BIOREMEDIATION OF XENOBIOTICS: USE OF DEAD FUNGAL BIOMASS AS BIOSORBENT

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Abstract

Biosorption is bioengineering where metabolism independent adsorption of xenobiotics to living or dead cells takes place.Microorganisms dead or alive are successfully exploited for bioremediation of xenobiotics by biosorption. In the present study bioremediation of xenobiotics of textile industry effluent was carried out by biosorption using dead fungus biomass of Aspergillus flavus. The dead biomass of fungus Aspergillus flavus shows maximum biosorption for three toxic components of textile industry effluent under different parameters. Methyl orange biosorption was found to be 53.62% at room temperature, at pH 5.5, with biomass concentration of 2g/L having contact time of 40 min and the dye concentration was 1ppm. Chromium biosorption was 72.18%, at pH 6, at room temperature with biomass concentration of 2g/L having contact time of 10 min and solution concentration 200ppm. Lead biosorption was found to be 76.12%, at pH 7, at room temperature with biomass concentration 2g/L having contact time of 40 min and solution concentration 1ppm. Desorption studies were also performed and was found that dead fungal biomass can be reused further.

Key words: *Bioengineering, Bioremediation, Biosorption, Textile industry effluent, Methyl Orange, Chromium, Lead, Aspergillus flavus .*

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1. INTRODUCTION

Textile industry is the second largest industry in India. It makes use of natural and synthetic dyes, heavy metals, mordants for manufacturing of textile fabrics. The compositions of the effluent are heavy metals (chromium, copper, lead, and arsenic), dyes (azo, reactive), surfactants, etc [1, 2]. It is characterized by high values of COD, BOD, suspended and dissolved solids [1]. Textile industry effluent causes ground water pollution which is of major concern as they exert harmful effect to environment and life forms by causing carcinogenicity, skin irritation, multi-organ failure, neurotoxicity etc. The conventional physical and chemical methods are less effective, expensive and use of chemicals leads to secondary pollution [3-5]. Activated carbon is found to be effective for adsorption but its generation requires high temperature which is not cost effective. An alternative to all these treatment methods is use of biological entities like microorganisms which can be used in live or dead forms for biosorption .[3,6 and 7]

Azo dyes have been found to be mostly used in the textile industries. They contain –N=N- group as chromophore group [8 and 9].They are resistant to microbial and chemical attack and are most recalcitrant compound which are carcinogenic, mutagenic, triggers allergic reactions in human. [1, and 10-15]. Heavy metal Cr is soluble in water, carcinogenic, neurotoxic leading to brain and nerves damage, multi-organ failure while Pb is known to interfere with enzyme activities and formation of RBCs, anemia, hepatitis, nephratic syndrome [16 and 17].

Adsorbates (xenobiotics) are solutes to be adsorbed while Adsorbent (living or dead biomass) is the solid material or support used for adsorption and hence it is not affected by microbial physiology [18 and 19]. The dried dead microbial biomass displays high affinity for metal ion [20].

In this study biosorption of azo dye Methyl Orange and heavy metals such as Chromium and Lead have been studied using dead biomass of fungal species Aspergillus flavus. Cr is used as metallic mordant while lead (acetate salt) is employed in textile imprinting [21]. For coloring of wools and silk fibers azo dye MO is used [22 and 23]. Various parameters for maximum sorption profile of these three compounds were studied such as pH of solution, contact time, concentration of solution, temperature, and biomass concentration.

2. MATERIALS AND METHOD

2.1 Fungus isolation and biosorbent preparation

Fungus Aspergillus flavus (identified by ARI, Pune) was isolated from soil. Aspergillus flavus was grown and maintained on GPYA (Glucose Peptone Yeast Extract Agar) medium [composition (g/L): glucose- 40; peptone- 5; yeast extract- 5; agar- 30; pH-5.6] incubated at room temperature for 48hrs. To obtain fungal biomass, Aspergillus flavus was inoculated in same liquid medium and incubated at RT, 150rpm for 20 days. The biomass was autoclaved at 1210 C, for 15min at 15lbs. Then it was filtered through Whattman filter paper No.1, dried at 550 C for one week and then crushed in mortar and pestle. This dried dead powdered

biomass was used for biosorption study. SEM analysis of dried fungi was studied.

