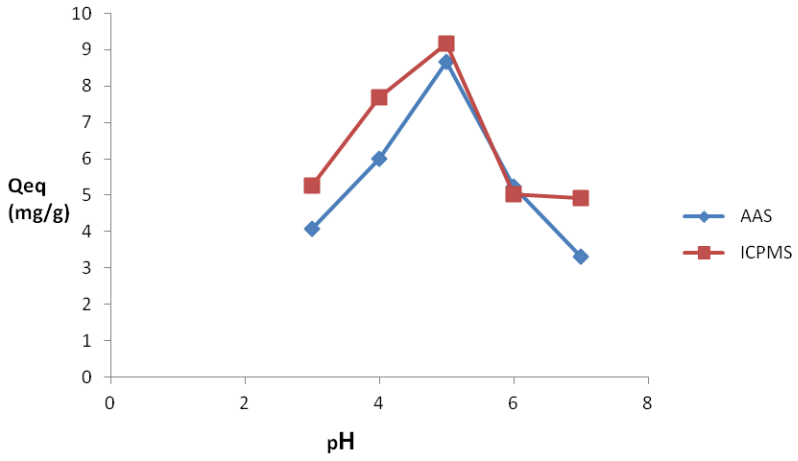

Figure 1. Effect of pH on biosorption of Cu(II) by *A.niger* NCIM 616 using AAS & ICPMS

Effect of Temperature on Biosorption

Removal of metal ions- Cu(II) by the biomass is carried out experimentally at different temperatures ranging from 20 to 35 ^{o}C are shown in Fig. 2. At low temperatures, the binding of Cu(II) ion to *A.niger* NCIM 616 was by passive uptake. Maximum initial adsorption rate of Cu(II) ion was obtained at temperature 35^{o}C. A decrease in reduction of metal concentration was observed when the incubation temperature was higher or lower than the observed optimum incubation temperature. At 35^{0}C, maximum amount of 11.34 mg/g(AAS) & 12.36 mg/g(ICPMS) of Cu(II) was adsorbed by dried microorganism. Maximum amount of adsorption was reported at 35^{0}C [18]. It was shown that the removal of copper metal ion increased with increasing temperature up to 35^{0}C.

ISSN: 0975-8585

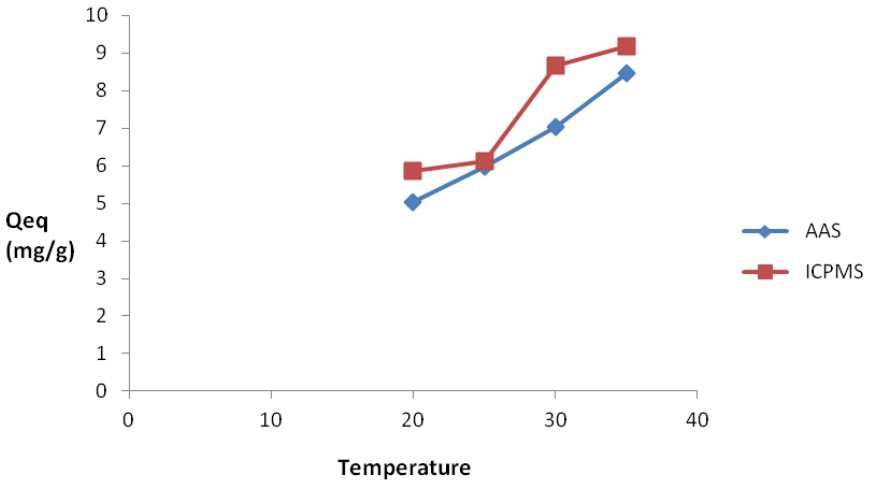

Figure 2. Effect of temperature on biosorption of Cu(II) by *A.niger* NCIM 616 using AAS & ICPMS

Effect of Time

To determine the optimal biosorption time, samples were periodically taken at one day interval. The results are presented in Fig 3. The results indicate that from 1-3hrs, there was very less reduction in the metal concentration. The rate of reduction of metal concentration increased by 2 fold on 4thhr, increased gradually and attained high reduction (mg/g) after 6hrs and continued upto 8 hrs of incubation with *A.niger* NCIM 616. Thereafter, further increase in time increased the metal concentration.

