IOSR Journal of Pharmacy
e-ISSN: 2250-3013, **p**-ISSN: 2319-4219, www.iosrphr.org
Vol. 2, Issue 6, Nov-Dec. 2012, PP. 60-64

A Comparative Study on Determination of Physico-Chemical parameters of Biosorption of Lead (II) by *Aspergillus niger* , NCIM 616 using AAS & ICPMS

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Abstract:- **Microorganisms can be important biosorbents for heavy metal remediation of contaminated soils and wastewaters.With different types and concentrations of heavy metals, strains display different resistance and removal abilities to the heavy metals. In the present study, biosorption of Pb(II) was carried out using Aspergillus niger NCIM 616 and also studied the effect of paramaters such as pH, temperature, incubation time, inial substrate concentration using AAS and ICPMS which were influenced more at the removal of Pb(II) and the comparision between the two instruments was also studied. The studies indicate that A.niger NCIM 616 is an effective biosorbent for Pb(II) removal. The maximum Pb (II) biosorption capacity has been found to be 60.7% Pb(II) of dry weight of biomass at an fungal dose of 20 mg/ dm^3 in contact time 8 days and optimum pH of 4.0 at temp 35^0 C. Accurate results of biosorption of pb(11) was obtained in ICPMS compared to AAS.**

Keywords*:- A. Niger NCIM 616, biosorption, fungal biomass, incubation time, Pb(II), pH , temperature.*

I. INTRODUCTION

1.1. Environmental pollution

Environment pollution is the undesirable change in physical, chemical and biological characteristics of air, land and water due to introduction of contaminants into natural environment. Heavy metals occur in immobilized form in sediments and as ores in nature. Waste waters from the industries include metal ions such as Pb, Cd, As, Hg have permanent effect on the environment, a major concern, because of their toxicity, non-biodegradable nature and threat to human, animal and plant life. Effect of lead on human health are major sources from Paint, pesticide, smoking, automobile emission, mining, burning of coal, manufacture and application of alkyl lead fuel additives. Some of the effects that are caused by lead on human healths like Liver, kidney, gastro intestinal damage, mental retardation in children, premature birth, anemia in both adult and children, inhibit biosynthesis of heme. Source of metal like lead is present in petrol-based materials and many other industrial facilities. Lead is a ubiquitous toxic metal which have mutagenic, carcinogenic, genotoxic, anthropogenic and phototoxic effects [1]. Environmental contamination by toxic metals is a serious problem world wide due to their incremental accumulation in the food chain and continued persistence in the ecosystem. Biotechnology has been investigated as an alternative method for treating the metal containing waste water of low concentrations. In response to heavy metals, microorganisms have evolved various measures via processes such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation- reduction reactions. It has been proved that they are capable of adsorption of heavy metals from aqueous solutions, especially for the metal concentration below 50 mg/l [2]. The metal-binding capacities of several biological materials have been identified to be very high, including marine algae, fungi and yeasts. It was reported that these microorganisms can accumulate a wide range of metal species.

In this paper the use of *A.niger* NCIM 616 as a biosorbent for biosorption from synthetic waste waters was studied and also determined physico-chemical parameters of biosorption of Pb(II) using AAS(Atomic Adsorption Spectrophotometer) & ICPMS(Inductively Coupled Plasma Mass Spectrometry).

II. MATERIALS AND METHODS

2.1. Microorganism

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.

2.2. Maintenance of Culture

The experiments decribed 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.

2.3. Spore Suspension /Inoculum

10ml 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.

2.4. 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, 15lbs, for 20 min. For the transfer of fungal cultures, spore inoculum was prepared by taking a loopsfull of spores from the slants of 5 day mother culture using inoculating loop in sterilized conditions. Tween 80 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.

2.5. 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 60^0C 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 4^0C.

2.6. 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.

2.7. 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 Pb(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.

III. RESULTS AND DISCUSSION

3.1. Optimization of fermentation process

Experiments were carried out by *A. niger* NCIM 616 with heavy metal such as Lead nitrate 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.

3.1.1. 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 [3], 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. At pH higher than 4.0, lead ions predicated and thus maximum adsorption of 11.07 mg/g (AAS) & 12.37 mg/g (ICPMS) was seen only at pH 4.

Adsorption of lead ion is over the pH range 4, 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 [4 & 5]. 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 water molecules upon binding [6]. 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 [6].

