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Biosorption of hexavalent chromium (VI) by *Aspergillus nomius* biomass and optimization of biosorption parameters

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Abstract
In this study, the hexavalent chromium biosorption capacities of a chromium tolerant fungal strain isolated from tannery effluent (Bantala Leather Complex, Kolkata, India) and having 99.82% similarity with *Aspergillus nomius,* was studied. Here we discuss, the removal of chromium (VI) by *Aspergillus nomius* biomass and scrutinize the practicality of exploiting them for bioremediation purposes. Therefore, to optimize biosorption, we tested biosorption potentials for the different parameters like temperature, ionic strength, pH, initial metal concentration, pre-treatment agents in varying concentrations, volume, exposure time, Amount of biomass, a combination of both biomass and volume. Biosorption studies were performed and the effectiveness of Cr (VI) biosorption was compared for each parameter. It was observed that biosorption was maximum (approximately): 95% when experimented with an initial Cr (VI) concentration of 1mg/L; 82% when sulfuric acid was used as a pretreatment agent; 98% at pH 6 in aqueous solution; 96% at a temperature of 25^0C; 96% when 1N sulfuric acid was used; 95% for 250ml solution; 98% when the exposure time was 24 hours and 97% when 1g of fungal biomass was used in 200ml of solution.

Keywords: biomass, biosorption, fungi, heavy metal, hexavalent chromium

Introduction

Rapid industrialization particularly in developing countries is leading to serious environmental pollution due to the discharge of a wide variety of toxic chemicals without proper treatment. Heavy metals such as Cr, Pb, Hg, Cu, Cd, as etc. are extensively used in a wide array of industrial processes and are considered the worst group of pollutants of the environment. According to WHO long-term exposure to chromium at levels exceeding the standard limit may lead to serious health issues like epigastric pain, nausea, vomiting, severe diarrhea, skin irritation and even cancer because chromium is mutagenic as well as carcinogenic.[1,3] More than 170,000 tonnes of Cr wastes are released into the environment annually as a waste product from various industries like steel, leather, electroplating, water cooling, electric, pigments, paint, battery, and many more.[2,3] About 80% of the tanneries in India use chrome tanning during which huge quantities of chromium-containing wastewater (usually beyond the maximum permissible limits) is generated and it is discharged into the environment causing serious ecological consequences.[4]

The conventional methods for removal of chromium from wastewater like ion exchange, chemical precipitation, lime coagulation, solvent extraction etc. are not much preferred because of high cost and toxic sludge generation. Biosorption is a new method that can be used for the removal of chromium and other heavy metals using the biomass of living and nonliving microorganisms such as bacteria, fungi, and algae.[3] This process of bioremediation is very cost-effective and it is considered to be a green technology as no toxic by-products are generated.[5] Moreover, biosorption processes have the potential to recover valuable metals from the wastewater as well.[6] The abilities of various microorganisms to remove metal ions from solutions have been extensively studied and among them, fungi have been recognized as a promising class of low-cost adsorbents for the removal of heavy metal ions from aqueous waste streams because of its high biomass production capability as well as high metal tolerance capacity [1, 6].

The main objective of this study, was to assess the biosorption of Cr (VI) ions onto the dead biomass of a chromium tolerant fungal strain isolated from tannery effluent (Bantala leather complex, Kolkata, India) and optimization of various process parameters to increase the efficiency of biosorption.

Materials and Methods

Microorganism

16 fungal strains (S1-S16) were isolated from tannery effluent of Bantala Leather Complex area (Kolkata, West Bengal, India) and their Cr (VI) tolerance capacity were tested at concentrations of 0.1-2 mg/ml. Among the 16 strains S12 showed the maximum Cr (VI) tolerance capacity of 2mg/ml and so this strain was subjected to fungal ITS sequencing analysis and phylogeny which revealed that the S12 strain has 99.82% similarity with *Aspergillus nomius* [7] So this S12 strain is being selected here for the biosorption study and it will be referred to as *Aspergillus nomius*. The strain is maintained on Czapekdox agar medium with regular subculturing after every 15 days and preserved at 4^0C.

