Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268	Vol. 12	No. 1	pp. 81-86	2013

Original Research Paper

Zinc(II) Removal by Chemically Treated Dead Biomass of Yeast Species

Geetanjali Basak and Nilanjana Das
School of Biosciences and Technology, Environmental Biotechnology Division, VIT University, Vellore-632 014, T.N., India

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 12-6-2012
Accepted: 27-8-2012

Key Words:
Zinc(II), removal
Yeast species
Dead biomass
Pretreatment

ABSTRACT

In the present study, the dead biomass of the two yeast species *viz., Candida rugosa* and *Cryptococcus laurentii* were subjected to various chemical treatments to assess the effects of pretreatment on zinc(II) removal from aqueous solution. Yeast biomass was pretreated with anionic surfactants *viz.,* sodium dodecyl sulphate (SDS), sodium dodecyl benzene sulphonate (SDBS) and dioctyl sulphosuccinate sodium (DSS), alkali (sodium hydroxide, sodium carbonate and sodium bicarbonate), acids (hydrochloric acid, sulphuric acid and acetic acid), and organic solvents *viz.,* methanol, formaldehyde and gluteraldehyde. Pretreatment of dead yeast biomass with anionic surfactants was found to improve the zinc(II) removal remarkably compared to all other treatments. Acid treatments resulted in significant reduction in zinc(II) removal efficiency. The pattern of zinc(II) removal efficiency of both the yeast species was found to follow the order: SDS (3 mM) > SDBS (3 mM) > DSS (3mM) > Na_2CO_3 (9 mM) ≥ NaOH (9 mM) ≥ untreated biomass > $C_2H_5O_8$ (7 mM) ≥ $NaHCO_3$ (9 mM) > CH_3OH (7 mM) > HCHO (7 mM) > CH_3COOH (5 mM) > HCl (5mM) > H_2SO_4 (5 mM). Maximum zinc(II) removal was noted in case of SDS treated *C. rugosa* and *C. laurentii* which exhibited 84.7 % and 74.5 % zinc(II) removal compared to the removal efficiency of 65.4 % and 54.8 % obtained by untreated *C. rugosa* and *C. laurentii* .

INTRODUCTION

Zinc is an essential metal required in trace quantities for growth and metabolism but may cause physiological and ecological problem when its concentration exceeds that required for correct biological functioning (Chapman et al. 1995). In the Dangerous Substances Directive (76/464/EEC) of the European Union, zinc has been registered as List 2 dangerous substance with environmental quality standards being set at 40µg/L for estuaries and marine waters and at 45-500 µg/L for freshwater depending on water hardness (The Council of the European Communities 1976). Zinc is commonly used in coating iron, wood preservatives, catalysts, photographic paper, ceramics, textiles, fertilizers, pigment and batteries (USDHHS 1993) and as a result it is often found in the wastewaters arising from these processes.

Conventional methods for removal of heavy metal ions from industrial wastewaters include chemical precipitation, chemical oxidation or reduction, reverse osmosis and ion exchange and adsorption, etc. (Janson et al. 1982, Grosse 1986). However, the application of these methods is often limited due to their inefficiency, high capital investment or operational cost. Therefore, exploitation of biological methods through utilization of biomaterials for uptake of heavy metal from dilute aqueous solution has been proposed by many researchers. Biosorption using cell biomass is getting due attention, because of the diversity and inexpensive materials used in this method (Kratchovil & Volesky 1998). Use of non-viable, dead biomass is advantageous in compared to the living microorganisms for biosorption process. Dead biomass can be easily regenerated after the recovery of adsorbed metal ions and can avoid the problem of toxicity of heavy metals in contrast to living cells. Moreover, pretreatment of biomass either by physical or chemical treatments (Javaid et al. 2011, Zhang et al. 2010, Goksungur et al. 2005) is known to improve the biosorption capacity of biomass. Chemical pretreatment methods such as using acids, alkalis and organic chemicals enhance or reduce metal removal depend on the adsorbents used and treatment procedures (Kapoor & Viraghavan 1998, Yan & Viraghavan 2000, Kiran et al. 2005, Bajwa et al. 2009). So far, no work is reported on zinc removal using chemically treated dead yeast biomass as adsorbent. Therefore, the aim of this study was to investigate the effect of chemical pretreatment of dead yeast biomass *viz., C. rugosa* and *C. laurentii* on removal of zinc(II) ions from aqueous solution in batch system.

