مجلة اتحاد الجامعات العربية

Biosorption of Heavy Metals onto Two Types of Fungi Biomass in Batch Experiments

Shahlaa Esmail Ebrahim.
Assist. Prof
Nadhem Hasan Hyder
Assist. Prof
Yousif Mohammed Yousif
Department of Environmental Engineering
University of Baghdad \Iraq

Abstract

Biosorption of lead, cadmium and zinc ions from synthetic wastewaters onto two types of fungi biomass were studied. Batch experiments for spent mushroom (macro-fungi) and cultured fungi (micro-fungi) Fusarium oxisporium biomass were performed. Spent mushroom biomass was found to have maximum uptake adsorption, 73.4, 34.38 and 24.48 mg/g for lead, cadmium and zinc ions, respectively, while cultured fungi biomass uptakes were 51.66, 24.52 and 20.46 mg/g for lead, cadmium and zinc ions, respectively. Optimum conditions were selected for biosorption uptake onto fungi biomasses. The best pH was found to be 4 for Pb(II), 5 for Cd(II) & Zn(II). The optimum contact time was found to be 2 hr for all metal ions. Optimum mean particle size was found to be 0.3 mm. Nine equilibrium isotherm models were used for representing the experimental data. Langmuir isotherm model gave the best fit model for all metal ions.

1-Introduction

Increased use of chemicals and metals in process industries has resulted in producing of large quantities of effluent that contain high level of toxic heavy metals which poses environmental-disposal problems due to their persistence and non-degradable nature. Environmental engineers are faced with the challenging duty to develop appropriate low cost methods for effluent treatment [**2**]. Conventional heavy metals removal has certain major disadvantages at concentrations lower than 100 mg/l. Moreover these processes may need expensive equipment and monitoring systems, and high energy or reagent costs, as a result producing toxic sludge and/or other waste products and incomplete metal removal. Therefore, effective new sustainable methods are required to diminish heavy metals concentrations to environmentally acceptable limits at a reasonable cost. Lately, biosorption has regarded as an alternative heavy metals removal method. The major advantages of the biosorption are low cost, high efficiency in reducing low concentrations of heavy metals, the recovery of heavy metals from the biosorbent could be easily accomplished and no toxic sludge generation [**1**]. The term "biosorption" is used to depict the accumulation of metal ions from a solution by biological materials. Biosorbents are prepared from materials naturally found in great quantity and/or waste biomass of fungi, algae or bacteria [**12**]. According to these types of biosorbents, fungal biosorbents have some advantages: *Firstly*, fungus shows excellent metal removal because of the diversity of functional groups existing in high percentages in the cell-wall material. *Secondly*, compared with some biosorbents such as algal biomass or plant products, fungus is easy to culture at large scales as it has a short reduplication cycle. Moreover, it can be easily grown using fermentation techniques and inexpensive growth media. *Thirdly*, fungal biomass can be obtained from industrial waste products e.g. (brewery industry waste or waste from citric acid production). *Fourthly*, a major portion of fungal biomass are non-pathogenic; thus they are generally considered as safe and are easily accepted by the public when implemented practically [**13,7**]. In this research batch experiments for spent mushroom (macro-fungi) and cultured fungi (micro-fungi) biomass were studied as biosorbents for the removal of lead, cadmium and zinc.

2. Materials and Experimental Procedure

2.1. Adsorbate

Stock solutions of lead, cadmium and zinc ions with a concentration of (1000 mg/l) were prepared by using $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_2O$ and $Zn(NO_3)_2.6H_2O$ (minimum purity 99 %). The desired concentrations were obtained from stock solutions after dilution. Dissolved metal concentrations were determined by atomic absorption spectrophotometer (GBC 933 plus, Australia) at Environmental Engineering Department/Collage of Engineering/University of Baghdad.