Biomass production

The fungus was cultured in a liquid medium (Czapekdox broth) in 100 mm glass Petri-plates. The growth medium consisted of (g/l of distilled water): Sucrose 30.000, Sodium nitrate 2.000, Di-potassium phosphate 1.000, Magnesium sulfate 0.500, Potassium chloride 0.500, Ferrous sulfate 0.010. The pH of the medium was adjusted to 5, before autoclaving by using 0.5M HCl. Once inoculated, the plates were incubated in an incubator for 2 weeks at 30 ˚C for the production of sufficient fungal mat.

Bio-sorbent preparation

The nonliving biomass of *Aspergillus nomius* was used as a biosorbent for the sorption of Cr (VI) from an aqueous solution. After incubation, the biomass was collected from the medium and washed with distilled water. The biomass was then inactivated and pre-treated by immersing it in 500ml 0.5 (N) solutions of various acids and alkali ((Sulfuric Acid, Hydrochloric Acid, Nitric Acid, Acetic Acid, and Sodium Hydroxide) and then kept in a water bath at 100^0C for 15 minutes. This, pretreated biomass was then washed with deionized water until the pH of the wash solution was in near-neutral range (pH 6.8 - 7.2). It was then dried at 60 ˚C in a hot-air oven for 12 hours, grounded using mortar and pestle and stored in an air-tight container [8].

Chromium (VI) solution preparation

Aqueous (stock) solution of chromium (VI) concentration 1000 mg/L was prepared by dissolving 2.83 g of potassium dichromate salt in 1000 ml distilled water.[6] Concentration of chromium was varied from 1 mg/L to 5 mg/L.

Optimization of various biosorption parameters

Effect of pre-treatment

Fungal biomass was modified by various pre-treatments with acids and alkali, 0.5 N, 500ml solutions of Sulfuric Acid, Hydrochloric Acid, Nitric Acid, Acetic Acid and Sodium Hydroxide were used for this purpose. Percentage removal of metal ion with this pre-treated fungal biomass was studied spectrometrically at 540 nm using Diphenylcarbazide (DPC) assay method [9].

Effect of pre-treatment agent concentration

The effect of pre-treatment agent concentrations were observed for biosorption by the biomass, in 1mg/L of Cr (VI) solution and using different concentrations of Sulfuric acid as pre-treatment agent (0.3, 0.5, 0.8 and 1 N). The adsorbent dosage was 0.5 g in 100 ml solution in 250 ml conical flask at 37˚C and adjusted to pH 2.0. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540nm.[6]

Effect of contact time

The effect of contact time was observed for different contact times (12, 24, 48 hours) at a concentration of 1 mg/L of Cr (VI). The adsorbent dosage was 0.5 g in 100 ml solution in 250 ml conical flask at 37˚C and pH 2.0. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540nm.[10]

Effect of biomass loading

The effect of biomass loading was observed for 100 ml of 1 mg/L concentration of chromium solution, loaded with different biosorbent dosages (0.5g, 1g, 2g) by keeping pH as 2.0 and temperature 37˚C. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540nm.[6]

Effect of pH

The effect of pH was observed for chromium (VI) concentration of 1 mg/L. The 1 mg/L concentration chromium solution was adjusted to various pH (2, 4, 6, 8, 10 and 12) and 0.5g of biomass at 37˚C was added. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540nm.[11]

Effect of temperature

The effect of temperature was observed for chromium (VI) concentration with 1 mg/L. The 1 mg/L concentration chromium solutions were exposed to various temperature (20, 25, 30, 35, 40 and 45˚C) in an incubator and 0.5g of biomass at 37˚C was added keeping pH 2.0. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540nm.[6]

Effect of initial metal ion concentration

The effect of initial chromium concentrations were observed with different chromium (VI) concentrations (1, 2, 3, 4 and 5 mg/L). The adsorbent dosage was 0.5 g in 100 ml solution in 250 ml conical flask at 37˚C and pH 2.0. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540nm.[6]

Effect of volume differences (keeping metal concentration constant)

The effect of varying volumes with constant metal concentration were observed for 1mg/L with different volumes used (50, 100, 150, 200 and 250 ml). The adsorbent dosage was 0.5 g in 100 ml solution in 250 ml conical flask at 37˚C and pH 2.0. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540nm.

