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Optimization of operating conditions for biosorption of chromium by *Aspergillus niger* isolated from contaminated soil

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Abstract

In recent years, the fungal biomass is used as a biosorbent for the removal of hazardous pollutants and heavy metal ions from various industrial effluents has gotten a lot of attention. The metal tolerant fungal isolates were isolated from the selected polluted sites and screened for their tolerance indices and minimum inhibitory concentration (MIC). The fungal isolate possessing highest tolerance and MIC was identified as Aspergillus niger was further used as a biosorbent to remove hexavalent chromium ions Cr(VI) in this investigation. From the present study, pH 6.0, temperature of 35°C and contact time of 120 minute showed highest removal of 78.8 percent from the aqueous metal solution containing chromium concentration of 150mg/L. The current study discovered that fungus from metal-polluted locations have better metal tolerance and biosorption effectiveness, and hence might be used for heavy metal biosorption.

Keywords: Biosorption, Heavy metal, Chromium, Biosorbent, Tolerance.

Introduction

Heavy metal pollution poses a serious hazard to both aquatic and terrestrial ecosystems. As a result, scientists are focusing their efforts on recovering heavy metals from watery media. Metal decontamination, on the other hand, is a difficult undertaking because none of the extraction processes are both cost-effective and ecologically friendly[1]. Chromium is a dangerous heavy metal that has become a serious health hazard among the various heavy metals (Cu, Ni, Zn, Pb, Hg, Cd, and Cr)[2]. One of the most prominent sources is the hexavalent form which is responsible for its toxic, carcinogenic, and mutagenic effects on people and other living things, and has been reported for its nephrotoxic malignant neoplastic illness[3,4]. Biosorption, a biological method of metal removal from aqueous solution, has been advocated as a less expensive and more effective procedure. The metal binding capacities of diverse biological materials, such as algae, fungi, and bacteria, are used in this process. It is envisaged that soil receiving long-term application of hazardous metal-containing wastewater/industrial effluents will produce selection pressure on soil fungus, resulting in higher metal tolerance and metal adsorption capability. The use of fungi as a source of biomass could be a cost-effective way to remove harmful metals. Previous studies show that different fungi are able to remove metal viz. *Aspergillus niger*[5], fungal biomass of *Mucor racemosus*[6] and industrial fungus *Rhizopus cohni*[7]. Studies found that the endophytic fungi viz., *A. fumigates, Rhizopus sp., Penicillium radicum* and *Fusarium proliferatum* pull out chromium from polluted water and soil and convert it to its trivalent form[8]. In the present study, heavy metal tolerant fungal species was isolated and biosorption was maximized by optimizing the process parameters. The biosorption potential of fungal isolates could be used for decontamination of polluted wastewaters and effluents.

Material and methods

Isolation of fungi and preparation of biomass: Metal tolerant fungal species were isolated from the composite soil samples collected from the nearby regions of Union carbide India Ltd. (UCIL), Bhopal. The samples were collected from different areas located in nearby area and outside the UCIL factory site and mixed thoroughly so that fungal cells could be uniformly distributed. To isolate the fungal strains, serial dilution method was used and fungal isolates were isolated on metal supplemented potato dextrose agar (PDA). The isolated fungal strains were screened for highest tolerance level against chromium metal ions and minimum inhibitory concentration. Tolerance index of isolated fungal strains was determined by comparing the growth of fungal isolates on control and metal supplemented PDA plates. The MIC was determined and the species showed higher MIC was further selected for biosorption studies.

On the basis of screening studies *Aspergillus niger* was selected for further biosorption studies. The selected fungal isolate was further allowed to grow under optimum growth conditions. The pure culture of *Aspergillus niger* was inoculated in potato dextrose broth and allowed to incubate for seven days at 30°C and pH 6.0. The fungal mycelium grown in the filamentous form was harvested by filtration through 150µm sieve and collected. The collected biomass was further washed with

distilled water several times and attached media particles were removed. After washing the biomass was used immediately for biosorption studies.

Optimization of process parameters affecting biosorption of Cr(VI) metal ion: The operating conditions were optimized for achieving maximized biosorption of Cr(VI) from aqueous metal solution by using live biomass of *A. niger*. The main operating conditions that were affecting the biosorption process were pH, temperature, contact time and initial metal ion concentration. All these parameters were optimized by performing biosorption experiments by varying individual parameters and keeping the other constant.

