International Journal of Basic and Applied Biology
Print ISSN: 2394-5820, Online ISSN: 2349-2539, Volume 2, Number 6; July-September, 2015, pp. 348-351

http://www.krishisanskriti.org/ijbab.html

Bioremediation of Cd in Liquid Media using Fungi Isolated from different Contaminated Sites

Daizee Talukdar[1] and Raman Kumar[2]

1Dept of Biotechnology MM University, Mullana Haryana
2Dept of Biotechnology MM University, Mullana Haryana
E-mail: [1]daizeetalukdar28@gmail.com, [2]ramankumar4@gmail.com

Abstract—*Wastewater particularly from cadmium-lead industry, fertilizer producing plants, thermal power plant contain enormous amount of cadmium metal. Cd is known to cause damage to living organism including human beings. Microorganisms including fungi have the capacity to absorb cadmium from a liquid medium. An attempt was, therefore, made to isolate fungi from sites contaminated with heavy metal for removal of Cd from wastewater. 6 fungal isolates tolerant to Cd were isolated from sewage, sludge and industrial samples containing heavy metals. The majority of fungi were able to tolerate upto 600ppm concentration of Cd. The most Cd tolerant fungi (upto 800ppm) were studied for removal of Cd from liquid medium at 100ppm concentration. This Cd tolerant fungus was identified as Aspergillus fumigatus by biochemical identification technique. Further removal of Cd in liquid medium was tested at different pH, incubation time and inoculum size. The results showed that this fungus could remove 76 % of Cd at pH 6 and inoculum size of 8% after incubation time of 144hrs. This indicated the potential of Aspergillus fumigatus as biosorbent for removal of Cd from wastewater containing higher concentration of heavy metals.*

1. INTRODUCTION

Industrial waste and sewage are serious and growing problems in most developing countries. Wastewaters particularly from industries contain toxic heavy metal which enters into human beings and animals through food chain. Management of environmental pollution and its control due to hazardous materials such heavy metals have been a great environmental concern during the last two decades [1]. Cd is mainly used in rechargeable batteries and for the production of special alloys. Although emissions in the environment have markedly declined in most industrialized countries, Cd remains a source of concern for industrial workers and for populations living in polluted areas, especially in less developed countries [2]. Soils normally contain 0.1–1.0 mg of Cd/ kg, while soils in industrial areas may accumulate up to 100 mg of Cd /kg [3]. Chronic cadmium exposure produces a wide variety of acute and chronic effects in humans. Cadmium accumulates in the human body and especially in the kidneys. According to the current knowledge, kidney damage (renal tubular damage) is probably the critical health effect. Other effects of cadmium exposure are disturbances of calcium metabolism, hypercalciuria and formation of stones in the kidney [4-7].

Although conventional physiochemical process like ion exchange, precipitation membrane technologies, electrochemical treatments, activated carbon adsorption, etc can be used for removal of heavy metals but they are not adequate to clean up environmental waste that has been contaminated by diluted metal solutions [8]. Hence biological methods are used for remediation strategies. Cadmium resistant fungus might be present in cadmium contaminated sites. These fungi have the capacity to bind cadmium with the help of cysteine rich protein known as metalloprotein [9-11]. Besides these many other efflux systems are also involved in metal remediation. Hence there is an immense need to isolate such heavy metal tolerant fungus and screen them from effluents which are contaminated with cadmium metal ion. The present study attempts to isolate and screen cadmium tolerant fungi and to evaluate their efficiency to remove cadmium from liquid media under laboratory conditions.

2. MATERIALS AND METHOD

2.1. Collection of sample

Samples of sewage, sludge and industrial effluents were collected in sterilized bottles from different battery industry, fertilizer industry, nuclear power plant and different sewage treatment plant located in Delhi, Chandigarh, Haryana, Punjab, Assam and Jammu. These samples were brought to laboratory and stored at 4^0C.

2.2. Isolation of fungi

Fungal isolates were isolated from samples of sewage, sludge and industrial effluents by serial dilution method using potato dextrose agar (Hi-Media, Mumbai, India) containing 100 ppm of Cd. The 1000 ppm stock solution of Cd was made in double distilled water using $CdCl_2$ (sd fine-chem limited, Mumbai, India). A serial dilution of each sample was made up to 10^6 and one ml of dilution 10^4 and 10^6 was added in sterilized petri plates in duplicate. 20ml of PDA medium containing 100ppm of Cd metal was poured in these petri plates and incubated at 30^0C for 96 hr.

2.3. Screening of fungal isolates for tolerance to Cd

Cd tolerant fungus (100ppm) was further screened for tolerance to Cd at 200, 300, 400, 500, 600, 700 and 800 ppm on PDA plates. Streaking of fungal isolates on normal PDA medium served as control (normal growth) for comparison of growth of fungal isolates on PDA medium containing different concentration of Cd. Observations on growth of fungal isolate were made after 96 hr of incubation.