2.2. Biosorbent

2.2.1 Biomass Preparation From Micro – Fungi

Six types of fungus; *Fusarium oxisporium, Rhizopus sp., Aspergillus terrous, Chysonilla sp., Alternaria alternata, and Aspergillus niger* were used in this study. The fungus species were isolated from agriculture soil (rhizospher zone of plants). The isolates were obtained and identified in the Department of Biotechnology/ College of Science/ University of Baghdad. They were maintained on Potato dextrose agar (PDA), stored at 4 ºC and renewed every month for further cultured experiments **[6]**. The isolated fungi were prepared in 2% (w/v) malt extract broth at pH= 5, all culture work was conducted aseptically. The biomass was carried out in 250 ml conical flask containing 50 ml of malt extract broth, the flasks were inoculated with 3 plugs of (8 mm) fungus isolates mycelium, excised from the margin of 7 days old culture PDA plates. The flasks were incubated at 28 ºC and 150 rpm in an orbital shaker incubator for 72 – 96 h. After fermentation; biomass was harvested by filtering the cultured medium through 0.45 µm membrane filter under vacuum pump. Then the biomass was dried in an oven at 60 ºC for 2 days, and stored in glass vials with screws **[22, 8]**. The biomass was utilized in further biosorption studies to select the best cultured fungus that gives the best metal removal.

2.2.2. Biomass Preparation from Macro – Fungi

Fruit bodies of spent mushrooms (*Agaricus bisporus*) were procured from the mushroom farm at College of Agriculture/ University of Baghdad. Spent mushrooms were dried at atmospheric temperature (37-45°C) for 5 days and washed thoroughly with distilled water to remove the dirt and impurities and later dried in an oven at 60 ◦C for 8 hr. The dried fruit bodies for both types of fungi were then pulverized in a mortar and sieved with different mean particle.

The geometric mean diameter for the different particle size is given by $d_{gm} = (d_1 d_2)^{1/2}$ where d_1 is the diameter of lower sieve on which the particles are retained and d_2 is the diameter of the upper sieve through which the particles pass **[4]**.

Physical characteristics of spent mushroom biomass are listed in **Table1.** The measurements of surface area and particle porosity were carried out in University of Missouri-Columbia and the other measurements in Ministry of Oil / Oil Research and Development Center, Baghdad, Iraq.

Table 1: Physical characteristic of fungi biomass

Spent Mushroom Biomass	
Physical characteristic (fungi biomass)	
Mean particle diameter, mm	0.3
Surface area (m^2/g)	4.63
Actual density (kg/m^3)	1480
Bulk density (kg/m^3)	637
Particle porosity	0.6876

Scanning Electron Microscopy (SEM) by (Hitachi S4700, Japan) and Transmission Electron Microscopy (TEM) by (JEOL JEM 1400, Japan) were used to characterize the spent mushroom biomass material and give a better image of the biomass at the libraries of Veterinary Medicine Collage/ University of Missouri-Columbia (USA) as shown in **Figures 1 & 2**.

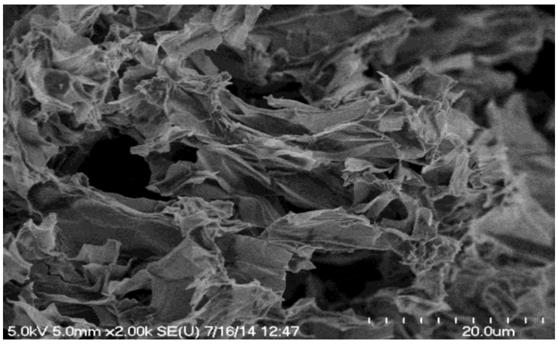

Figure1: SEM test image at 2000 magnification

SEM technology shows the surface morphology of the biosorbent, while TEM technique is used for visualization of biomass where an internal view of the biomass can be obtained **[14]**.

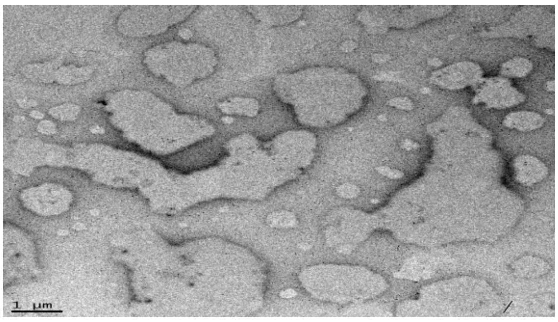

Figure 2: TEM test image at 2000 magnification

2.3. Experimental Procedure

2.3.1. Effect of pH

The effect of pH on Pb(II), Cd(II) and Zn(II) biosorption onto fungi biomass was studied. Different pH values ranging from 2 to 8 were maintained by using 0.1 M NaOH or HNO_3. A fungi biomass of 0.1 g was mixed with 100 ml of single metal ion solutions; concentration of 50 mg/l of metal ions agitated at 200 rpm for a period of 4 h and at room temperature (25°C). Samples (10 ml) were taken from each volumetric flask and filtered using filter paper (type: WATMAN No.42,) the remaining concentration of the metals measure by using Atomic Absorption (AA). The biosorption efficiency η was calculated by the difference of the initial and equilibrium concentration of the metal ions according to the following equation **[24]**:

$$\eta = \frac{(C_o - C_e)}{C_o} \times 100 \qquad (1)$$

where C_o : initial metal concentration mg/l.