Effect of varying combinations of biomass load and volume

The combined effect of biomass loading and volume differences was observed for chromium (VI) concentration with 1 mg/L. The 1 mg/L concentration chromium solutions were exposed to various combinations of biomass load and volume (0.5g in 100 ml, 1g in 200 ml and 1.5g in 300ml) in an incubator at 37˚C was added. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540nm.

Biosorption efficiency calculation

Biosorption efficiency (%) was calculated using the following equation: [12]

$$E= (C_i-C_f /C_i) \times 100$$

Where,

E=Percentage removal of hexavalent chromium

C_i= initial metal ion concentration, mg/L

C_f= final metal ion concentration, mg/L

Each experiment was repeated three times in order to get accurate results and the average was taken for the calculation of biosorption efficiency.

Results and Discussion

Effect of pre-treatment

Metal adsorption to biomass can be manipulated by pre-treating the biomass with alkali, acid, and heat which may increase the amount of metal sorbed. The comparison for chromium adsorption capacities by *Aspergillus nomius* biomass when pre-treated with acids and alkali is shown in Figure 1. It is seen that when the biomass was pre-treated with sulfuric acid the adsorption efficiency was maximum (82%) whereas when pre-treated with alkali (NaOH) the adsorption efficiency was minimum (1%). Adsorption of chromium may vary because of the action of chemical agents contributing to modifications in the cell wall structure. Similar observation was reported by Reya *et al* where they used *Aspergillus oryzae* and *Aspergillus sojae* biomass and the acid treatment yielded better results than alkali treatment [6].

Effect of pre-treatment agent (Sulfuric acid) concentration

Effect of various concentration of sulphuric acid on biosorption by dead *Aspergillus nomius* biomass was studied and results are shown in Figure 2. It was observed that the percentage removal of chromium increased with increasing the concentration of the pre-treatment agent and was maximum at 96.5% when 1N solution was used.

Effect of contact time

Effect of contact time using dead *Aspergillus nomius* biomass was studied and results are shown in Figure 3. Initially there was a gradual increase in the adsorption efficiency as contact time increased followed by a decrease in efficiency after 24 hrs. The percentage removal was around 98% and 90% respectively for durations of 24 hrs and 48 hrs. As time passed, the metal uptake by the adsorbent surface slowed down, due to the competition for decreasing availability of active sites occupied by the metal ions remaining in the solution. Santhi and Guru reported synonymous results where they recorded maximum biosorption at 24 hours, using *Aspergillus niger* biomass as the biosorbent [8].

Effect of biosorbent dosage

The biosorbent dosage is also one of the significant factors to be considered for effective biosorption. It determines the sorbent/sorbate equilibrium of the system. Chromium biosorption with varying biosorbent loads is shown in Figure 4. From the figure it is observed that as the concentration of biosorbent increases from 0.5 g to 2 g there is increase in the percentage removal. This may be due to the increase in the number binding sites on the biomass. Santhi and Guru reported similar results where they used *Aspergillus niger* biomass as the biosorbent.[8]

Effect of pH

Effect of pH on biosorption was studied and results are shown in Figure 5. It was observed that the percentage removal of chromium was maximum at pH 6.0 and was found to be 98%. As the pH increases from pH 2.0 to pH 6.0, there is increase in the removal efficiency and after pH 6.0 it starts to decrease. The cell wall of *Aspergillus* species contains a large number of surface functional groups. Biomass has active sites capable of binding metal ions and such bond formation could be done by displacement of protons which can be determined by pH [3].

Effect of temperature

Metal adsorption to biomass maybe manipulated by temperature variations. Results are shown in Figure 6. The percentage removal of chromium was maximum at 25˚C (95.9%) and gradually decreased with increasing temperature, as for exothermic nature of the adsorption process, with a minimum at 45^0C [13]. Santhi and Guru reported similar results where they achieved maximum biosorption at 27˚C, using *Aspergillus niger*biomass as the biosorbent.