2.4. Removal of Cd by fungal isolates in liquid medium

The highly Cd tolerant fungal isolates were evaluated for removal of Cd in potato dextrose broth medium containing 100ppm concentration of Cd. Potato dextrose broth containing 100 ppm of Cd was dispensed in 100 ml lots to 250 ml conical flasks and sterilized at 15 lbs/psi for 15 min. These flasks were inoculated with 1 ml of freshly prepared spore suspension (10^6–10^7 spores/ml) of each fungal isolate and put on shaker for 96 hr at 30^0C at 100 rpm. Un-inoculated flasks containing PD broth of 100 ppm concentration of Cd served as control. Fungal growth was harvested after 96 hr through filtration using Whatman filter No. 42**.** Cd concentration in filtrate was estimated by Atomic Absorption Spectrophotometer (GBC932, Semiautomatic) [12]. All the experiments were conducted in triplicate.

2.5. Removal of Cd in liquid medium by fungal isolate at different process conditions

The fungal isolate showing highest removal was evaluated for removal of Cd in PDB medium containing 100ppm Cd at different pH gradients from 2 to 7. PDB medium containing 100ppm Cd was dispensed in 100 ml lots to 250ml conical flask. These flasks were inoculated with 1 ml of freshly prepared spore suspension (10^6–10^7 spores/ml) of each fungal isolate and put on shaker for 96 hr at 30^0C at 100 rpm. Fungal growth was harvested after 96 h through filtration using Whatman filter No. 42. Cd concentration in filtrate was estimated. Similarly, the removal study of Cd in PDB medium containing 100ppm Cd was done at different incubation time. (48, 72, 96, 120, 144 and 196 hrs) and inoculums size (1%, 2%, 4%, 6%, 8%, 10%) . The removal of Cd by fungal biomass was calculated by the following equation:

$$\% \text{ Removal} = \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}} \times 100$$

3. RESULT AND DISCUSSION

3.1. Isolation of Tolerant Fungi

6 fungal isolates (IF1, IF2, IF3, IF4 IF5 and IF6) tolerant to Cd were isolated from samples of sewage, sludge and industrial effluent contaminated with Cd.

3.2. Screening of Fungal Isolates for Tolerance to Cd

6 fungal isolates were further screened for their tolerance to Cd at 200, 300, 400, 500, 600, 700 and 800ppm of Cd. Data indicated decrease in number of isolates tolerant to Cd at higher concentration of Cd metal. Out of 6 fungal isolates tolerant to Cd at 100 ppm, only 2 isolates (IF2 and IF 3) could tolerate Cd at 800 ppm and the rest (IF1, IF4, IF5, IF 6) could tolerate Cd at 600ppm. Isolate IF2 was identified as *Aspergillus fumigatus* by the cultural and biochemical characteristic at Indian Culture Collection, Department of Pathology, Indian Agricultural Research Institute, PUSA, New Delhi.

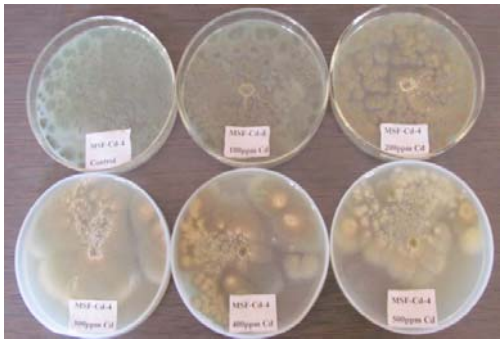


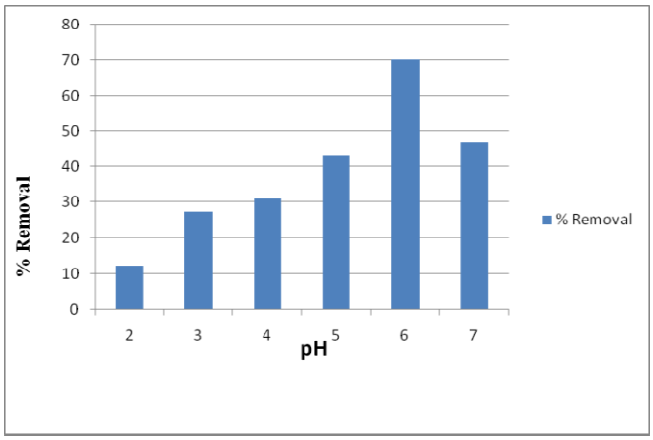
Fig. 1: Growth of isolate IF2 at different Cd concentration

3.3 Removal of Cd by fungal isolates in liquid medium.

Removal studies of all the 6 fungal isolates were carried out in PDB medium containing 100ppm of Cd. The maximum removal (70%) was observed by the fungal isolate IF2 in PD broth containing 100ppm of Cd. Similar results with respect to removal of Cd and other heavy metals by fungi have been reported earlier [13].