MATERIALS AND METHODS

Chemicals: Zinc(II) stock solution was prepared (1000 mg/L) by dissolving 4.55 g of powdered $Zn(NO_3)_2.6H_2O$ (Hi Media, Mumbai, India) in 1000 mL of deionised water. The working solutions of metal were prepared by diluting the stock solution to desired concentrations.

Biosorbent preparation: Two yeast species *viz., Candida rugosa* and *Cryptococcus laurentii* were isolated from

Common Effluent Treatment Plant (CETP), Ranipet, Vellore, Tamilnadu, India. The yeasts were phenotypically characterized and identified to a species level by VITEK 2 Compact Yeast card reader with software version V2C 03..01 at the Council for Food Research and Development (CFRD), Kerala, India. The isolates were maintained in yeast extract peptone dextrose (YEPD) agar slants at 4°C. Mass cultivation of yeast isolates were carried out in inexpensive sugarcane bagasse extract medium as reported in our previous study (Basak et al. 2011). The yeast biomass was harvested by centrifugation at 10,000 rpm for 5 min and subjected to successive washings with double distilled water to remove the culture broth.

Chemical pretreatments of the yeast biomass: Stock solution of various chemicals *viz.*, alkali (NaOH, Na_2CO_3 and $NaHCO_3$), acid (HCl, H_2SO_4 and CH_3COOH), anionic surfactants (SDS, SDBS and DSS) and organic solvents (HCHO, CH_3OH and $C_2H_5O_8$) were prepared separately. The effect of pretreating reagent concentration on removal of zinc(II) ions was studied by treating the biosorbents (5 g/L of dead yeast biomass of yeast species) with 100 mL solution *viz.*, alkali (NaOH, Na_2CO_3 and $NaHCO_3$), acid (HCl, H_2SO_4 and CH_3COOH), anionic surfactants (SDS, SDBS and DSS) and organic solvents (HCHO, CH_3OH and $C_2H_5O_8$) each at concentration ranging from 1-21 mM. All the pretreated samples were agitated in shaker at 120 rpm for 24 h. Then they were extensively washed with distilled water until the pH of the wash solution reached neutral range (pH 6.8 to 7.2) and subjected to oven drying at 60°C for overnight. All the pretreated dried yeast biomass was powdered and used for the zinc removal studies.

Batch studies for removal of zinc(II) ions by pretreated biomass: Batch removal experiments were conducted using zinc(II) ion added in the form of $Zn(NO_3)_2.6H_2O$. Each type of pretreated biomass (0.15 g) was added to 100 mL of 90 mg/L of zinc(II) ion at pH 6.0. The reaction mixture along with biomass was agitated in orbital shaker at room temperature and 120 rpm. After 4h of contact time, the samples were withdrawn and subjected to centrifugation at 10,000 rpm for 5 min. The residual metal ion concentrations were determined using Atomic Absorption Spectrophotometer (Varian AA- 240, Australia). Negative controls (without sorbents) were taken to ensure that removal was only by dried biomass of yeast species *viz.*, *C. rugosa* and *C. laurentii*. Batch experiments were conducted in triplicate and average values were used in the analysis.

The zinc(II) removal percentage using yeast biomass was calculated by using the following equation:

$$\text{Zinc(II) removal \%} = \frac{C_i - C_f}{C_i} \times 100 \qquad ...(1)$$

Where C_i is the initial concentration of zinc(II) ion (mg/L). C_f is the final concentration of zinc(II) ion (mg/L).

RESULTS AND DISCUSSION

A series of experiments in batch mode were carried out using raw dead biomass of yeast and the biomass pretreated with different chemicals in order to study the effect of chemical pretreatments on zinc(II) removal.