Effect of Initial Metal Ion Concentration

Effect of initial metal ion concentration on biosorption of Cr (+6) is shown in Figure 7. The percentage removal of chromium was around 95% to 72.8% with initial metal ion concentrations of 1mg/L to 5mg/L respectively. The percentage removal decreased as the concentration increased and this may be due to saturation of active sites.[14] Santhi and Guru reported synonymous results where they also achieved maximum biosorption at 0.1mg/L Cr (VI) concentration, using *Aspergillus niger* biomass as the biosorbent.

Effect of volume differences (keeping metal concentration constant)

Metal adsorption to biomass maybe manipulated by variations in solution volumes. Results are shown in Figure 8. The percentage removal of chromium was maximum when 250ml of solution volume was taken (94.9%) and gradually decreased with decreasing volumes. This may be due to unavailability of space for optimal interactions with the biomass.

Effect of varying combinations of biomass load and volume

The combined effect of biomass loading and volume differences were studied for chromium removal and results are shown in Figure 9. It was observed that the most effective combination was 0.5g of biomass in 100ml aqueous solution with an efficiency of 98%.

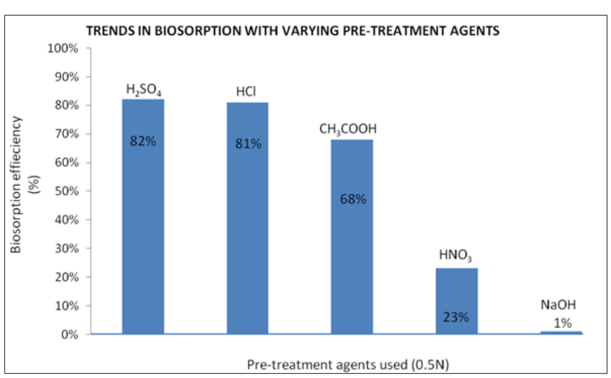