3.4. Removal of Cd in liquid medium by fungal isolate at different pH.

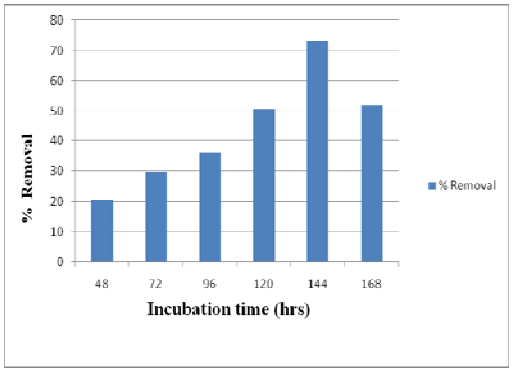
pH value influences the chemistry of heavy metal solution, and it represents one of the most important factor in metal ion adsorption. Consequently, the effect of varying pH between 2 to 7 on removal efficiency of Cd by *Aspergillus fumigatus* was examined. The maximum removal (70.20%) of Cd was found at pH 6. The removal of Cd by *Aspergillus fumigatus* was found to be strongly influenced by pH 6 of the liquid medium. At pH 6 the living cells of fungi could grow significantly. With increase in pH beyond 6, the Cd removal rate decreased, which might be due to the osmotic changes and hydrolyzing effect [14-16]. Most of the microbial surfaces are negatively charged due to the ionization of functional group, thereby contributing to metal binding. Fungal surfaces have a negative charge on pH range two to six. At low pH, cell walls ligands were closely associated with the hydronium ions [H_3O^+] and restricted the approach of metal cations as a result of the repulsive forces [17].



Graph 1: Removal of Cd from liquid medium by *Aspergillus fumigatus* at different pH"

3.5. Removal of Cd in liquid medium by fungal isolate at different incubation time

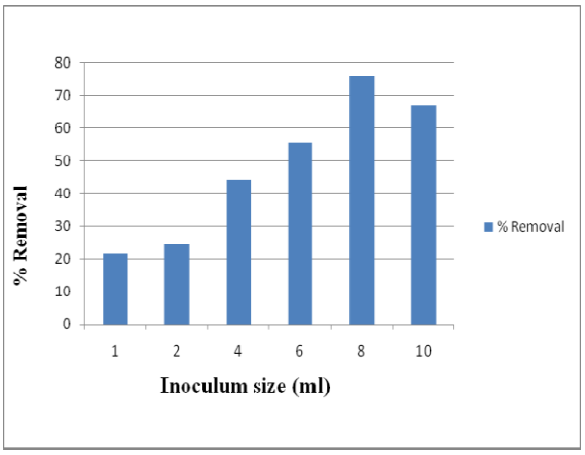
Cd removal efficiency as a function of time of fungal biomass from liquid media is demonstrated**.** *Aspergillus fumigatus* was further evaluated for removal of Cd in liquid medium at different incubation time (hrs). At time interval of 144hrs, *Aspergillus fumigatus* coud remove 73.4% of Cd. It can be seen that the Cd removal by fungi is a relatively lent process and almost reached the maximum level after 6 days. As a result as the incubation period is increased, the rate of removal also increased. After 144hrs of incubation, there was a decrease in removal of Cd. Many researchers have reported that the rate of removal was observed in two phases. An initial phase of faster absorption followed by a phase of slower absorption. This may be due to the abundance of metal species and avaibility of Cd binding sites in the microb [18].



Graph 2: Removal of Cd from liquid medium by *Aspergillus fumigatus* at different incubation time (hrs)

3.6. Removal of Cd in liquid medium by fungal isolate at different inoculums size (ml).

The effect of inoculum size was evaluated for the biosorption process of Cd by fungal biomass in experiments carried out at inoculums size of 1%, 2%, 4%, 6%, 8% and 10% of fungal biomass. Results showed that the maximum removal of Cd was obtained at inoculums size of 8% of fungal biomass. *Aspergillus fumigatus* could remove 76% of Cd in liquid medium.