2.2 Reagents Preparation and lambda max determination

Three components methyl orange, chromium and lead were used in this study. Lambda max of aqueous solution of methyl orange and chromium was determined by using spectrophotometer Visican 167 while for lead UV/Vis spectrophotometer was used. The lambda max of methyl orange, chromium and lead was found to be 460nm, 420nm and 210nm respectively. Aqueous stock solution of 10ppm for Pb and MO and 1000ppm for Cr were prepared in distilled water.

2.3 Study of sorption profile

Sorption profile was studied by using 0.1g of biosorbent in 50ml of each solution in 100ml Erlenmeyer flask. The contents were kept at static conditions, centrifuged at 4000rpm after 10min time interval to completely sediment biosorbent, supernatant was used for estimation of biosorption efficiency. Initial and final absorbance was determined for each solution which was used for estimation of biosorption. Biosorbent was dried and used for desorption studies.

Effects of different parameters on sorption process were studied including biosorbent concentration, initial concentration of dye / heavy metal solution and pH of solution. To observe initial concentration at which maximum biosorption was achieved, concentration of MO in range of 1 to 8 ppm, Pb in range of 1 to 5ppm and Cr from 100 to 600ppm were studied. Similarly to see the effects of pH various pH were studied for each of the solution. Biosorbent concentration was investigated in the range of 1 to 3g of biosorbent per liter of solution.

2.4 Calculation of % Biosorption by dead biomass

Biosorption efficiency of biosorbent was calculated by using following formula:

$$\% \text{ Biosorption} = \frac{\text{Initial Absorbance} - \text{Final Absorbance X100}}{\text{Initial absorbance}}$$

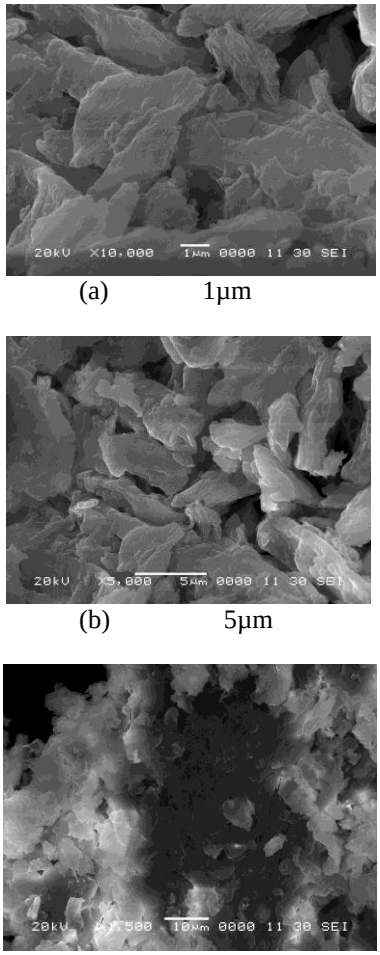
2.5 Desorption and recycling of biosorbent (dead biomass)

After determining the biosorption profile, the reuse and recycling possibility of biosorbent was investigated. For desorption of biosorbent 0.1M HCl was used for MO, Cr and Pb. MO being soluble in water, 0.1M HCl was used for desorption of heavy metals as well as MO.

3. RESULTS

3.1 SEM Analysis

SEM of dried dead *Aspergillus flavus* biomass was performed to investigate the mesh size, shape and particle size of the biosorbent (Fig. 1). It has been found that particle size of dead *Aspergillus flavus* biosorbent varies between 1µm to 10µm.



(a) 1µm

(b) 5µm

© 10µm

Fig 1 - SEM images of dead *Aspergillus flavus* biosorbent

3.2 Effect of initial concentration

The effect of initial concentration of solution of MO, Cr and Pb on biosorption was studied. It was seen that *Aspergillus flavus* shows maximum biosorption for MO, Pb and Cr at concentration of 1ppm, 1ppm and 200 ppm within 40min, 40min and 30min respectively (Fig. 2, 3, 4). Percent biosorption was found to be 18.90% for MO, 52.57% for Pb while for Cr 39.19%.