The decrease in reduction of metal concentration after an optimum incubation was probably owing to a reduced growth rate from fast depletion of nutrients available to the organism, and also could be owing to the production of secondary metabolites resulting in lower enzyme activity. Fig.3 shows the biosorption kinetics of Cu(II) ion removal at various intervals of time at previously optimized conditions i.e., at pH 4 & 5 at 35 ^{o}C by plotting the metal ion uptake capacity (*q*) versus time. The biosorption capacity increased with increasing contact time and a larger amount of metal ions were adsorbed by 8 hrs of contact time. Equilibrium was established after 8 hrs of contact time. After an equilibrium was established, no more Cu(II) is adsorbed.

ISSN: 0975-8585

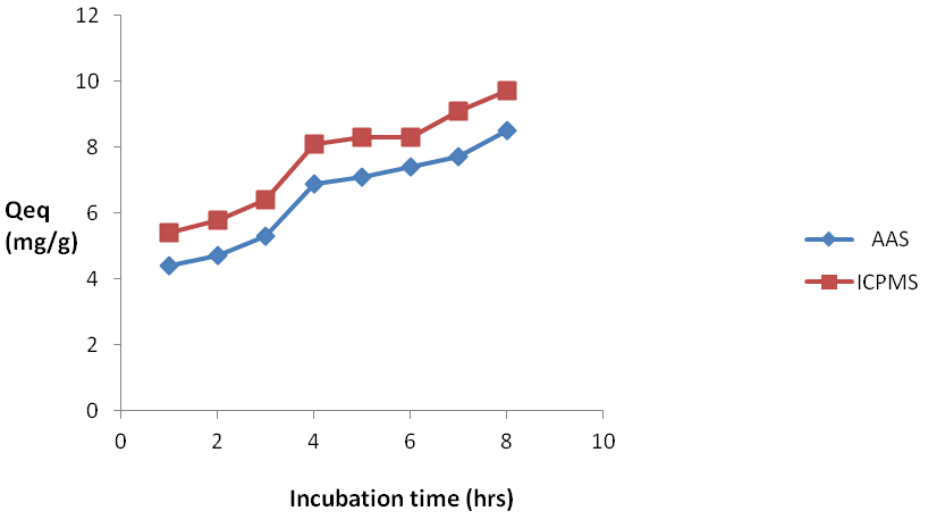

Figure 3. Effect of incubation time on biosorption of Cu(II) by *A.niger* NCIM 616 using AAS & ICPMS

Effect of Initial Metal Ion Concentration on Cu(II) Biosorption

The effect of initial Cu(II)concentration on the sorption capacity of biomass was investigated at different temperatures, pH 5 for Cu(II).As a rule, increasing the initial metal concentration results in an increase in the biosorption capacity because the initial metal concentration provides a driving force to overcome mass transfer resistances between the biosorbent and biosorption medium. So higher sorption capacities were obtained at higher initial concentrations for the metal ions at all temperatures studied. Increasing the metal ion concentration generally caused a decrease in the biosorption yield and maximum Cu(II)biosorption yield is determined as 48.37 % an initial concentration of 20 mg /dm^3 at 30^0C. In the case of lower concentrations, the ratio of initial number of metal ions to the available sorption sites was low and higher biosorption yields were obtained. At higher concentrations, the available sites of biosorption became fewer and the saturation of the sorption sites was observed. So biosorption yields decreased.