Kapoor and Viraraghavan, (1997) [7] 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 lead is observed under AAS & ICPMS.

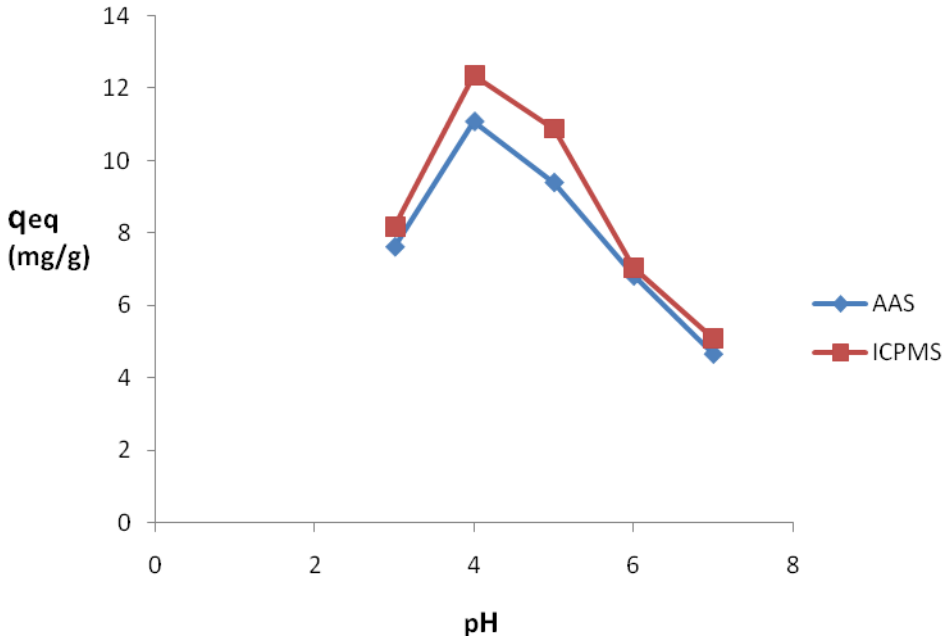

Fig 1. Effect of pH on biosorption of Pb(11)

3.1.2. Effect of Temperature on Biosorption

Removal of metal ions- Pb(II) by the biomass is carried out experimentally at different temperatures ranging from 20 to 35 ^oC are shown in Fig.2. At low temperatures, the binding of Pb(II) ion to *A. niger* NCIM 616 was by passive uptake. Maximum initial adsorption rate of Pb(II) ion was obtained at temperature 35^oC. A decrease in reduction of metal concentration was observed when the incubation temperature was higher or lower than the observed optimum incubation temperature. At 35^0C, maximum amount of 11.34 mg/g(AAS) & 12.36 mg/g(ICPMS) of Pb(II) is adsorbed by dried microorganism. This adsorption data is further fitted to two adsorption models to find out the suitable model. [4] Maximum amount of adsorption was reported at 35^0c. [8] It was shown that the removal of lead metal ion increased with increasing temperature up to 35^0C.

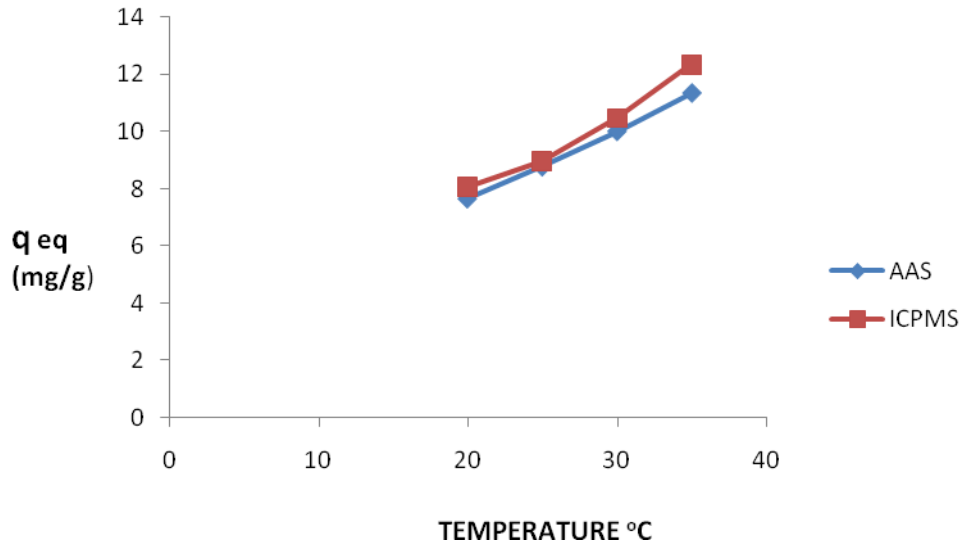

Fig 2. Effect of temperature on biosorption of Pb(11)

3.1.3. Effect of incubation 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. There after, 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 Pb(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 Pb(II) was adsorbed.