Optimization of pH: The metal solutions of 100mg/L were prepared and 150 ml of metal solution was transferred to 250ml capacity Erlenmeyer flasks. The pH of each flask was maintained in the range of 3.0 to 10.0. To the metal solution with specific pH, 1g of live biomass was added and shaken at 120rpm at 30 °C for 60 minutes. After 60 minutes the biomass was removed and residual metal was determined in aqueous solution.

Optimization of temperature: In an Erlenmeyer flask of 250 ml capacity, one gram of live biomass was added to 150ml of 100mg/L aqueous metal solution and pH was maintained to 5.0. Each flask was kept under different temperature viz., 25, 30, 35, 40 and 45°C. Flasks were allowed to shake at the speed of 120 rpm for 60 minutes. The residual metal ion concentration was determined in aqueous metal solution.

Optimization of Initial metal ion concentration: The initial concentration of metal ion was optimized by inoculating one gram of live biomass in aqueous solution of varied concentration of metal ions. The concentration of Cr(VI) was maintained in the range of 50ppm to 250ppm. The pH of all the metal solutions was maintained to 5.0 and kept at 35°C for biosorption process for 60 minutes. Afterwards, the residual concentration of metal ion was determined in aqueous solution.

Optimization of contact time: One gram of live biomass were inoculated in batch of metal ion solution containing 100mg/L of Cr(VI) metal ion. The samples were withdrawn at specific intervals viz. 30, 60, 90, 120, 150, 180 and 210 minutes. The residual metal ion concentration was determined after specific time interval to assess the biosorption potential.

Results and discussion

Fungal colonies were grown on all plates containing 10mg/L of Cr(VI) metal ion concentration and inoculated with different dilutions prepared from soil suspension. Total of eleven fungal isolates were obtained and they were screened for their tolerance against higher levels of Cr(VI) metal ion concentration. Out of eleven fungal isolates, five fungal isolates had shown the tolerance index higher than that of 0.75. Further, these fungal isolates were screened for the minimum inhibitory concentration. The highest MIC of 400ppm was obtained for the fungal isolate FI-03. The morphological and microscopic examination of the selected fungal isolate was conducted to identify the fungal species. Fungal colonies are of olive color changing to brown color producing black conidia at the centre and white mycelia towards edge. Microscopically, hyphae were septate and conidiophores were long and smooth terminating in a globose vesicle. Vesicle was entirely covered by metulae and phialides. Conidia of brown to black originated which are rough and globose. Based on the morphological and microscopic characteristic, FI-03 was identified as *Aspergillus niger*.

pH optimization: Live biomass was subjected to biosorption process under different pH conditions and the pH value was selected at which highest removal of metal ion was observed. The pH of the solution affects the speciation of metal ions, it also influences the surface properties of the sorbent[9].

The highest biosorption of 56 percent was observed at pH 6.0. The lowest biosorption was observed at acidic and alkaline pH conditions as evidenced from Figure-1.