C_e: final metal concentration mg/l.

2.3.2. Effect of Contact Time

The removal of metal ions was also studied as a function of contact time at optimum pH. The rate at which biosorption takes place is an important factor when designing biosorption systems. Consequently, it is necessary to establish the time dependence of metal removal for maximizing the rate of metals uptake by the biosorbent during equilibrium. Using the same procedure used in section (2.3.1) at time interval 0-240 min. The time in which no further metal ions removal can be attained, was considered as the optimum contact time.

2.3.3. Equilibrium Isotherm Experiments

Different weights of micro/macro fungi biomass were used, (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, and 0.6) g dry biosorbents were placed in 12 volumetric flasks of 250 ml using the same procedure in section (2.3.1). The pH of the metal solutions was adjusted to the optimum values. The flasks were then placed on an Incubator shaker (Lab S1-600R, Korea) and agitated continuously for 2 h at 200 rpm at room temperature. The samples filtered by filter paper, few drops of 0.1M HNO_3 were added to samples to decrease the pH value below 2 in order to fix the concentration of the heavy metals during storage before analysis **[5]**. The adsorbed amount is then calculated by the following equation:

$$q_e = \frac{V_l(C_o - C_e)}{W_{(wet/dry\ base)}}. \qquad (2)$$

Where v_1: volume of the metal solution (L).

The adsorption isotherms were obtained by plotting the weight of solute adsorbed per unit weight of biomass (q_e) against the equilibrium concentration of the solute in the solution (C_e) **[24]**.

3. Adsorption Isotherm Models

Nine models for single system have been tested in the present work for both types of fungi biomass and are represented in **Table2:**

Table (2): Adsorption isotherm models

Isotherm model	Equation	References
Langmuir	$q_e = \frac{q_{max} b C_e}{(1 + bC_e)}$	**[13,14,15]**
Freundlich	$q_e = KC_e{}^{1/n}$	**[16,17]**
Temkin	$q_e = \frac{RT}{b_{Te}} \ln(a_{Te} C_e)$	**[18,19]**
Redlich and Peterson	$q_e = \frac{K_{RP} C_e}{1 + a_{RP} C_e^{\beta_{RP}}}$	**[20,21]**

Slips	$q_e = \dfrac{K_s C_e^{\beta_s}}{1 + a_s C_e^{\beta_s}}$	**[22,23]**
Khan	$q_e = \dfrac{q_{max} b_k C_e}{(1 + b_k C_e)^{a_k}}$	**[23,24]**
Radke–Praunsitz	$q_e = \dfrac{K_{RP} C_e}{1 + \left(\dfrac{K_{RP}}{F_{RP}}\right) C_e^{1-N_{RP}}}$	**[15]**
Toth	$q_e = \dfrac{q_{max} b_T C_e}{\left[1 + (b_T C_e)^{\frac{1}{n_T}}\right]^{n_T}}$	**[23]**
Dubinin and Radushkevich	$q_e = Q_D exp[-K_D(\mathcal{E}_D)^2]$	**[25]**

4. Results and Discussion

4.1. Selection the Best Cultured Micro Fungus

Six types of cultured fungus (*Rhizopus sp., Aspergillus terrous, Fusarium oxisporium, Chysonilla sp., Alternaria alternata, and Aspergillus niger*) were tested for the best heavy metals removal as shown in **Figure3.** It's clear from this figure that *Fusarium oxisporium* showed the best removal efficiency than the other types, and it was 69.42, 43.64 and 37.90 % for lead, cadmium and zinc ions, respectively, therefore these results encourage using this type in the following experimental work.