Table 1. Effect of initial Cu(II) concentration on the sorption capacity at equilibrium and adsorption yields of the biomass at different temperatures (*X*: 1.0 g dm–3, pH 5 and agitation rate: 100 rpm)

C_0 ($_{mg/dm^3}$)	T= 20°C		T= 25°C		T= 30°C		T= 35°C	
	qeq(mg/g)	% AD	qeq(mg/g)	% AD	qeq(mg/g)	% AD	qeq(mg/g)	% AD
20	3.16	15.8	5.09	25.45	7.12	35.6	9.67	48.37
40	5.28	13.2	7.35	18.37	12.06	30.15	13.78	34.45
60	6.92	11.53	9.08	15.13	15.81	26.35	15.12	25.27
80	9.17	11.46	11.61	14.51	19.18	23.97	20.03	25.03
100	11.89	11.89	17.63	17.63	20.02	20.02	21.46	21.46

ISSN: 0975-8585

CONCLUSION

The ability of *A.niger* NCIM 616 to adsorb Cu(II) is investigated in a batch system. It was seen that pH, temperature and initial metal ion concentration highly affected the biosorption capacity of the sorbent. The studies indicate that *A.niger* NCIM 616 is an effective biosorbent for Cu(II)removal. The maximum Cu(II) biosorption capacity has been found to be 48.37% Cu(II) of dry weight of biomass at an fungal dose of 20 mg/dm^3 in of contact time 8 days and optimum pH of 5.0 at temp 35^0 C. This shows that *A.niger* NCIM 616 has greater adsorbing capacity for Cu(II). Accurate results of biosorption of metals were obtained in ICPMS. Consequently, fungal biosorption technologies are still being developed and much more work is required. Some practical applications have been achieved, and the fundamentals look promising: fungi have the potential to remove metal ions to very low concentrations and to accumulate large amounts of specific toxic elements. Very little comparative or comparable information, especially economical analysis, are available.

REFERENCES

[1] Dnmez G, Aksu Z. Water Res 2011; 35 (6): 1425–1430.
[2] Patterson JW, Ann Arbor, MI, USA, 1977.
[3] Kim YH, Yo YJ, Lee HY. Biotechnol Lett 1995; 17 (3): 345–350.
[4] Friis N, Myers-Keith P. Biotechnol Bioeng 1986; 28: 21–28.
[5] Norberg AB, Persson H. Biotechnol Bioeng 1984; 26: 239–246.
[6] Ozçayan G, Ozen C, Sag Y, Kutsal T, Caglar A. Istanbul, Turkey; 1996: 1033.
[7] Nourbakhsh MN, Sag Y, Ozer.D, Aksu Z, Kutsal T, Çaglar A. Process Biochem 1994; 29:1-5.
[8] Aksu Z, Ozer D, Ekiz H, Kutsal T, Caglar A. Environ Technol 1996; 17 (2): 215-220.
[9] PK, CM So. Microbiology 1993; 73: 113–121.
[10] Gutrick DL, Bach H. Appl Microbiol Biotechnol 2001; 54 (4): 451-460.
[11] Gadd GM, White C.Trends Biotechnol 1993; 11 (8): 353-359.
[12] Sherma DC, Foroter CF. Water Res 1993; 27 (7): 1201-1208.
[13] Lezcano JM, Gonzalez F, Perez I, Blazquez ML, Munoz JA, Ballester A, Hammaini A. Fundamentals, Technology and Sustainable Development. 2001; Part B: 217-226.
[14] Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H. Current Sci 2001; 78: 967-973
[15] Aldor I, Fourest E, Volesky B. J Chem Eng 1995; 73: 516-522.
[16] Schiewer S, Volesky B. J Environ Sci Technol 1995; 29: 3049-3058.
[17] Kapoor T. Viraraghavan. J Bioresour Technol 1997; 61: 221-227.
[18] Dursun AY, Uslu G, Cuci Y, Aksu Z. J Biochem Eng 2003; 38: 1647-1651.