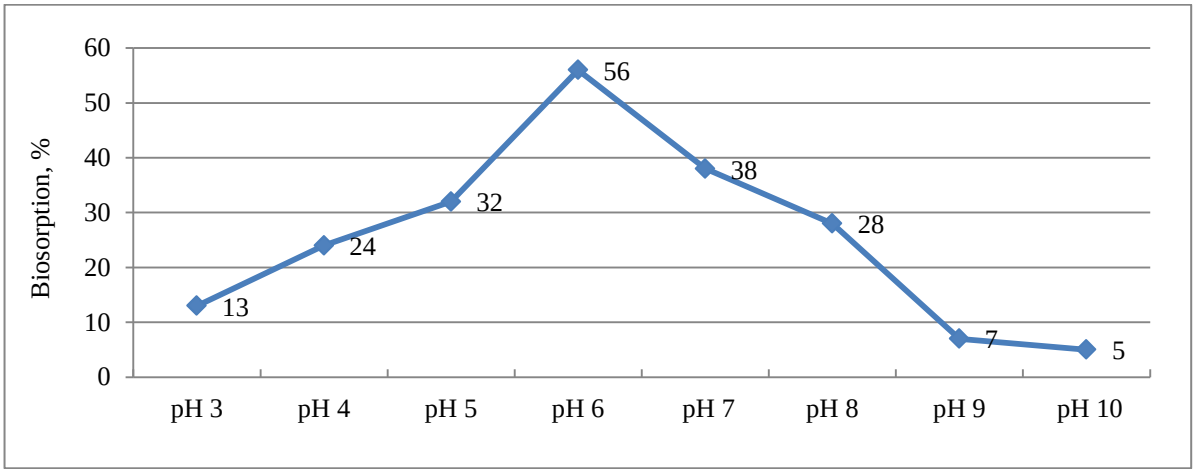

Figure-1: Percentage biosorption of Cr(VI) metal ion at different pH conditions.

At pH values greater than pKa, the functional groups gets deprotonated which leads to the binding of reduced Cr(VI) ions while functional groups protonated at pH values less than pKa do not facilitate the binding of metal ions. The increase in pH was observed when Cr(VI) is reduced, since reduction of chromium (CrVI) process consumes proton[10]. Indeed, the chromium biosorption is a favored process as the carboxyl group and amino group present on the surface of the fungal biomass electro-statically attracts chromium anions, which gets protonated under slightly acidic pH conditions[11].

Temperature Optimization: Temperature influences the stability of cell wall composition and configuration, thus affecting the biosorption process[12]. Biosorption process was found to be increased as the temperature rises from 25°C to 35°C. The maximum biosorption of 62.8% was observed at the temperature of 35°C (Figure-2). As the temperature is increased from 35°C, the biosorption of metal ions get decreased because at higher temperatures the enzymatic activity and integrity of the cell wall may be affected which in turn reduces the uptake of metal ion. Tahir *et al*. suggested that if biomass is exposed to temperatures higher than 30°C the number of active sites may increase[13].

Optimization of Initial metal ion concentration: The initial concentration of metal ions imparts a driving force which controls the mass transfer resistance of metal ions between the solid and liquid phases[14]. The highest biosorption of 71.13mg/g of metal ion was observed at 150mg/L initial metal ion concentration (Figure-3). The uptake of metal ion remains constant or varied slightly when initial concentration was increased from 150mg/L to 250mg/L.

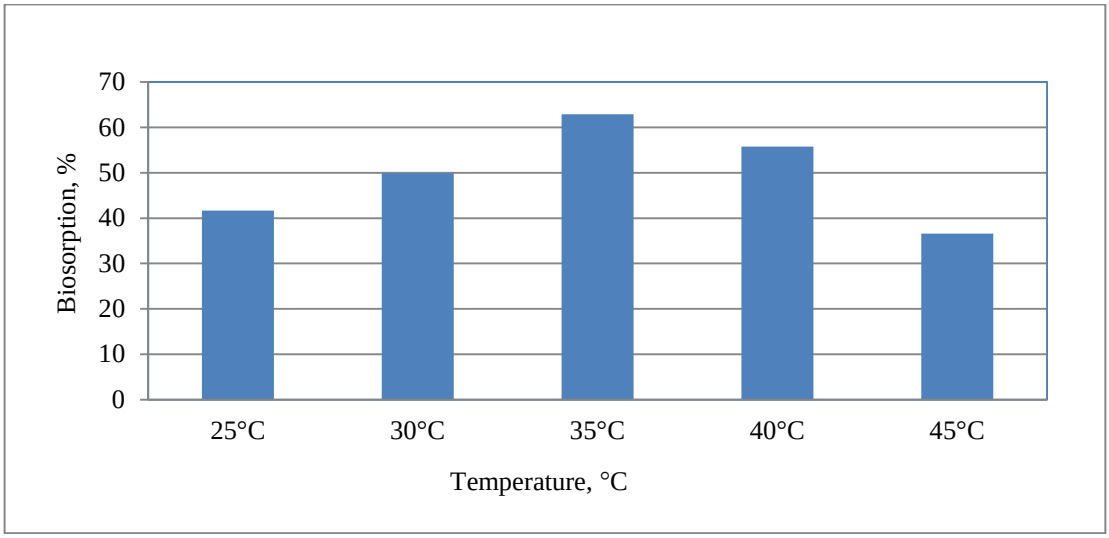

Figure-2: Percentage biosorption of Cr(VI) metal ion under different temperature.

