ORIGINAL ARTICLE

Biosorption of Heavy Metals in Industrial Effluents by Agaricus Bisporous

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ABSTRACT

Aim: To investigate the extraction of the heavy metals (Cu^2 and Cd) from wastes of paper, leather, textile, medical and chemical factories using Agaricusbisporous.
Methods: Growth rate of Agaricusbisporous were carried out in a selected medium as well as in the effluent. The biosorption of metal ions increased as the concentration of substrate decreased in the medium. The optimum pH for the fungal growth was 6.0. The optimum temperature for the maximum extraction of heavy metals by Agaricusbisporous was 25°C.
Key words: Agaricusbisporous, Biosorption, heavy metals, effluents, copper, cadmium.

INTRODUCTION

Industrialization in Pakistan started in the 1950s and manufacturing processes were not environmentally friendly, as the technology had not yet been developed. Despite all the best intentions to push Pakistan towards development and self-sufficiency, the cost of progress had a heavy burden on the industry[1]. Most of the wastes generated by industries, be it in the form of solids, effluents or gases, are directly discharged into the air, water bodies and adjacent lands without any prior treatment or detoxification [2, 3].

The recent shift from the agriculture to manufacturing industry in Pakistan has further blighted the environmental degradation. Numerous textile mills, tanneries, cement, edible oil, fertilizer, paint and other chemical factories have been established throughout Pakistan, in pockets of what have been termed as industrial areas. These areas are largely located near urban centers, along rivers and waterways. According to the Environment Protection Agency (EPA) - Pakistan, the industrial effluents being dumped into the waterways and on the land by the electroplating and tanner industries alone, contains toxic metals such as Chromium (Cr), Nickel (Ni), Arsenic (As), Mercury (Hg), Copper (Cu^2) and Lead (Pb), which have contaminated the soil and the biota residing around in such soils in Pakistan[4]. In Faisalabad studies have reported that the city effluents, which comprises of both domestic as well as industrial waste, contain Pb, Cu, Cadmium (Cd) and other hazardous heavy metals[5].Biosorption is a physical process carried through ion exchange, surface complexion and precipitation and has proved as a promising biotechnology for pollutant removal from fluids[6]. The effect of contact time, pH, Ionicmedium, initial metal concentration were studied for Cd biosorption in industrial effluents by using Agaricus bisporous at different pH and temperatures by Heng [7] who reported Agaricus bisporous as effective source of biosorption of Cd and Pb in industrial wastes.

The potential use of microorganisms in treatment of hazardous materials and metals from their aqueous environments by biosorption is considered as a preferred method. The term biosorption has been used to describe the passive non-metabolically mediated process of metal ion binding by living or dead biomass[8].

Although there are many studies on biosorption and bioaccumulation process in model systems but there is lack of information on trace metal ion removal from real industrial effluents. Therefore, the main objective of this study was to evaluate trace elements removal efficiency in a real multi-component system, in order to establish operating strategies capable of achieving permissible discharge levels of trace elements in effluents. This study also investigated the workable optimum temperature and pH for Agaricusbisporous.

MATERIALS AND METHODS

Effluents samples were collected from outlet pipes of leather, paper, textile and chemicals industries. To ensure accuracy and precision, triplicate effluents

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samples were drawn from the sampling point. The samples were stored at 4 °C[7].

The pure cultures of test fungal specie viz., *Agaricusbisporous* was obtained from Agricultural university Faisalabad Pakistan. The pure culture was maintained on MEA and Kirk media.

MEA was prepared by adding malt extract (1.5g), yeast extract (0.6g), agar (2.25g), calciumchloride (0.06g), magnesium sulfate(0.045g), glucose(1g) in 150 ml distilled water and autoclaved at 121^{o}C for 30 minutes. After autoclaving it was poured insterilized Petri plates. Kirk media was prepared by adding potassium dihydrogen phosphate, calcium chloride, ammonia sulfate, yeast extract, peptone, glucose, agar, magnesium sulfate, malt extract in distilled water and autoclaved for 15 minutes and was poured insterilized Petri plates.

Mycelia of the test fungi was grown in malt extract (ME) media by different concentrations of glucose i.e. 0.9, 1.8, 2.7, 3.6gmsin conical flasks by taking active inoculate from preserved stock culture. Inoculated flasks were incubated at 24-25°C in stationary phase for 72 hours. The flasks were removed at an interval of 8 hours. After the complete growth of mycelia A. bisporous was separated from culture broth by filtration through Whitman filter paper No.1 and successive washings with deionized water checked by the wet and dry weight. The biomass of the fungus was dried in oven at 60°C for 24 hours and homogenized in a blender to break the cell aggregates into smaller fragments of 0.5-1 mm diameter. The dried biomass was preserved in airtight jars. Experiment was performed by taking 0.2g of oven dried biomass of test fungal in test tubes containing 5ml of Cd and Cu^2 contaminated solution and was centrifuged at 150 rpm at 25°C for 12 hours. After each experiment, the mixture was filtered through Whitman filter paper No.1 and the sorption of metals was determined using atomic absorption spectrophotometer.

pH: To100 ml of effluent in each conical flask 1.5 g glucose was added. Samples were adjusted at different pH values (4.5,5.0,5.5,6.0 and 6.5). The flasks were then autoclaved at 1atm and 120^{o}C for 20 minutes. Following autoclaving, each flask was inoculated under aseptic conditions with an already preserved culture of Agaricus Bisporous. The flasks were shaken on shaker at 25^{0}C at 120rpm for 20 days. Each flask material was filtered with Whitman filter paper No. 1.The filtrate was then used for analysis of metals (Cd and Cu^2) by atomic absorption pectrophotometer.