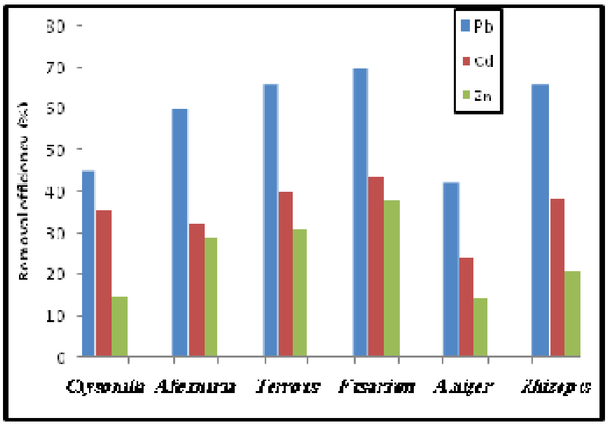

Figure 3: The removal efficiency of different types of cultured fungi to remove Pb(II), Cd(II) and Zn(II)

4.2. Effect of pH

In order to evaluate the influence of pH on sorption of the metal ions, the experiments were carried out in pH range of 2–8. For both *Fusarium oxisporium* and spent mushroom biomass the results are shown in **Figures4and 5.**

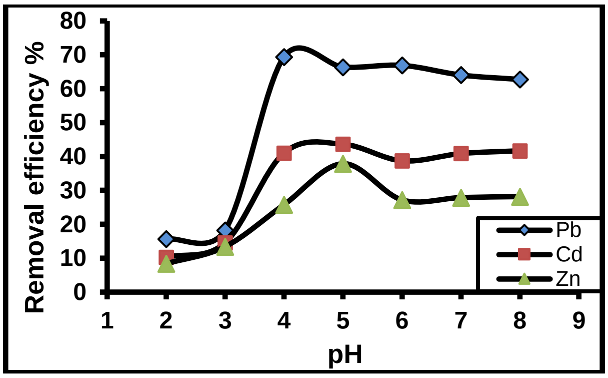

Figure 4: Effect of different pH on lead, cadmium and zinc ions removal efficiencies by cultured fungi biomass

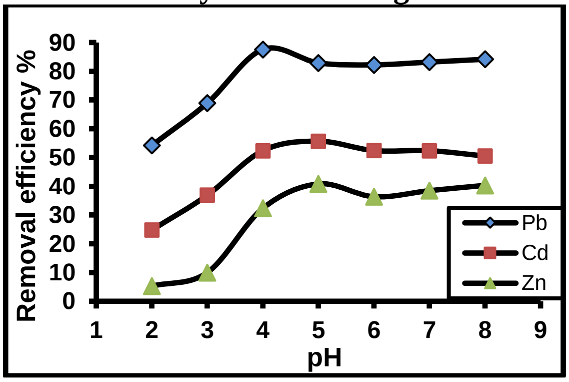

Figure 5: Effect of different pH on lead, cadmium and zinc ions removal efficiencies by spent mushroom biomass

From these Figures the maximum sorption was observed within pH ranges from 4 to 7 which might be due to partial hydrolysis of metal ions. Further increase in pH i.e., above 7 causes precipitation of metal ions on the adsorbent; this is due to the insoluble metal hydroxides which starts to precipitate at high pH values and makes the true sorption studies impossible. To achieve high removal efficiency without metal hydroxide precipitation, pH of 4.0 for Pb (II) and pH of 5.0 for Cd (II) and Zn (II) were selected for both types of fungi biomass for subsequent experiments.

4.3. Effect of Contact Time

The removal of metal ions (Pb, Cd and Zn) from aqueous solution was studied as function of contact time in the time interval (0-240) min. From **Figures6 and 7,** it was observed that the rate of metal ions removal was high at the beginning until 120 min as the sorption rate become practically very slow. It can be concluded that 2 h contact time is sufficient to reach equilibrium conditions for all metals.

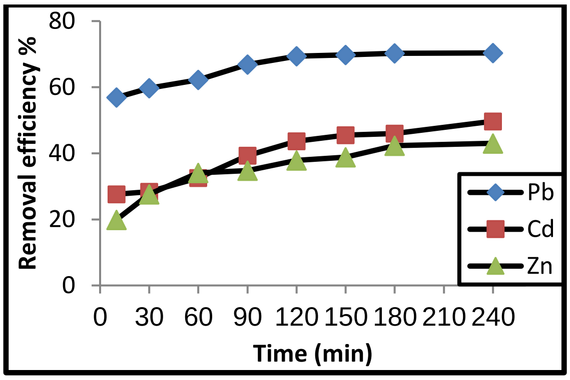

Figure 6: Effect of contact time on lead, cadmium and zinc ions removal efficiencies by cultured fungi biomass