Fig 1: Effect of the different pre-treatment agents on biosorption

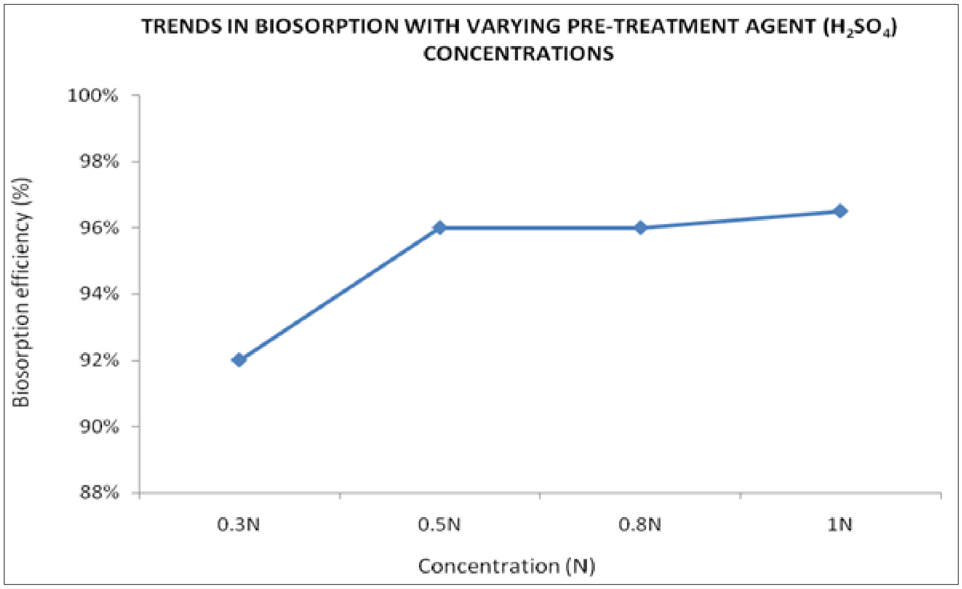

Fig 2: Effect of various concentrations of H_2SO_4 on biosorption

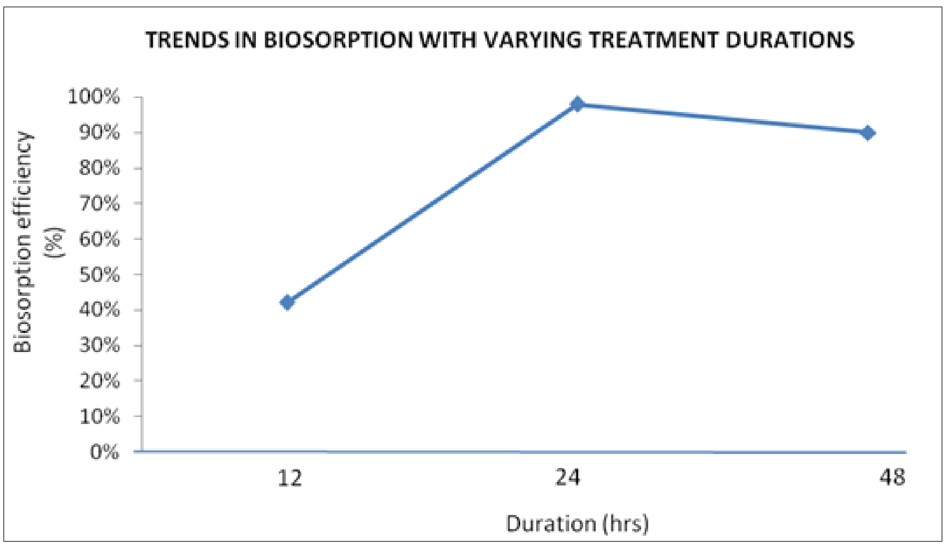

Fig 3: Effect of duration of exposure on biosorption

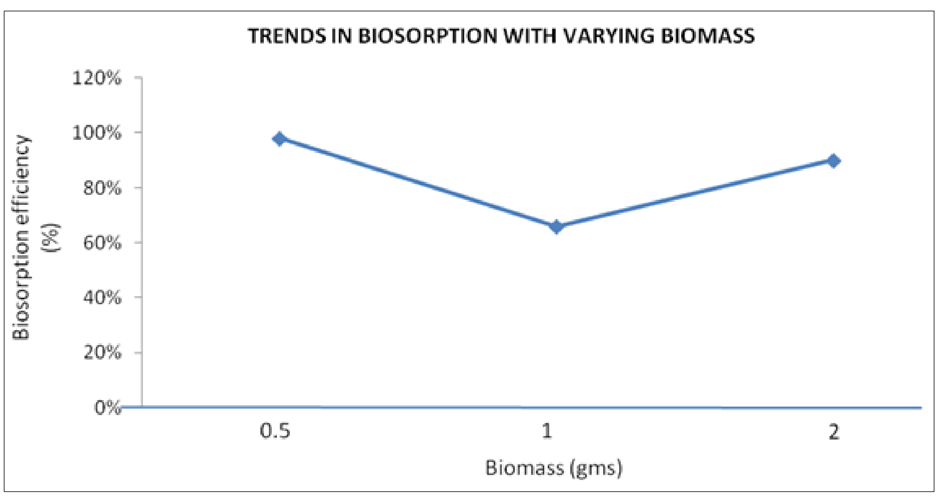

Fig 4: Effect of the biomass dosage on biosorption

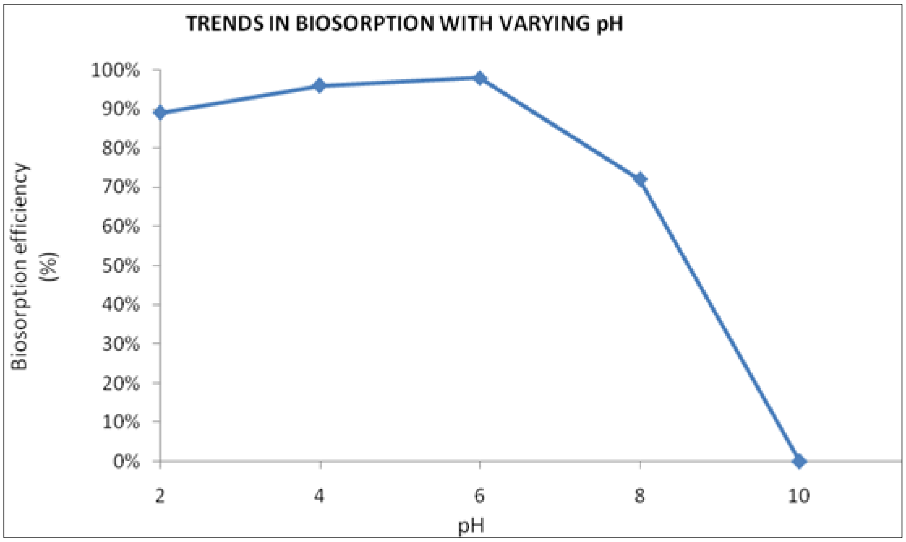

Fig 5: Effect of pH on biosorption

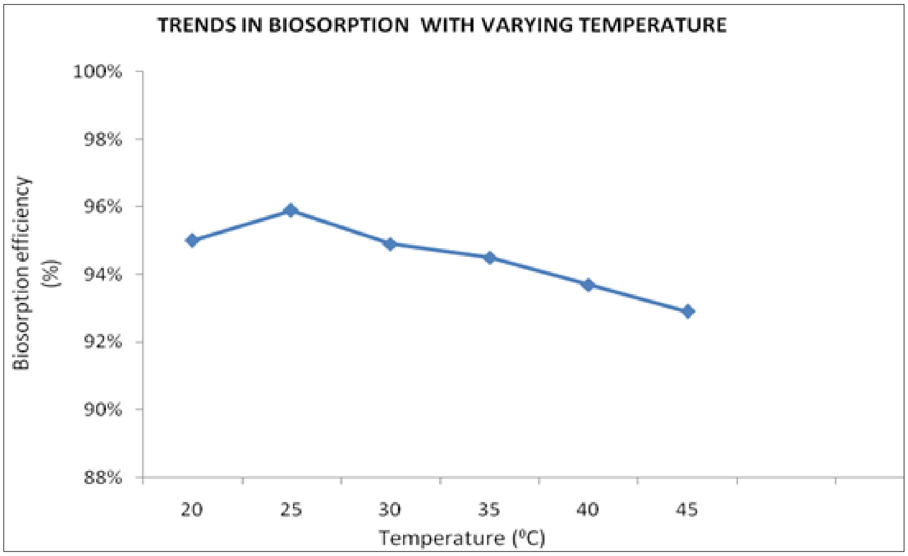

Fig 6: Effect of temperature on bisorption efficiency

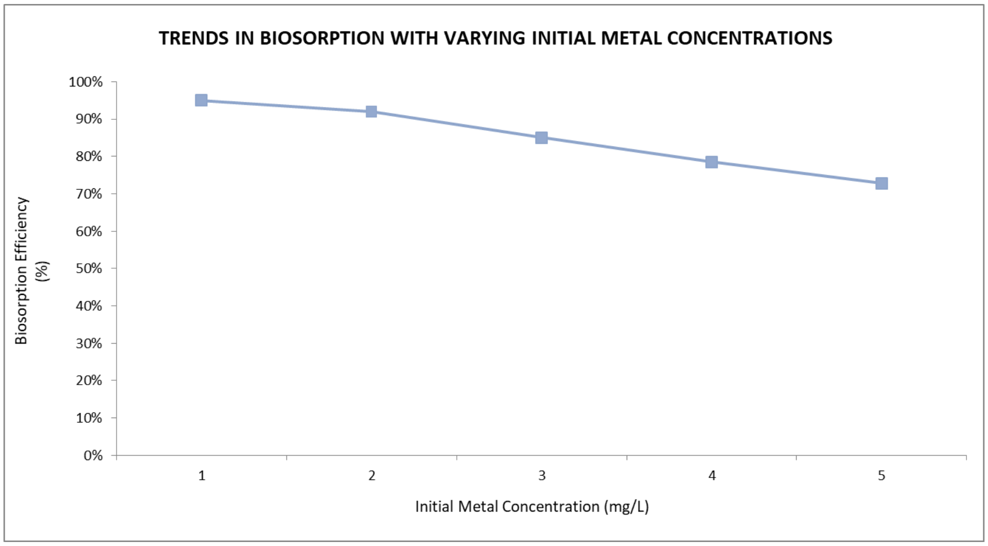

Fig 7: Effect of initial metal concentration on bisorption efficiency

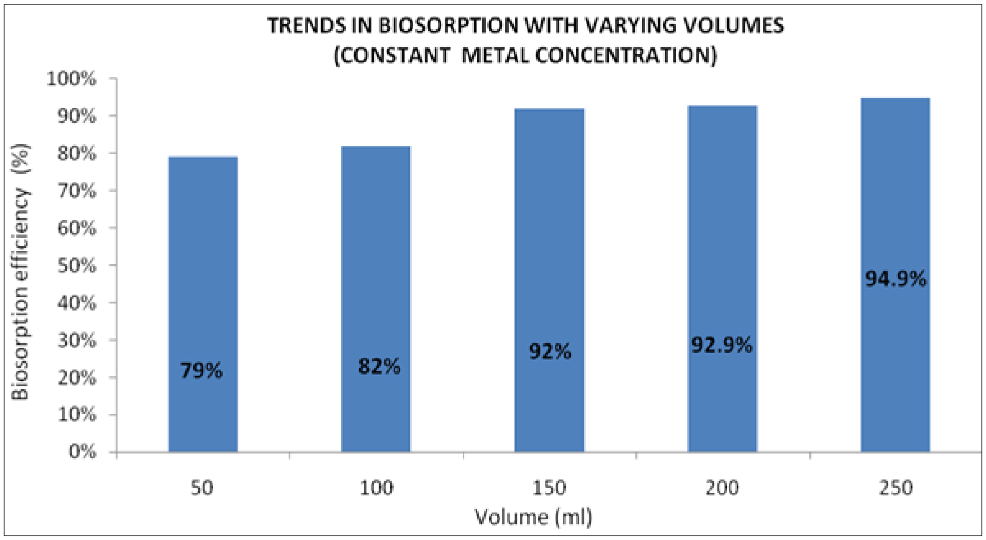

Fig 8: Effect of different volumes tested on bisorption

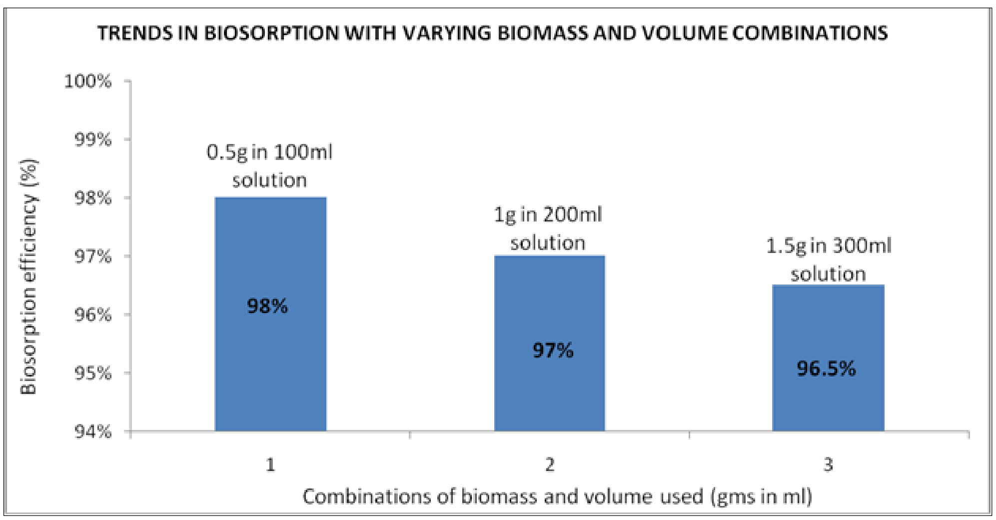

Fig 9: Effect of different biomass and volume combinations tested on biosorption

Conclusions

The results show that the hexavalent chromium can be effectively removed up to 82% from aqueous solution using sulfuric acid as the pre-treatment agent using dead *Aspergillus nomius* biomass at 37°C. The results also showed that as the concentration of the sulfuric acid was increased, the percent removal was also found to increase linearly with a maximum of 96.5% at 1N. Afterwards, the fungus was always pre-treated using sulphuric acid to inactivate the biomass and also