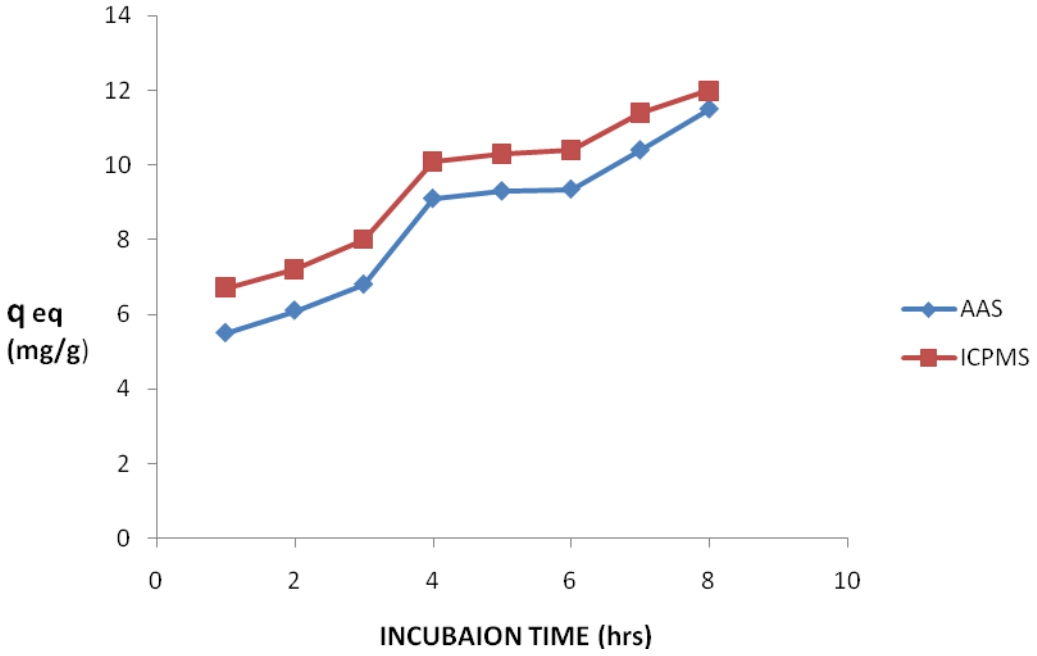

Fig 3. Effect of incubation time on biosorption of Pb (11)

3.1.4. Effect of initial metal ion concentration on Pb(II) biosorption

The effect of initial Pb(II) concentration on the sorption capacity of biomass was investigated at different temperatures, pH 4 for Pb(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 Pb(II) biosorption yield is determined as 60.15 % an initial concentration of 20 mg /dm^3 at 35^0C. 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 (Table.1). So biosorption yields decreased.

Table 1. Effect of initial Pb(II) concentration on the sorption capacity at equilibrium and adsorption yields of the biomass at different temperatures (*X*: 1.0 g dm−3, pH 4 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	5.2	26	6.03	30.15	8.01	40.05	12.03	60.15
40	8.08	20.2	9.07	24.25	13.48	33.7	16.41	41.02
60	11.1	18.5	13.28	22.13	18.03	30.05	19.06	31.76
80	13.04	16.3	17.35	21.68	21.62	27.05	22.36	27.95
100	17.8	17.8	19.21	19.21	23.05	23.05	25.72	25.72

IV. CONCLUSION

The ability of *A. niger* NCIM 616 to adsorb Pb (II**)** was 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 Pb(II) removal. The maximum Pb (II) biosorption capacity has been found to be 60.7% Pb(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 4.0 at temp 35^0 C. This shows that *A. niger* NCIM 616 has greater adsorbing capacity for Pb(II). Accurate results of biosorption of matlas 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.

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