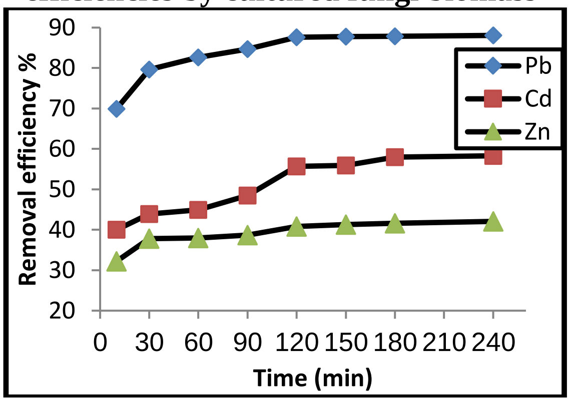

Figure 7 Effect of contact time on lead, cadmium and zinc ions removal efficiencies by spent mushroom biomass

4.4. Effect of Particle Size

The effect of particle size was investigated for both types of fungi biomass at three different particles diameter (0.15-0.6, 0.6-1, and 1-2 mm: mean particle diameter is 0.3, 0.775 and 1.414 mm) according to (2.2.2). **Figures8 and 9** show the removal efficiencies at different fungal biomass particle diameter. It is found that the value of fungal biomass particles diameter has a great effect on the biosorption removal efficiency. Decreasing the mean particles diameter from 1.414 to 0.3 mm, cause an increase of biosorption removal, this increase in biosorption capacity may be attributed to the larger surface area of the smallest particle size of biosorbents.

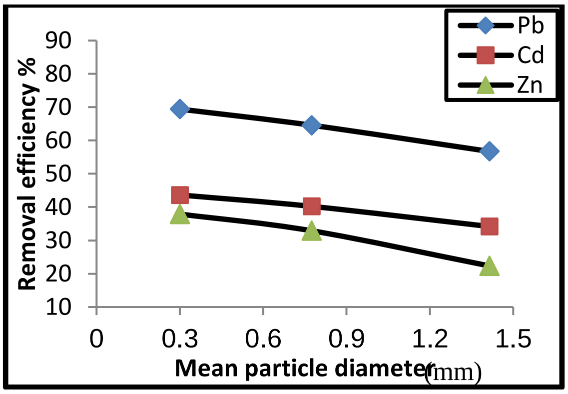

Figure 8: Effect of particle diameter on lead, cadmium and zinc ions removal efficiencies by cultured fungi biomass

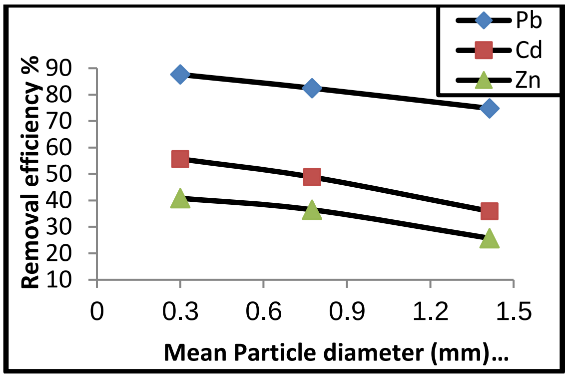

Figure 9: Effect of particle diameter on lead, cadmium and zinc ions removal efficiencies by spent mushroom biomass

4.5. Estimation of Adsorption Isotherms Constants

The adsorption isotherm for single component systems lead, cadmium and zinc ions onto both types of fungi biomass were correlated with nine models illustrated in section (3). The parameters for each model obtained from non-linear statistical fit of the equation to the experimental data using ***(Statistica-v.8)*** program. Table (3) shows the parameters of single solute isotherm uptake onto both fungi biomasses. The Langmuir model gave the best fit for the experimental data as high value of (R^2) was obtained; this model has been used successfully to describe the equilibrium biosorption.

4.6. Equilibrium Isotherm

A comparison between the biosorption capacity of spent mushroom and cultured fungi biomass for removal of metal ions in single metal systems were conducted in order to determine the state of biomass to be the best for metal removal. **Figures10 to 12** show the biosorption isotherm capacity for lead, cadmium and zinc ions using Langmuir model by applying equation (3).