for obtaining maximum efficiency of hexavalent chromium removal. Maximum Cr (VI) removal of about 98 % was obtained, when the biosorption was allowed to take place for 24 hours at 37ºC, pH 2.0 and 150 rpm. When 0.5g of biomass was used for biosorption, in 100ml volume solution, maximum chromium removal was achieved at 98% efficiency. The removal efficiency was also checked for pH variations and it was found that removal increased with increasing pH up to 6, and then the efficiency declined. A maximum removal of about 96% was obtained when biosorption took place at 25^0C temperature, keeping other conditions same but, it declined gradually at higher temperatures. Maximum Cr (VI) removal of about 95% was obtained when initially the concentration of Cr (VI) was 1mg/L and declined gradually with higher concentrations. Maximum Cr (VI) removal of about 95 % was obtained when the biosorption was allowed to take place in 250ml volume of solution keeping 1mg/L Cr(VI) concentration constant and other conditions same. It was also found among the various biomass dosage-volume combinations tested, the combination of 0.5g biomass in 100 ml, 1mg/L solution, was optimum at 98% for Cr (VI) removal at pH 2.0 and 37°C.

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References

1. Sivakumar D. Biosorption of hexavalent chromium in a tannery industry waste-water using fungi species. Global Journal of Environmental Science Management. 2016; 2(2): 105-124.

2. Anyanwu CU, Ezaka E. Growth Responses of chromium tolerant bacteria to different concentrations of chromium. International Journal of Basic & Applied Sciences, 2011, 11(5).
3. Marandi R. Biosorption of Hexavalent Chromium from Aqueous Solution by Dead Fungal Biomass of *Phanerochaete crysosporium*: Batch and Fixed Bed Studies. Canadian Journal on Chemical Engineering & Technology, 2011, 2(2).
4. Verma T, Srinath T, Gadpayle RU, Ramteke PW, Hans RK, Garg SK *et al*. Chromate tolerant bacteria isolated from tannery effluent. Bioresource Technology. 2001; 78:31-35.
5. Bennett RM, Cordero PRF, Bautista GS, Dedeles GR. Reduction of hexavalent chromium using fungi and bacteria isolated from contaminated soil and water samples. Chemistry and Ecology. 2013; 29(4):320-328.
6. Reya II, Rajamehala M, Lakshmi PM, Emilin RR. Comparitive Study On Biosorption Of Hexavalent Chromium Using *Aspergillus Oryzae* NCIM 637 And *Aspergillus Sojae* NCIM 1198 From Electroplating Effluent. International Journal of ChemTech Research. 2012; 4(4):1708-1719.
7. Guha S, Debnath S, Gayen S. Isolation of hexavalent chromium (VI) tolerant fungi from Tannery effluent and their growth responses to different concentrations of chromium (vi). Sustainability, Agri, Food and Environmental Research, 2020, 8(X).