To 100 mL of effluent in each conical flask 1.5 g glucose was added. Samples were placed at different temperatures (25,30and45^{0}C).

To 100 mL of effluent in each conical flask, 1.5 g glucose was added. The flasks were then autoclaved at 1atm and 120^{0}C for 20 minutes. Following autoclaving, each flask was inoculated under aseptic conditions with an already preserved culture of Agaricus Bisporous with different concentration with the help of discs (1 disc, 2 disc and 3 disc).The flasks were shaken on shaker at 25 ^{0}C at 120rpm for 20 days. Each flask material was filtered with Whitman filter paper no 1.The filtrate was then used for estimation of metals (Cd and Cu^2) by atomic absorption spectrophotometer[2].

RESULTS

The growth profile of the organisms shown in terms of wet weight and dry weight (Table 1). Maximum fungal growth was observed between 64-72 hrs of incubation in malt extract medium. The relative decrease in the substrate concentration in the MEM with increase in biomass concentration was observed at optimum temperature of 25°C & pH 6.5 (Table 1).

Table 1: Results of biomass wet weight and glucose concentration with time of incubation

Time(Hrs)	8	16	24	32	40	56	64	72
Wet weight(g)	0	0	2	4	8	20	28	22
Glucose conc (g)	10	9	8	7	6	6	5	4

The concentration of various heavy metal ions i.e., Copper(Cu^2) and Cadmium(Cd) in effluents were found significantly higher($P<0.01$) than the levels permitted by the environmental legislation of National environmental quality standard for liquid industrial effluents (NEQS). The wastewater had pinching odor, acidic in nature with pH ranging from 4.5 to 5, and of dark yellow coloration. The Biological Oxygen Demand (BOD) of 70 mgL^{-1} levels was within the desirable limits. However, the level of Chemical Oxygen Demand (COD) of 2800mgL^{-1} was significantly higher ($P<0.05$) than that of tolerance limits. The biological analysis indicated the absence of biological flora in all samples (Table: 2).

Table 2: ResultsofBiologicalandChemical Oxygen Demand.

	BODmg/l	**COD mg/l**
Leather	180	549
Textile	164	488
Paper mill	100	298
Chemical Industry	44	137
Medical Laboratory	28	80
NEQS	80	150

pH is an important parameter affecting the biosorption process.Table3 shows the affect of pH in the range of 4.5-6.5 on the biosorption rate of heavy metal (Cu) from the effluent samples by

Agaricusbisporous. The biosorption of Cu^2 by *A. bisporous* was observed to increase significantly (P<0.05) with increasing pH values.An increase in pH resulted in significant (P<0.05) increase in the biosorption of the ions. The increase in biosorption levels with increased pH can be explained by the influence of the number of negative surface charges, which depend on the dissociation of functional groups.

Table 3: Effect of pH on Cu from the effluents sample by *Agaricusbisporous*

Effluents pH for Copper (Cu^2)					
	4.5	**5**	**5.5**	**6**	**6.5**
Medical	0.03	0.029	0.023	0.021	0.02
Chemical	0.06	0.057	0.052	0.049	0.037
Textile	0.04	0.035	0.032	0.03	0.029
Leather	0.013	0.01	0.01	0.009	0.007
Paper	0.03	0.027	0.025	0.024	0.02

Table 4: Effect of pH on Cd in effluents sample by *Agaricusbisporous*

Effluents pH for Cadmium(Cd)					
	4.5	**5**	**5.5**	**6**	**6.5**
Medical	0.022	0.019	0.017	0.016	0.013
Chemical	0.018	0.017	0.015	0.013	0.01
Textile	0.02	0.02	0.019	0.016	0.015
Leather	0.015	0.015	0.014	0.012	0.01
Paper	0.025	0.021	0.02	0.02	0.018

Table 3 showed that at pH 6.5 the maximum rate of biosorption was observed in all five effluents by *Agaricusbisporous* .At pH 4.5 the minimum rate was observed. The extraction rate of copper by Agaricusbisporous significantly (P<0.05) increased with increasing pH, as shown in (Table 3). The extraction of Cu also increased significantly (P<0.05) by Agaricusbisporous with increase in pH 4.5 to 6.5.