Figure13 shows the experimental uptake capacity was 73.4, 34.38 and 24.48 mg/g for lead, cadmium and zinc ions, respectively, onto spent mushroom biomass, compared with 51.66, 24.52 and 20.46 mg/g for lead, cadmium and zinc ions, respectively, onto cultured fungi biomass. **Figure14** shows that the removal efficiencies 97.37, 90.48 and 88.24 % for lead, cadmium and zinc ions, respectively, onto spent mushroom biomass, while it was 93.58, 84.30 and 83.2 % for lead, cadmium and zinc ions, respectively, onto cultured fungi biomass. It was demonstrated that a concentration of 6 g/l of biomass for both fungi biomass could achieve very good removal when the solution concentration was 50 mg/l for lead, cadmium and zinc ions. These results and availability of spent mushroom in large quantities may encourage using it as a biomass if will used in continuous experimental systems.

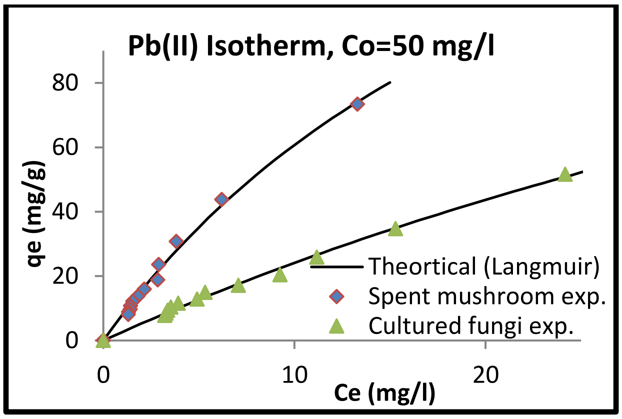

Figure 10: Biosorption isotherm of lead onto spent mushroom and cultured fungi by Langmuir model

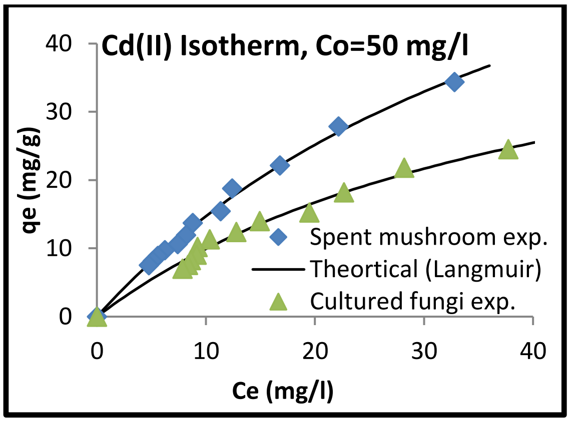

Figure 11: Biosorption isotherm of cadmium onto spent mushroom and cultured fungi by Langmuir model

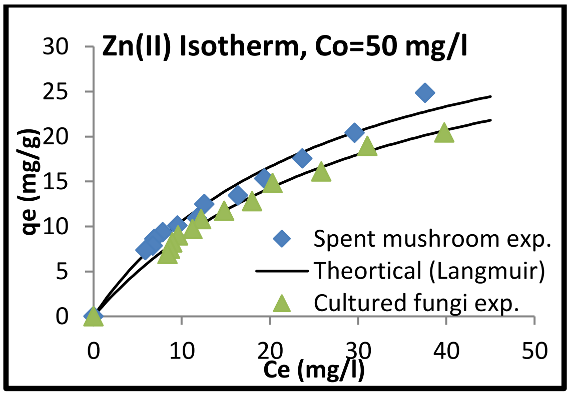

Figure 12: Biosorption isotherm of zinc onto spent mushroom and cultured fungi by Langmuir model

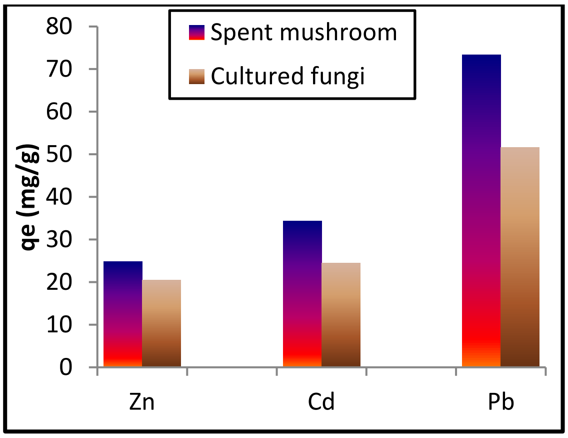

Figure 13: Maximum experimental uptake capacity for Pb(II), Cd(II) and Zn(II) onto spent mushroom and cultured fungi biomass