Effect of alkali treatments on Zn(II) removal: In the present study, yeast biomass was pretreated with different alkalis (NaOH, Na_2CO_3 and $NaHCO_3$) at different concentration to evaluate the zinc(II) removal from aqueous solution. Fig. 1a and Fig. 1b showed the effect of alkali pretreatment of yeast biomass *viz.*, *C. rugosa* and *C. laurentii* respectively on the removal of zinc(II) ion. It was observed that zinc(II) removal efficiency increased when the alkali concentration was increased from 1 to 9 mM. However, the zinc(II) removal efficiency of the biomass treated with alkali at a concentration above 9mM reduced significantly. This implied that the pretreatment with NaOH, Na_2CO_3 and $NaHCO_3$ above 9mM concentration caused serious destruction of cell structure, which resulted in a lower removal of zinc(II) ions (Junlian et al. 2010). Zinc(II) removal efficiency of *C. rugosa* pretreated with NaOH, Na_2CO_3 and $NaHCO_3$ was found to be 65.4 %, 66.1 % and 64.5 % respectively. Whereas, *C. laurentii* showed efficiency of 54.8 %, 55.4 % and 53.4 % respectively.

Effect of acid treatments on Zn(II) removal: Experiments were performed to study the effect of different concentration of acids (HCl, H_2SO_4 and CH_3COOH) on removal of zinc(II) ions. Zinc removal efficiency was increased with the increase in acid concentration ranging from 1 mM to 5 mM for both *C. rugosa* and *C. laurentii* respectively (Fig. 2a and Fig. 2b respectively). However at a concentration above 5mM for all the acids, there was a decrease in zinc(II) removal efficiency for both the yeast species. This might be due to serious destruction of the cell surface structure leading to the loss of some of the negatively charged groups (Junlian et al. 2010). In case of *C. rugosa* pretreated with HCl, H_2SO_4 and CH_3COOH at 5 mM concentration, zinc(II) removal was noted as 33.1 %, 26.2 % and 43.1 % respectively, whereas *C. laurentii* pretreated with HCl, H_2SO_4 and CH_3COOH showed less zinc(II) removal efficiency of 27.8 %, 19.1 % and 35.5 % respectively.

Effect of anionic surfactant treatments on Zn(II) removal: A remarkable increase in zinc(II) removal efficiency was noted at 3mM of anionic surfactants (SDS, SDBS and DSS) concentration which was found to be 84.7 %, 76.2 % and 70.8 % respectively for *C. rugosa* and 74.5 %, 69.2 % and 63.7 % respectively for *C. laurentii* (Fig. 3a and Fig. 3b

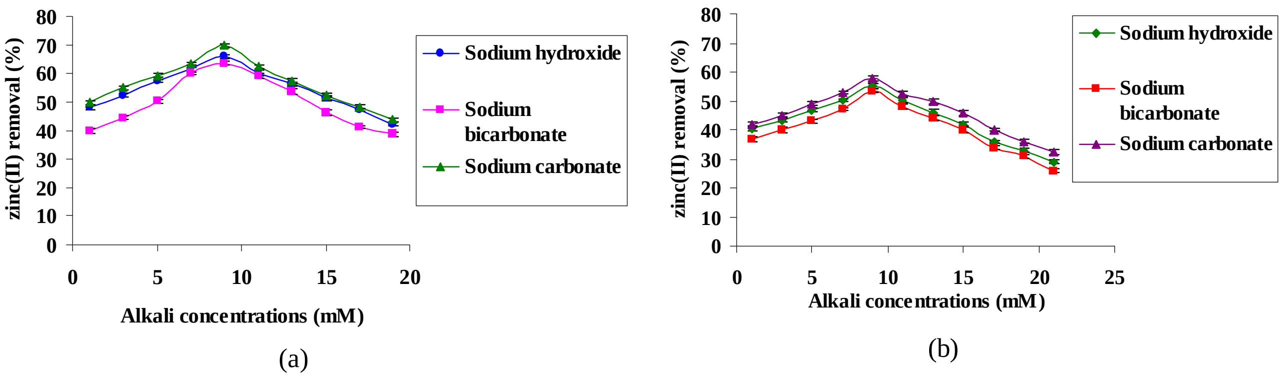

Fig. 1: Effect of alkali treatment on zinc(II) removal using two yeast species: (a) *C. rugosa* and (b) *C. laurentii.*