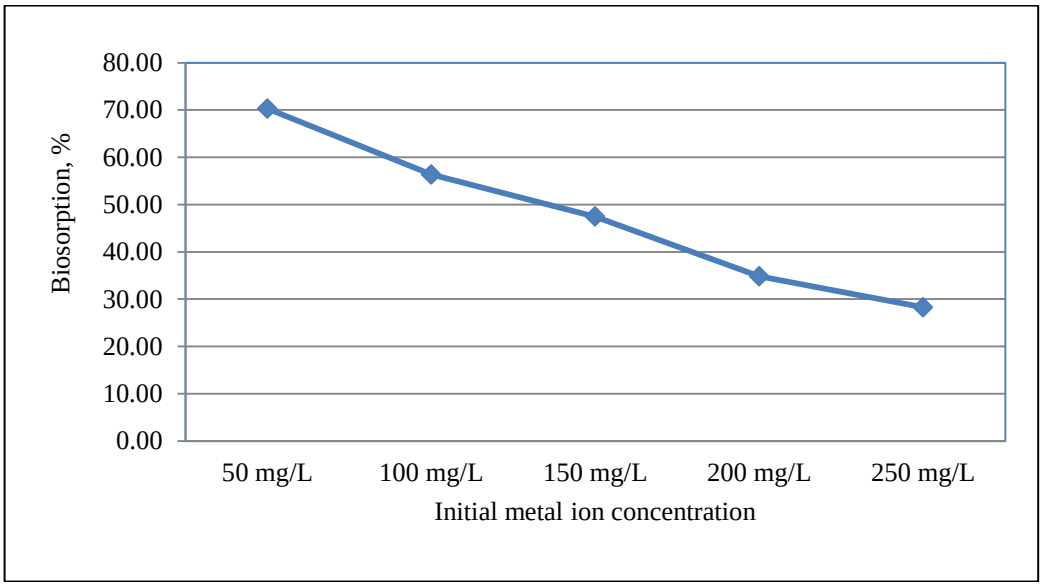

Figure-3: Percentage biosorption of Cr(VI) at different initial concentration of chromium metal ion.

The uptake of metal ion by per unit mass of live biomass was found to be increases as the initial concentration of chromium was increased in the aqueous solution. This may occur due to higher probability of collisions between chromium metal ions and the live biomass of *A. niger*. At the same time, at higher concentrations more amount of chromium Cr(VI) ions are not sorbed and left in metal solution because all the binding sites get saturated[11]. Jobby *et al*., also found the similar trend of decrease in percentage removal as the metal ion concentration increases from 100mg/L to 500mg/L but the uptake capacity of *Sinorhizobium sp. SAR1* was increased from 6.27mg/g to 28.95mg/g[15].

Optimization of contact time: Removal of metal ion was observed to be increases as the contact time was increasing and remains constant after equilibrium was achieved. The percentage biosorption increases from 28.87% to 73% when contact time was increased from 30 minutes to 120 minutes (Figure-4). Further the percentage biosorption remains more or less constant as the contact time increases from 120 minutes to 210 minutes. Due to the availability of active sites on the surface of biomass of *A. niger*, the higher biosorption rate was observed for initial stages. The process of biosorption is fastened if equilibrium is established within few hours[16]. When the maximum biosorption capacity of biosorbent reaches under certain conditions, the binding sites may become fully occupied and further increase in contact time would not affect the rate of biosorption process[17].

Batch Biosorption of Chromium metal ion: After optimization of all the process parameters, batch studies were conducted to assess the removal of metal ions under all the optimized conditions. The obtained live biomass of 1g was inoculated into 150ml metal solution containing 150mg/L of chromium metal ion. The operating conditions were pH of 6.0 and temperature of 35°C. After completion of contact time of 120 minutes, the residual concentration of chromium ion was assessed. The process results into removal of chromium metal ion in the range of 74.21 percent to 78.78 percent. The process was conducted in replicates.