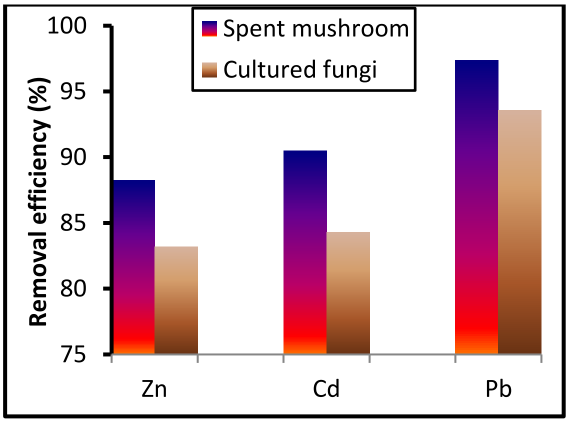

Figure 14: Maximum removal efficiency for Pb(II), Cd(II) and Zn(II) onto spent mushroom and cultured fungi biomass

5. Conclusion:

The biosorption isotherms of lead, cadimium and zinc ions on the cultured fungi and spent mushroom biomass have been investigated in this study. For cultured fungi, *Fusarium oxisporium* showed maximum removal efficiency than other species, so it was used for the comparative with spent mushroom.

Optimum conditions were selected for biosorption uptake onto both types of fungi biomass. The best pH was found to be 4 for Pb(II) and 5 for Cd(II) & Zn(II). The optimum contact time was found to be 2 hr for all metal ions. Optimum mean particle size was found to be 0.3 mm.

Nine equilibrium isotherm models were used for representing the experimental data. Langmuir isotherm model was found to be the most suitable for describing the biosorption equilibrium for heavy metals system onto both biosorbents.

Finally, Spent mushroom biomass was found to have higher uptake capacity than cultured fungi biomass. From these results and availability of spent mushroom in large quantities may encourage using it as a biomass if used in continuous experimental systems.

7. References:

1. Abdul-Talib S., Tay C., Abdullah S., and Liew H., (2013), "Fungal Pleurotus Ostreatus Biosorbent for Cadmium (II) Removal in Industrial Wastewater", Journal of Life Sciences and Technologies, Vol. 1, No. 1, 65-68.

2. Ahluwalia S.S., Goyal D., (2007), "Microbial and plant derived biomass for removal of heavy metals from wastewater", Bioresource Technology, 98, 2243–2257.
3. Aksu Z., Gönen F., Demircan Z., (2002), "Biosorption of chromium(VI) ions by Mowital (R)B30H resin immobilized activated sludge in a packed bed: comparison with granular activated carbon", Process Biochem, 38,175–186.
4. Alexander P.M., Zayas I., (1989), "Particle size and shape effects on adsorption rate parameters", J. Environmental Engineering, V.115, No.1, Feb., pp 41-55.
5. APHA, (1995),"Standard methods for the examination of water &wastewater", 19th Edition, American Public HealthAssociation, Washington, DC.
6. Cocero B.M., (2004), "Modeling and Prediction of Specific Compounds Adsorption by Activated Carbon and Synthetic Adsorbents", Water Research, 14, pp 1719-1728
7. Dhankhar R. and Hooda A., (2011), "Fungal biosorption-an alternative to meet the challenges of heavy metal pollution in aqueous solution", Environmental Technology, 32:5, 467-491.
8. Foo K.Y., Hameed B.H., (2010), "Insights into the modeling of adsorption isotherm systems", Chemical Engineering Journal, 156, 2–10.
9. Freundlich H., (1907), "Ueber die Adsorption in Loesungen", Z.; Phisik. Chem., 57, 385-470.
10. Huang H., Cao L., Wan Y., (2012), "Biosorbtion behavior and mechanism of heavy metals by the fruiting body of jelly fungus from aqueous solutions", Appl microbial biotechnol., 96, 829-840.
11. Jianlong W., Xinmin Z., Decai D. and Ding Z., (2001), "Bioadsorption of lead (II) from aqueous solution by fungal biomass of Aspergillus niger", J. of Biotechnology., 87, 273-277.
12. Joshi N., (2003), "Biosorption of heavy metals", M.Sc thesis in Biotechnology, Department of Biotechnology and Environmental Sciences, Thapar Institute of Engineering and Technology Patiala.
13. Kapoor A. and Viraraghavan T., (1995), "Fungal biosorption – an alternative treatment option for heavy metal bearing waste water: A review", Bioresour. Technol., 53, pp. 195–206.
14. Khan A. R., Ataullah R., Al-Haddad A., (1997), "Equilibrium

adsorption studies of some aromatic pollutants from aqueous solutions on activated carbon at different temperatures", J. Coll. Int. Sci., 194, 154–165.