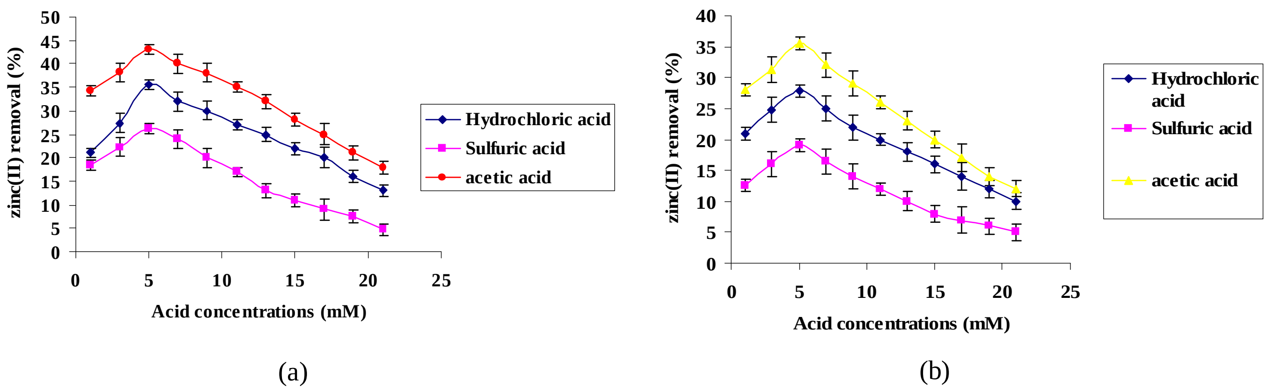

Fig. 2: Effect of acid treatment on zinc(II) removal using two yeast species: (a) *C. rugosa* and (b) *C. laurentii.*

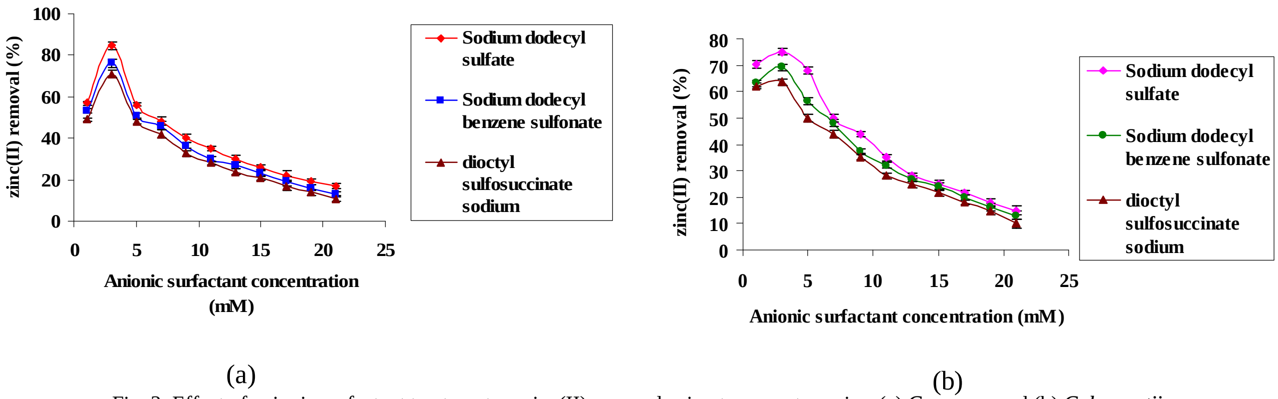

Fig. 3: Effect of anionic surfactant treatment on zinc(II) removal using two yeast species: (a) *C. rugosa* and (b) *C. laurentii.*

respectively). Further increase in the concentration of anionic surfactant decreased the removal percentage of zinc(II) ions. Among all the anionic surfactants, SDS showed the maximum removal of zinc(II) ions, which might be due to the increased basicity of SDS treated yeast biomass compared to the SDBS and DSS treated biomass (Ahn et al. 2009). The critical micelle concentration (CMC) of SDS is 8 mM. The percentage of zinc(II) removal increases in presence of SDS below its CMC value (8mM) (Lin et al. 1990). A decrease in adsorption above CMC may be due to slow transfer of micelles-metal complex from bulk to the surface of the adsorbent. These micelles possess a hydrophobic interior and exterior, causing them to behave like dispersed oil drops. The interaction between the micelles and ion species occurs mainly through hydrogen bonding and electrostatic forces (Shimamoto & Mima 1979).