Graph 3: Removal of Cd from liquid medium by *Aspergillus fumigatus* at different inoculums size

4. CONCLUSION

6 fungal isolates isolated from sewage, sludge and industrial effluents and they were screened for their tolerance to Cd in PDA medium containing Cd from 100 to 800ppm. There was decrease in number of fungi for their tolerance to Cd with increase in concentration of the heavy metal. Majority of the fungal isolate was able to tolerate till 600ppm. All the 6 fungal isolates were further screened for removal in PD broth containing 100ppm of Cd metal. Data revealed that only one fungi could remove substantial amount of Cd (70%) from liquid medium. This fungi was identified as *Aspergillus fumigatus* and further removal of Cd in liquid medium using *Aspergillus fumigatus* at different pH, incubation time and inoculums size was done. Although further studies are needed, these results are very promising as a starting point for a potential application of these microorganisms in bioremediation, including those scenarios affecting agricultural soils, which can be polluted with Cd metal consequence of the incorporation of amendments such as sewage, sludge or municipal solid wastes. This study may be helpful to develop affordable Ecofriendly technology for the treatment of sewage, sludge and industrial effluents before being released in to the environment.

5. ACKNOWLEDGEMENT

The authors are thankful to the Management, MM University, Mullana and Head, Department of Biotechnology, MM University, Mullana, for providing all the laboratory facilities for this work.

REFERENCE

[1] Diels, L., Van Roy, S., Mergeay, M., Doyen, W., Taghavi, S., and Leysen, R., "Immobilization of bacteria in composite membranes and developments of tubular membrane reactors for heavy metals recuperation", 1993, pp. 275–293.

[2] Sethi, P, K. and Khandelwl, D, J., "Cadmium exposure: health hazards of silver cottage industry in developing countries", *Med Toxicol*, 2006, pp. 14-5.

[3] Kabata-Pendias, A. and Pendias, H., "*Trace Elements in Soils and Plants*", 2nd edn. CRC Press Inc,

[4] Nordberg, G., Nogawa, K., Nordberg, M., and Friberg, L., "Cadmium*", In: Handbook on toxicology of metals"*, 2007, pp. 65-78.

[5] Bernard, A. and Lauwerys, R., "Effects of cadmium exposure in humans", *In: Handbook of experimental pharmacology*, 1986, pp. 135-77.

[6] Bernard, A., Roels, H., Buche,t J,P., Cardenas, A., and Lauwerys, R., "Cadmium and health: the Belgian experience", *IARC Sci Publ*, 1992, pp. 15-33.

[7] Hagino, N. and Yoshioka, Y, A., "Study of the etiology of Itai-Itai disease", *J Jpn Orthop Assoc*, 1961, pp. 812-5.

[8] .Matheickal, J.T. and Yu, Q., "Biosorption of lead (II) and copper (II) from aquoues solution by pretreated biomass of Australian marine algae", *Bioresour. Technol*, 1999, pp. 223-229.

[9] Sigel, A., Sigel, H., and Sigel, R.K.O., "Metallothioneins and Related Chelators", *Metal Ions in Life Sciences*, 2009, pp. 7966-7967.

[10] Margoshes, M. and Vallee, B,L., "A cadmium protein from equine kidney cortex", *Journal of American Chemical Society*, 1957, pp. 4813**.**

[11] Turner, J, S., Morby, A, P., Whitton, B, A., Gupta, A., and Robinson, N, J., "Construction of $Zn^{2+/}Cd^{2+}$ hypersensitive cyanobacterial mutants lacking a functional metallothionein locus", *J Biol Chem* , 1993, pp. 4494-8.

[12] Greenberg, A, E., Trussel,l R,R., and Clesceri, L,S., "Standard methods for the examination of water and wastewater", 1985,pp 146-150.

[13] Liu, Y., Fan, T., Zeng, G., Li, X., Tong, Q., Ye, F., Zong, M., Xu, W., and Huang, Y., "Removal of cadmium and zinc from aqueous solution by *Aspergilus niger*", *Trans. Nonferr Met Soc China*, 2006, pp. 681-686.

[14] Qba,l M. and Edyvean, R.G.J., "Biosorption of lead, copperand zinc ions on loofa sponge immobilized biomass of Phanerochaete chrysosporium", *Miner. Eng*, 2004, pp. 217- 223.

[15] Melgar, M.J., Alonso, J. and Garcia, M.A., "Removal of toxicmetals from aqueous solutions by fungal biomass of *Agaricus macrospores". Sci. Total. Environ*, 2007, pp. 16 – 19.

[16] Mungasavalli, D.P., Viraraghavan, T. and Jin, Y., "Biosorption of chromium from aqueous solutions by pretreated *Aspergillus niger, Batch* and column studies", *Colloids Surf. A. Physicochem.Eng.Asp*, 2007, pp.214 – 223**.**

[17] Hasan,S., Mohd Ali, H., Gupta, B.S., " Adsorption of Ni(SO4) on Malaysian rubber–wood ash", *Biores. Technol*, 2000, pp. 153–158.

[18] Garnham, G.W., Godd, G.A., and Godd, G.M., "Accumulation of Co, Zinc, manganese by the estuarine green microalgae *Chlorella Salina* immobillized in alginate beads", *Environ Sci Technol*, 1992, pp.1764-1770.