15. Koner S., Pal A., and Adak A., (2012), "Use of Surface Modified Silica gel factory Waste for Removal of 2,4-D Pesticide from Agricultural Wastewater: A case study", Int. J. Environ. Res., 6(4):995-1006.

16. Langmuir I., (1918), "The adsorption of gases on plane surfaces of glass", mica and platinum. J. Am. Chem. Soc., Vol. 40, pp.1361–1403

17. Lucas S., Cocero M. J., (2004), "Adsorption isotherms for Ethyllacetate and Furfural on activated carbon from Supercritical Carbon Dioxide", Fluid Phase Equilbria, Vol.219, pp. 171-179

18. Redlich O., Peterson D.L., (1959), "A useful adsorption isotherm", J. Phys. Chem. 63:1024

19. Slips R., (1948), "On the structure of a catalyst surface", J. Chem. Phys., 16, 490-495.

20. Sulaymon A.H., Ebrahim S.E., (2011), "Evaluation of adsorbent for removal of phenol and methylene blue from wastewater", J. environmental application and science, 6 (2), 173-186.

21. Temkin M., (1940), "Die gas adsorption und der nernstsche wärmesatz", Acta. Physicochem. URSS, Vol. 1, pp. 36–52.

22. Vijayaraghavan K., Yun Y.S., (2008), "Bacterial biosorbents and biosorption", Biotechnology Advances, 26, 266–291.

23. Yan G., and Viraraghavan T., (2000), "Effect of the pretreatment on the bioadsorption of heavy metals on Mucor rouxii" ISSN 0378-4738. Water SA. 26(1): 119-123.

24. Yang K., Zhu L., Xing B., (2006), "Adsorption of polycyclic aromatic hydrocarbons by carbon nanomaterials’", Environmental Science Technology, 40, 1855-1861.

25. Zhou D., Jianlong W., (1992), "Study on breeding of citric acid – producer and its fermentation", Food Ferment. Ind., (in Chinese) 5, 45-49.

الإمتزاز الحيوي للمعادن الثقيلة بواسطة نوعين من الكتلة الحيوية للفطريات في تجارب الدفعات

د. شهلاء اسماعيل ابراهيم
استاذ مساعد
ناظم حسن حيدر
يوسف محمد يوسف
قسم هندسة البيئة
كلية الهندسة- جامعة بغداد / العراق

الخلاصة

تمت دراسة عملية الإزالة للمعادن الثقيلة (الرصاص، الكادميوم والخارصين) من المياه المحضرة مختبرياً بواسطة نوعين من الكتلة الحيوية للفطريات (fungi biomass). تم القيام بسلسلة من تجارب الدفعات (batch experiment) للكتلة الحيوية للفطر المستهلك وكذلك للفطريات التي تم تنميتها مختبريا (Fusarium oxisporium). وجد أن الكتلة الحيوية للفطر المستهلك أفضل من الكتلة لفطر (Fusarium oxisporium) لإزالة المعادن الثقيلة من المياه المحضرة مختبريا بصورة منفردة. حيث كانت سعة الازالة 73.4، 34.38و 24.48 ملغم/غم لكل من ايونات الرصاص ، الكادميوم والخارصين على التوالي بواسطة الكتلة الحيوية للفطر المستهلك مقارنة مع 51.66، 24.52 و 20.46 ملغم/غم لكل من ايونات الرصاص ، الكادميوم والخارصين على التوالي بواسطة الكتلة الحيوية لفطر (Fusarium oxisporium). تم اختيار أفضل الظروف التشغيلية لعملية الإزالة بواسطة الكتلة الحيوية للفطريات. حيث وجد إن أفضل رقم هيدروجيني pH كان 4 لايونات الرصاص, 5 لايونات الكادميوم والخارصين. كذلك وجد إن أفضل وقت للاتصال بين الملوث والمادة الممتزة هي ساعتين. كما وجد إن أفضل قطر لدقائق الكتلة الحيوية كان 0.3 ملم. تم استخدام 9 موديلات رياضية لتوصيف النتائج العملية, ووجد أن موديل لانكمير (Langmuir) أفضل موديل رياضي يمثل النتائج العملية للملوثات..