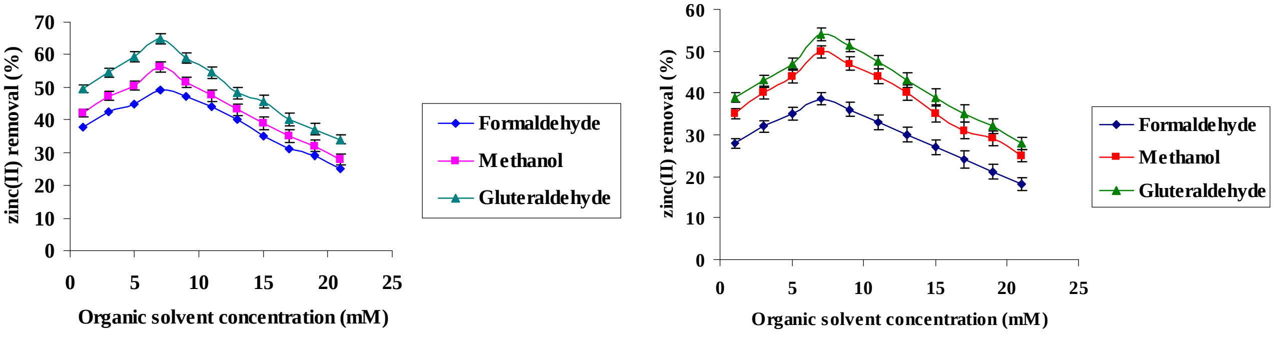

Fig. 4: Effect of organic solvent treatment on zinc(II) removal using two yeast species: (a) *C. rugosa* and (b) *C. laurentii.*

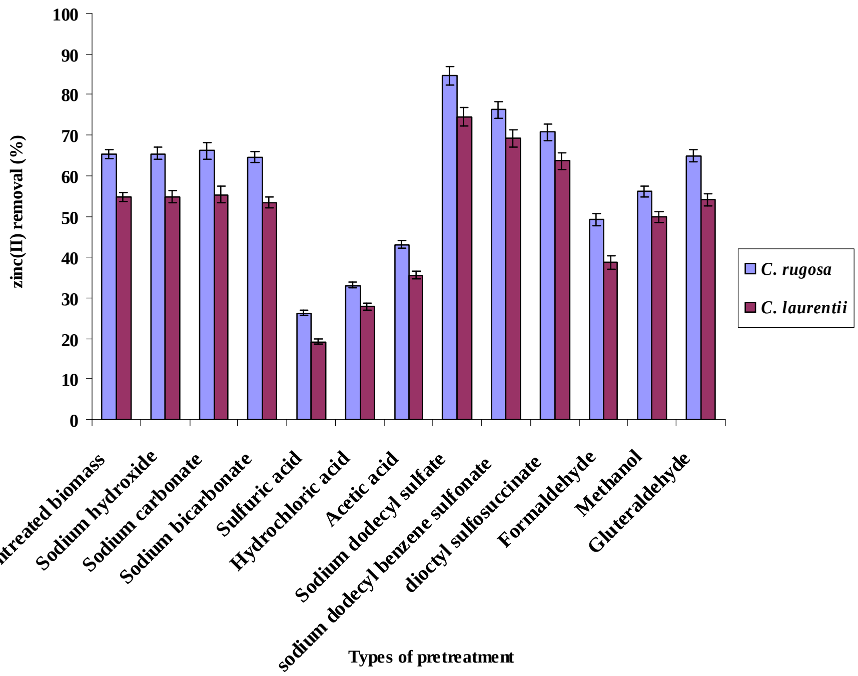

Fig. 5: Comparative studies on zinc(II) removal using pretreated dead biomass of *C. rugosa* and *C. laurentii.*

Effect of organic solvent treatments on zinc(II) removal: Experiments were performed to evaluate the effect of different organic solvents (HCHO, CH_3OH and $C_2H_5O_8$) on removal of zinc(II) ions. Zinc removal efficiency was increased with the increase in solvent concentration ranging from 1 mM to 9 mM for both *C. rugosa* and *C. laurentii* respectively (Fig. 4a and Fig. 4b respectively). *C. rugosa* pretreated with 9mM of HCHO, CH_3OH and $C_2H_5O_8$ showed zinc(II) removal efficiency of 49.2 %, 56.2 % and 64.9 % respectively. *C. laurentii* pretreated with 9mM of HCHO, CH_3OH and $C_2H_5O_8$ showed zinc(II) removal efficiency of 38.7 %, 49.9 % and 54.1 % respectively.