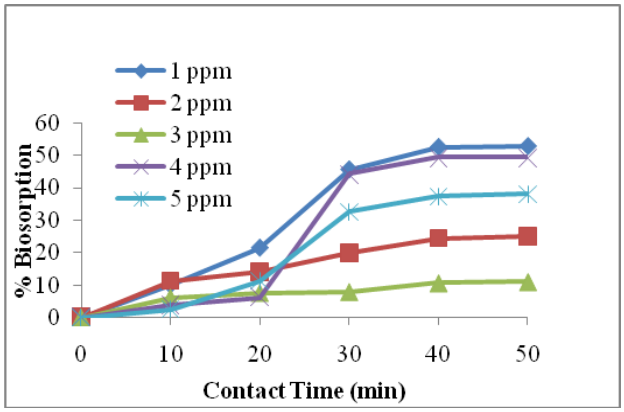


Fig. 2: Biosorption of Pb by *Aspergillus flavus*

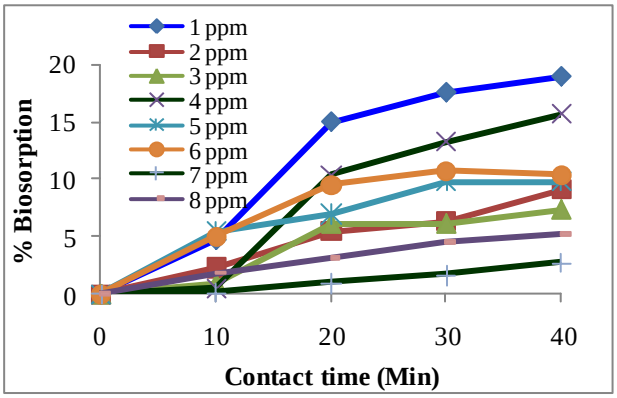


Fig. 3: Biosorption of Methyl Orange by *Aspergillus flavus*

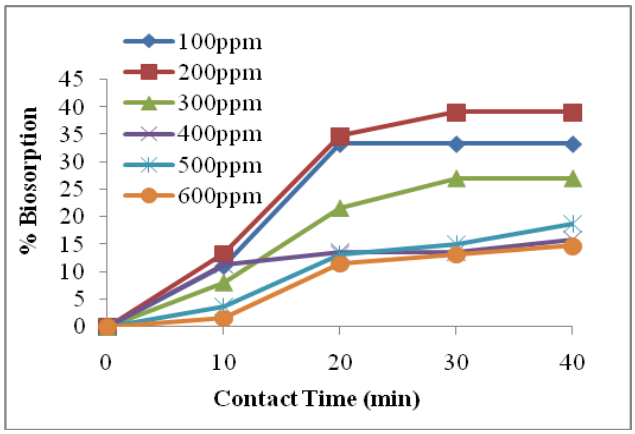


Fig. 4: Biosorption of Cr by *Aspergillus flavus*

3.3 Effect of pH of solution

Maximum biosorption was recorded at pH 5.5, 6 and 7 within 40 ,10 and 40 for MO, Cr and Pb respectively. At pH 5.5 MO was adsorbed 53.62%, while at pH 6 Cr was found to be 72.18% and 73.35% Pb was adsorbed (Fig. 5, 6 and 7). It has been found that increase in pH leads to decrease in biosorption of MO and other dyes.