Conclusion

Heavy metal pollution in different ecosystems impose a greater threat to living systems than heavy metal itself. The ability of fungal resistance to heavy metal provides a useful tool for heavy metal monitoring and bioremediation in the environment. The biosorption technique is a microorganism-based technology for removing chromium from the aquatic environment that is cost-effective, safe, and simple to use, and it has a lot of potential for future uses[18]. In the present study, the chromium biosorption ability of *Aspergillus niger* isolated from local polluted sites was assessed. It was observed that 10mg/ml of adsorbent was found to be optimum for sorption of 150mg/L of chromium with the initial pH of 6.0 at 35°C during 120 minutes of contact time. The findings suggest that the fungal species present in the polluted sites develops the tolerance against the pollutants and these species can be screened for removal or bioremediation of the pollutants.

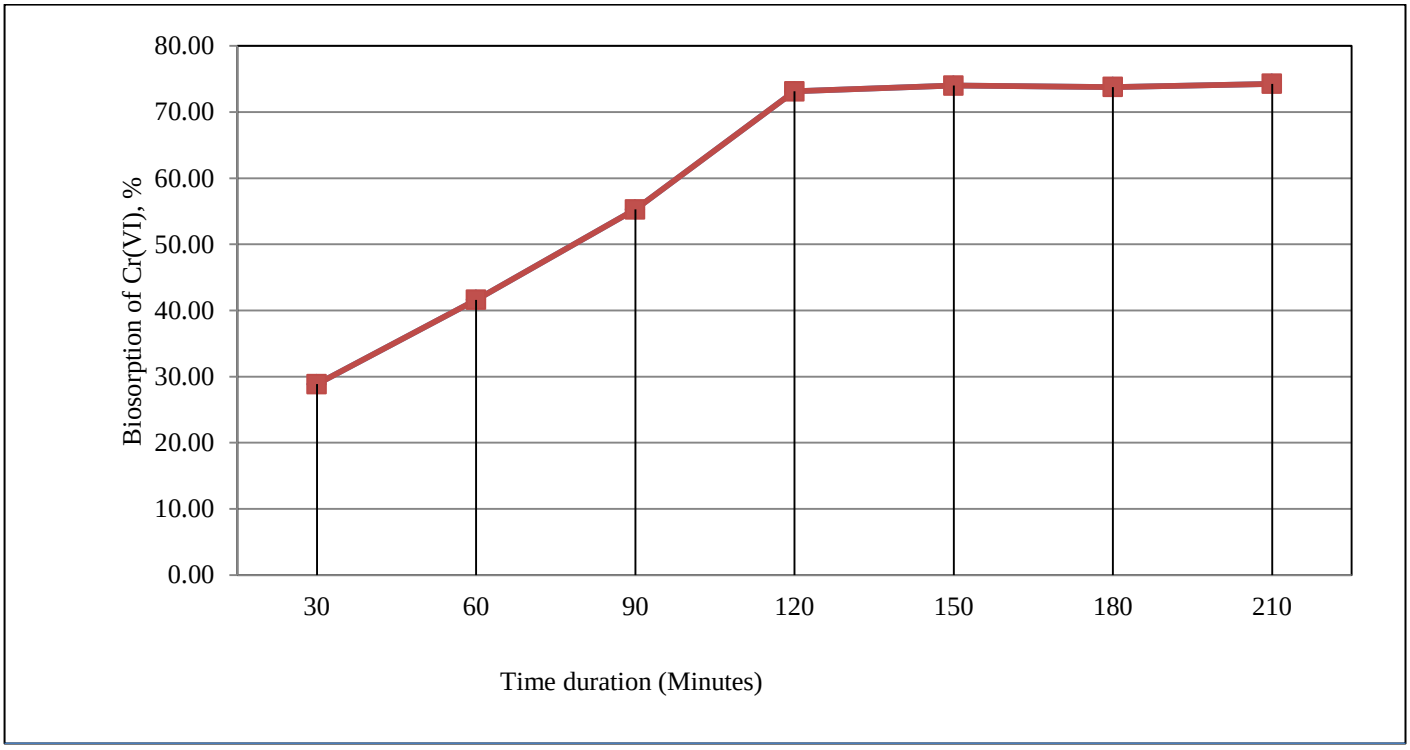

Figure-4: Effect of contact time on biosorption of chromium metal ion onto the biosorbent.

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