Comparative studies on zinc(II) removal using chemically pretreated yeast biomass: Batch experiments were performed to compare the zinc(II) removal potential of pretreated yeast biomass and untreated biomass at optimized concentration for each chemical. The effect of pretreatments using different chemicals on zinc(II) removal by both the yeast species is shown in Fig. 5. The results showed that the dead yeast biomass *viz., C. rugosa* and *C. laurentii* treated with Na_2CO_3 (9 mM) showed slight increase in zinc(II) removal 66.1 % and 55.4 % compared to the untreated *C. rugosa* (65.4 %) and untreated *C. laurentii* (54.8 %) compared to other chemicals. *C. rugosa* treated with NaOH and $NaHCO_3$ (9 mM) respectively showed zinc(II) removal efficiency of 65.5 % and 64.5 % respectively. Pretreatment using alkali chemicals i.e., NaOH, did not show any improvement and $NaHCO_3$ showed reduction in zinc(II) removal. It could be due to chemical modifications of the cell wall components. The modification of biomass due to NaOH treatment probably destroys autolytic enzymes that cause putrification of biomass and remove lipids and proteins that mask the reactive sites (Muraleedharan & Venkobachar 1990). Reduction in zinc(II) removal efficiency due to $NaHCO_3$ treatment could be the result of more affinity of active chemical groups (HCO_3^-) ion to the cell wall components of the adsorbent (Javaid et al. 2011).

Pretreatments of yeast biomass using acids like H_2SO_4, HCl, CH_3COOH showed significant reduction in the zinc(II) removal efficiency in case of both the yeast species (Fig. 5). Similar results were reported in case of *Aspergillus niger* (Kapoor & Viraraghavan 1998) and *Aspergillus fumigatus* (Saleh et al. 2009). It could possibly be explained in terms of H^+ binding to the biomass after acid treatment being responsible for the decrease in removal of zinc(II) ions. This indicated that the acid destroyed the absorbing groups and their positive ions (H^+) may covalently bonded to the absorbing surface. According to Bux & Kasan (1994), removal of heavy metal cation depends on electronegativity of biomass. Thus, the remaining H^+ ions on the acidic pretreated yeast biomass may change electronegativity for both the yeast isolates thereby reducing the biosorption efficiency.

Pretreatment with anionic surfactant enhanced the removal of zinc(II) ion to a greatest extent compared to the untreated biosorbents. Yeast biomass *viz., C. rugosa* and *C. laurentii* treated with SDS (3 mM) showed the highest zinc(II) removal efficiency (84.7 % and 74.5 % respectively) followed by treatment with SDBS (3 mM) and DSS (3 mM) for *C. rugosa* and *C. laurentii* respectively. In this study, treatment of yeast biomass with anionic surfactants reduced its total acidity and increased its total basicity. This result implied that these anionic surfactants successfully covered the surface of the yeast biomass. Specifically, total acidity of the yeast biomass was decreased because the surfactants covered surface acidic groups, especially carboxylic groups. The hydrophilic head(s) of the surfactants can act as basic functional groups. In aqueous solutions, the bound anionic surfactants can be dissociated, and then the protons bind to the hydrophilic head(s), resulted an increase of the adsobents total basicity (Ahn et al. 2009).

Among the various organic solvents used for zinc(II) removal, gluteraldehyde treated biomass showed improvement in zinc(II) removal was noted by gluteraldehyde treatment followed by methanol and formaldehyde. The treatment of biomass with methanol caused esterification of the carboxylic acid present on the cell wall. The metal binding ability of carboxyl groups was reduced as a result of esterification (Drake et al. 1996). In case of formaldehyde treatment, the results revealed that amino groups present on the cell wall of yeast biomass gets methylated due to chemical modification with formaldehyde. Thus, the methylation of amino groups reduced the metal ions binding on the biomass residue (Loudon 1984).

CONCLUSIONS

Based on the results of the present study, it can be concluded that anionic surfactant (SDS) treated dead yeast biomass used as adsorbent showed maximum removal of zinc(II) ions from the aqueous solution compared to all other pretreatments.

ACKNOWLEDGEMENT

Authors of this article would like to thank VIT University for providing Lab facility and financial support for the smooth conduct of the work.

REFERENCES

Ahn, C.K., Park, D., Woo, S.H. and Park, J.M. 2009. Removal of cationic heavy metal from aqueous solution by activated carbon impregnated with anionic surfactants. J. Hazard. Mater., 164: 1130-1136.