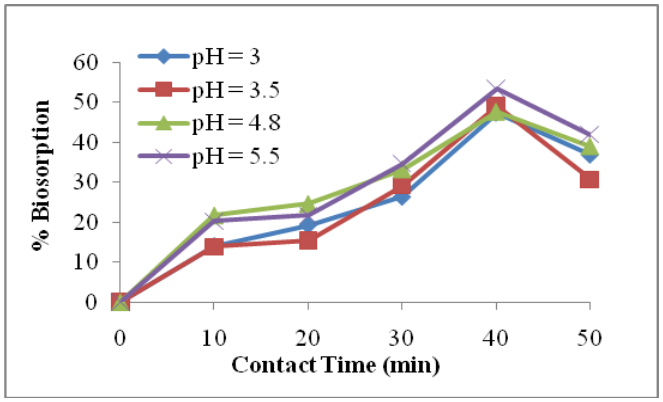


Fig. 5: Effect of pH on biosorption of MO by *Aspergillus flavus*

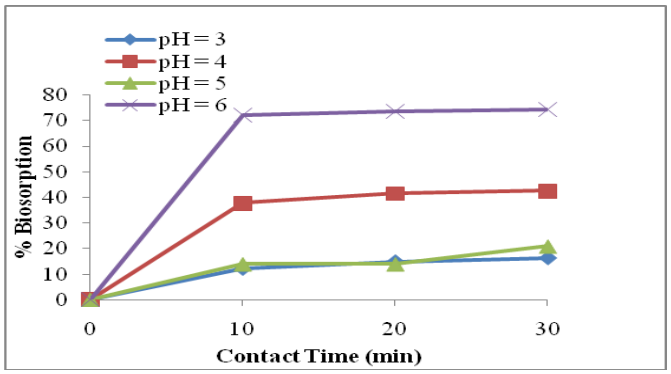


Fig. 6: Effect of pH on Biosorption of Cr by *Aspergillus flavus*

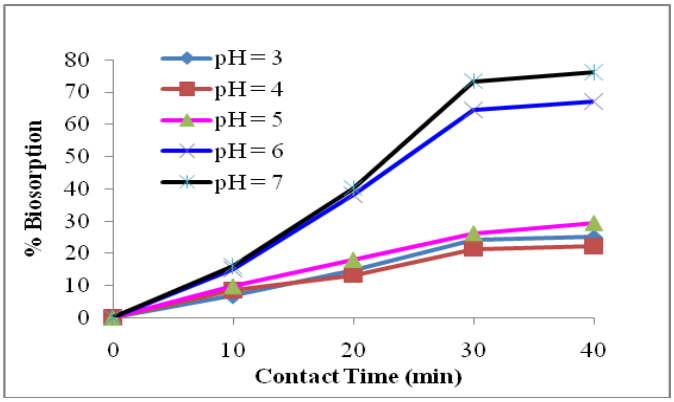


Fig. 7: Effect of pH on Biosorption of Pb by *Aspergillus flavus*

3.4 Effect of Biomass concentration

In our study we have found that 2g biosorbent shows maximum biosorption for 1L of all three component solutions. That is 2g/L biomass concentration is found to be effective (Fig. 8, 9, 10).

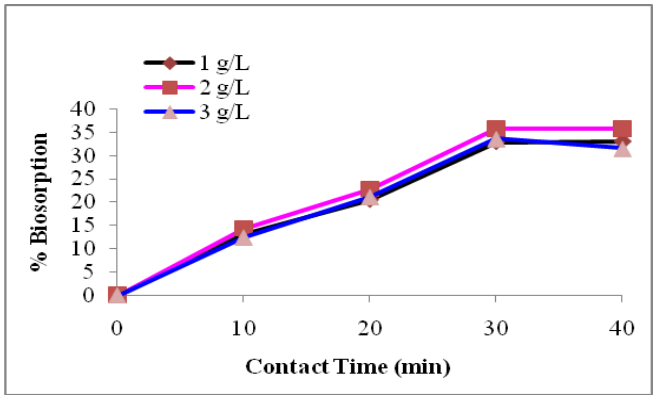


Fig. 8: Effect of biomass concentration on biosorption of Methyl orange by *Aspergillus flavus*

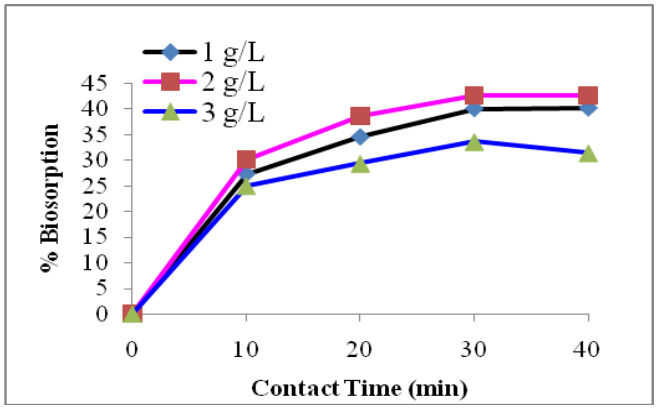


Fig. 9: Effect of biomass concentration on biosorption of Cr by *Aspergillus flavus*