Table 4 shows the effect of pH in the range of 4.5-6.5 on the biosorption rate of heavy metals (Cd) in the effluent samples by *Agaricusbisporous*. The biosorption activity on the effluents significantly (P<0.05) increased with high acidity. At pH 6.5 there was maximum biosorption of Cd. It should be noted that the biosorption of Cd was relatively low at pH 4.5 in comparison with those obtained for fungi at pH 6.5. The increase in biosorption levels with an increase in pH can be explained by the influence of the number of negative surface charges, which depend on the dissociation of functional groups. Heng et al,2013, reported that initial biosorption starts at pH 5.5 ranges to 6.5 which correlate with the findings of present study. They further reported that presence of NaCl and other metals reduces biosorption of Cd and Pb is not affected.

Table 5 shows the decreased rate of biosorption of Cd with increasing temperature in effluents. At 25°C biosorption rate was maximum in effluents. Minimum extraction of Cd was observed at 45°C. 25°C was optimum temperature for the growth of *Agaricusbisporous(P<0.01).*

Table 6 shows significantly (P<0.05) decreased rate of biosorption of Cu with increasing temperature in effluents. At 25°C biosorption rate was maximum in effluents. Minimum extraction of Cu was observed at 45°C. 25°C was optimum temperature for the growth of *Agaricusbisporous.*

Table 5: Effect of temperature on the biosorption of Cd

Effluents Temp for (Cd)			
	25°C	**35°C**	**45°C**
Leather	0.01	0.07	0.014
Medical	0.01	0.012	0.02
Chemical	0.01	0.017	0.019
Paper	0.01	0.018	0.023
Textile	0.01	0.016	0.02

Table 6: Effect of temperature on the biosorption of Cu

Effluents Temp for (Cu)			
Leather	0.02	0.025	0.03
Medical	0.004	0.005	0.009
Chemical	0.027	0.035	0.052
Paper	0.008	0.01	0.02
Textile	0.025	0.03	0.035

Table 7: Effect of concentration on the biosorption of Cd

Effluents Conc. for (Cd)			
	1Disc	**2Disc**	**3Disc**
Leather	0.005	0.002	0
Paper	0.02	0.01	0.005
Medical	0.004	0.001	0
Textile	0.026	0.021	0.01
Chemical	0.025	0.015	0

In contrast to temperature it was observed that biosorption rate significantly (P<0.05) increased with increasing concentration as shown in tables 6 and 7. The extraction rate of Cd, was recorded to increase by Agaricusbisporous fungi with increasing concentration as shown in Table 7.When three discs were added it was recorded that fungi absorbed maximum Cd from all effluents. In all effluents the extraction of Cd was maximum by *Agaricusbisporous*at high concentration. While at low concentration the extraction of Cd was low in paper and chemical effluents as compared to other effluents as shown in Table 7.

Table 8: Effect of concentration on the biosorption of Cu^2

Effluents Conc. for (Cd)			
	1 Disc	**2 Disc**	**3 Disc**
Leather	0.01	0.05	0
Paper	0.04	0	0
Medical	0.01	0.01	0
Textile	0.02	0.02	0
Chemical	0.1	0.05	0

DISCUSSION

The extraction rate of Cu increased by Agaricusbisporous fungi with increasing concentration as shown in table 8,When three discs were added it was recorded that fungi absorbed significantly ($P<0.05$) higher Cu from all effluents.

In leather, medical and chemical effluents the extraction of Cu^2 was maximum by *Agaricusbisporous* at high concentration. While in paper and textile effluents the extraction of Cu was lower than other effluents at high concentration .At low concentration the extraction of Cu was low in paper, chemical and textile effluents as compared to other effluents as shown in table 8.

The biosorption potential assays revealed the selectivity order i.e., $Cu^2 \geq$ Cd by the test fungi. This indicates their variability in metal ions binding affinities for the same or different functional groups (amino, carboxylate, phosphate, sulpha hydrate and thiole) on cell walls. Since in solution all the metal ions are in competition with each other for the available binding sites, a metal that has a higher affinity for particular functional group would bind in greater concentration. Earlier workers have also observed that fungal biosorbents carrying high affinity for Cu^2 and Cd as compared to other metal ions[4].

The low uptake of heavy metals probably arises due to antagonistic effect of other heavy metals ions in biosorption mixture. This may also be related to different electrode potential of various metal ions, resulting in different biosorption affinities[1].Similar concept of stronger chemical and physical affinity for metal ion at greater electronegative bonds and ionic radii has been suggested in other studies[8].

This might be explained by the fact that an excessive complex interaction was involved among several factors such as, different charge/mass ratios,availability of varied functional groups on the fungal cell walls and a variety of cations competing for the same binding sites.Thebiosorption of metals on to the biosorbent are due to electrostatic attraction between the positively charged metal ions and the negatively charged groups of cells wall constituents of the fungal mycelia[2].In biosorption processes, several parameters determine the biosorption rate including structural properties of biosorbents. In our experiment the biosorptive capacity of dead cells was relatively higher indicating the occurrence of both surface biosorption and bioaccumulation mediated by enzymes, which may be active in complexion and binding the metal and its transport eventually depositing the metals into the vacuoles[1]. The present investigation showed that AgaricusBisporous are capable of biosorption of heavy metals from effluents.

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