Bajwa, R., Javaid A. and Manzoor, T. 2009. Ni(II) and Cu(II) removal by chemically treated biomass of *Rhizopus arrhizus*. Pak. J. Phytopathol., 21(1): 45-48.

Basak, G., Charumathi, D. and Das, N. 2011. Combined effects of sugarcane bagasse extract and Zinc(II) ions on the growth and bioaccumulation properties of yeast isolates. Int. J. Eng. Sci. Tech., 3(8): 6321-6334.

Bux, F. and Kasan, H.C. 1994. Comparison of selected methods for relative assessment of surface charge on waste sludge biomass. Water SA., 20: 73-76.

Chapman, M., Peter, H, Allen, E., Godtfredsen, K. and Zgraggen, M.N. 1995. Evaluation of bioaccumulation factors regulating metals. Environ. Sci. Technol., 30: 448A- 451A.

Drake, L.R., Lin, S., Rayson, G.D. and Jackson, P.J. 1996. Chemical modification and metal binding studies of *Datura innoxia*. Environ. Sci. Technol., 30: 110-114.

Goksungur, Y., Uren, S. and Guvenc, U. 2005. Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass. Biores. Technol., 96: 103-109.

Groose, D.W.J. 1986. A review of alternative treatment processes for metal bearing hazardous waste streams. Air Pollut. Control Assoc., 36: 603-614.

Janson, C.E., Kenson, R.E. and Tucker, L.H. 1982. Treatment of heavy metals in wastewaters. Environ. Prog., 1: 212-216.

Javaid, A., Bajwa, R. and Manzoor, T. 2011. Biosorption of heavy metals by pretreated biomass of *Aspergillus niger*. Pak. J. Bot., 43(1): 419-425.

Junlian, Q., Lei, W., Hua, F.X. and Hong, Z.G. 2010. Comparative study on the Ni^{2+} biosorption capacity and properties of living and dead *Pseudomonas putida* cells. Iran. J. Chem.Chem. Eng., 28(1): 159-167.

Kapoor, A. and Viraraghavan, T. 1998. Biosorption of heavy metals on *Aspergillus niger*: Effect of pretreatment. Biores. Technol., 63: 109-113.

Kiran, I., Akar, T. and Tuneli, S. 2005. Biosorption of Pb(II) and Cu(II) from aqueous solutions by pretreated biomass of *Neurospora crassa*. Process Biochem., 40: 3350-3358.

Kratchovil, D. and Volesky, B. 1998. Advances in the biosorption of heavy metals. Tibtech., 16(7): 291-300.

Lin, S.Y., Keigue, K. and Malderelli, C. 1990. Diffusion controlled surface adsorption studied by pendant drop digitization. AIChe J., 36: 1785-1795.

Loudon, G.M. 1984. Organic Chemistry Reading, Massachusetts, USA, pp. 1196.

Muraleedharan, T.R. and Venkobachar, C. 1990. Mechanism of biosorption of Cu^{2+} by *Ganoderma lucidium*. Biotechnol. Bioeng., 35: 320-325.

Saleh, M.A.G., Khaled, M.G. and Abdulaziz, S.B. 2009. Biosorption characteristics of *Aspergillus fumigatus* in removal of cadmium from an aqueous solution. Afr. J. Biotechnol., 8: 4163-4172.

Shimamoto, T. and Mima, H. 1979. Effect of polyols on the interaction of p-hydroxybenzoic acid esters with polyoxyethylene dodecyl ether. Chem. Pharmacol. Bull., 27: 2602-2617.

The Council of the European Communities 1976. Directive-76/464/EEC-on pollution caused by certain dangerous substances discharged into the aquatic environment of the community. Off. J. Eur. Commun., L., 129: 23-29.

USDHHS 1993. Toxicological Profile for Zinc. US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, Georgia.

Yan, G. and Viraraghavan, T. 2000. Effects of pretreatment on the bioadsorption of heavy metals on *Mucor rouxi*. Water SA., 26(1): 119-123.

Zhang, Y., Liu, W., Xu, M., Zheng, F. and Zhao, M. 2010. Study of the mechanisms of Cu^{2+} biosorption by ethanol/caustic-pretreated baker's yeast biomass. J. Hazard. Mater., 178: 1085-1093.