8. Santhi R, Guru V. Biosorption of Hexavalent Chromium Using *Aspergillus Niger* dead biomass and its optimization studies. International Journal of Current Microbiology and Applied Sciences. 2014; 3(10):669-678.
9. Method 7196A chromium, hexavalent (colorometric), 2015 https://www.epa.gov/sites/production/files/2015-12/documents/7196a.pdf
10. Sethuraman P, Balasubramanian N. Removal of Cr (VI) from aqueous solution using *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*. International Journal of Engineering Science and Technology. 2010; 2(6):1811-1825.
11. Goyal N, Jain SC, Banerjee UC. Comparative studies on the microbial adsorption of heavy metals. 2013; 7(2):0-319.
12. Bajpai AK, Rai L. Removal of chromium ions from aqueous solution by biosorption on toternary biopolymeric microsphere. Indian Journal of Chemical Technology. 2010; 17(1):17-27.
13. Mameri N, Boudries N, Addour L, Belhocine D, Lounici H, Grib H *et al*. Zinc biosorption by a bacterial non-living *Streptomyces rimosus* biomass. Water Resource. 1999; 33:1347-1354.
14. Saleh M, Al-Garni, Khaled M. Ghanem and Ahmed Ibrahim S. Biosorption of mercury by capsulated and slime layer forming Gram-negative Bacilli from an aqueous solution. African Journal of Biotechnology. 2010; 9(38):641-642.