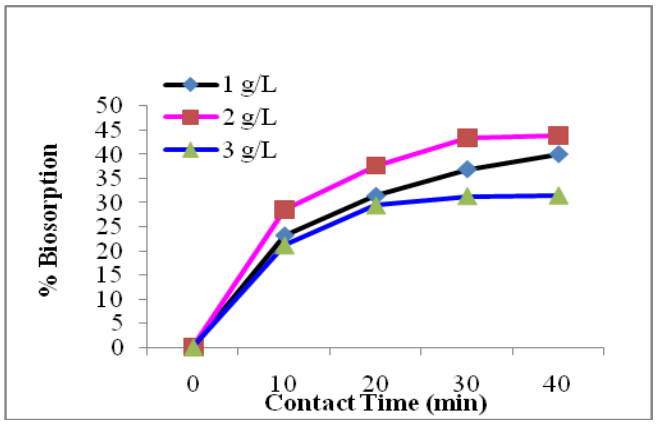


Fig. 10: Effect of biomass on biosorption of Pb by*Aspergillus flavus*

3.5 Desorption experiments

In this study, desorption of MO, Cr and Pb were carried out by using 0.1M HCl solution where complete desorption for MO, Pb and Cr at 50 min, 30 min and 50 min were seen respectively(Fig. 11)

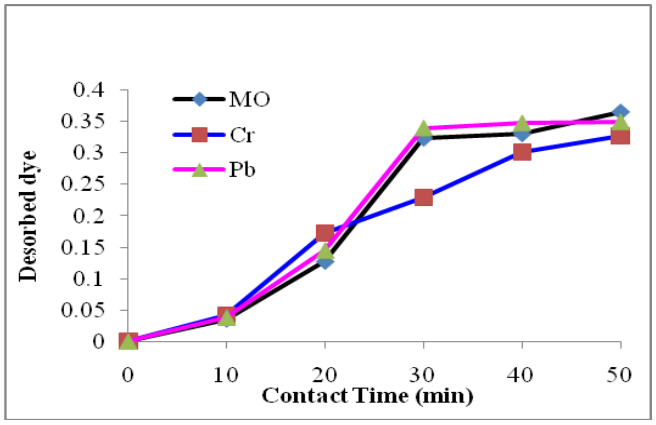


Fig. 11: Desorption of MO, Cr and Pb from *Aspergillus flavus*

4. DISCUSSION AND CONCLUSION

Increase in pH leads to decrease in biosorption of MO and other dyes due to characteristics of biomass and MO [21].It has been found that increase in biomass concentration decreases the biosorption efficiency [16, 22]. This occurs due to decrease in surface area on biosorbent for binding of adsorbent [16]. Our findings are in sync with the previous findings.

Humicola fuscoatra adsorbs methyl orange, though it shows biosorption of 62.5% it requires a long contact time of 6 hrs [26 and 28] whereas dead fungal biomass of Aspergillus flavus adsorb 53.62 % MO with contact time of only 40 min.. Percentage of adsorption by Aspergillus niger of Cr was found to be more by base treated as compared to acid treated and untreated Cr solution [29].While untreated dead fungal biomass of Aspergillus.flavus adsorb 72.18%, of Cr with contact time of 10min. Aspergillus terreus have shown biosorption of Pb upto 100mg/g FFB (Free Fungal biomass) in 10 min contact time [30]. Our study shows biosorption of 76.12% of Pb which is comparable.

It has been found that 0.1M HCl is effective in desorption of heavy metals [16]. The reuse of dead fungal biomass can be done for 4-5 cycles of biosorption and desorption without significant loss in biosorption capacity. Aspergillus terreus is known to show biosorption potential 78% of Pb after five continous cycles of desorption [30]. Aspergillus niger shows biosorption of Cr upto 40% after five cycles of desorption [29]. Comparitively in our study dead fungal biomass of Aspergillus flavus shows biosorption capacity of Pb, Cr and MO at 76%, 72% and 53% respectively after 5 cycles of desorption processes. This dead fungal biomass of Aspergillus flavus can be used for biosorption upto 5-6 times and after desorption it can be discarded into the soil to increase the fertility of the soil and it is not harmful to soil flora.

Biosorption capacity of the dead biosorbent is due to high surface to volume ratio [24]. Being metabolic independent process, there are no restrictions of enzymatic activities of adsorption [25]. It is harmless, cheap, eco-friendly, highly

efficient [26 and 27]. Recover and reuse of bioadsorbent is possible [18]. Biomass generated at fermentation industries can be used as biosorbent after killing it thereby making it a best practice for GLP of fermentation industries [29]. Thus better alternative for conventional methods of adsorption is biosorption and it is necessary to make use of biosorption at large scale for treatment of wastewater.

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