Testing the efficiency of local isolates of *Aspergillus niger* for Bioremediation

Khalil I. Bander , Qanat Mahmmoud Atya and Shemaa Fatih Ali
Department of Biology, College of Science, University of Tikrit, Tikrit , Iraq
(Received 15 / 10 / 2008, Accepted 1 / 3 / 2009)

Abstract :
Two locals isolates of *Aspergillus niger* from two pollutated sites were studied for their ability to take up lead from simple mineral salt medium. The results showed that the two isolates had the ability remove lead ions from the medium, although there were differences between the two isolates in takeing up of lead from the medium. The results revealed that the pH of medium plays an important role on the ability of the two isolates in removing lead during growth on mineral-salt medium

Introduction
The presence of heavy metals in the environment is known to cause severe damage to our life in general. Besides, these metals kill microorganism during biological treatment of waste water , with a consequent delay of the process of water purification.[1].
The presence of toxic metals such as lead, antimmy, mercury and cadmium in the environment cause a great concern to their health implications. Physico-chemical method.[2]. such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange and membrane technologies have been widely used to remove heavy metal ions from industrial waste water .These processes are either in effective or expensive.[3].
It was well documented that the presence of metals can inhibit a broad range of microbial processes including methanogesis ,acidogensis ,enzymatic activity , growth , nitrogen and sulfur conversions ,dehalogenenation and reductive processes in general.[4].
Biological methods such as biosorption, bio accumulation may provide an attractive alternative to Physico-chemical methods for the removal of heavy metal ions .[5].
Microorganisms take up metal either by an active transport (bio accumulation) and /or as a passive transport (biosorption). [6,7].Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metal waste. Most studies of biosorption for metal removal have involved the usage of either laboratory-growth microorganism or biomass generated by the pharmacology and food processing industries of waste water treatment units.[8, 9].
The cell walls of microorganism mainly composed of polysaccharides, proteins, lipids and often containing functional group. such carboxy, hydroxyl sulphate, phosphate and amino acid group that bind metal ions Number of microorganism have been proposed to tolerate and remove heavy metals . Microorganisms modulate metal toxicity by maintaining a low intracellular concentration of toxic metal via extracellular complexation and precipitation, adsorption to the cell surface, or accumulation in the pericellular or endocellular regions of the cell.[10].

Material and methods:
Isolation
The fungus *A. niger* was isolated from pollulated (soil & water) by serial dilution methods (2).
The isolates were mainted on potato sucrose agar slants incubated at (25ºC) and then stored at (5ºC). The inoculation was made from 3-5 days old cultures under sterilized conditions. [12].
Growth medium
A simple mineral salt medium used for growth and maintenance of fungus contained the following constituents: 200mg KH_2PO_4, 600mg K_2HPO_4, 500mg $(NH_4)_2SO_4$, 100mg $MgSO_4$, 10mg $CaCl_2$, 5mg $FeCl_2$, 1.0 mg $ZnSO_4$, 0.25 mg $MgMoO_4$, 100mg $MnCl_2$, 5mg $Pb(NO_3)_2$ and 10.000mg glucose; they dissolved in a liter of tap water. Flask (250 ml capacity) containing 50 ml sterile medium were inoculated by o.1ml of conidial suspension prepared from 3-5days old culture fungal mycelium and incubated at 25°C for 7 days in a cooling incubator .The pH was 5.5 . [12].
Assay method
The fresh and dry cell mass was determined by filtering the culture medium through weighed Whatmann filter paper no. (44). Mycelium were thoroughly washed with tap water and dried (105ºC) over night and mycelial dry weight was determined by subtracting the weight of the filter paper .[12].
Analytical methods
The total lead concentration in the solutions was determined using atomic absorption Spectrophoto- meter in the air acetylene (reducing) flame mode. The excitation wave length was set at 357.9nm., and the slit width was adjusted to 0.2nm. Changes in Pb concentration during the growth of the fungus were measured using the diphenylcarbazide method.
Diphenylcarbazide forms a red-violet complex selectively with Pb, and the intensity of this complex was read at 542. 6nm. using a shimadzu UV-visible spectrophotometer. [18].
The effect of pH on fungal growth
The effect of different pH (3, 3.5, 4, 4.5, 5, 5.5) on lead uptake by the two isolate of *Aspergillis niger* grown on a simple mineral salt medium were also determined under the same conditions , as mentioned above .

Table 1: Ability of two isolates of *Aspergillis niger* to remove lead during growth on a mineral salt medium containing 5 gm /L at pH 5.5

Location of isolate	Mycelium weight (gm.)		Concentration of Remaining non-absorbed lead ion mg.
	Wet	Dry	
Soil	1.719 *	0.121 *	2.4 *
Polluted water	1.167	0.125	2.9

* Average of three readings

Table 2:The isolate of *Aspergillis niger* from polluted water

pH	Fresh weight gm.	Dry weight gm.	Remaining of lead mg. in growth medium
3	1.117	o.116	3.6
3.5	1.123	0.109	3.9
4	1.100	0.1	3.5
4.5	1.099	0.094	3.5
5	1.101	0.96	2.8
5.5	1.097	0.094	2.2

Table 3:The isolate of *Aspergillis niger* from polluted soil

pH	Fresh weight gm.	Dry weight gm.	Remaining of lead mg. in growth medium
3	1.075	0.087	1.1
3.5	1.102	0.111	3.5
4	1.084	0.084	3.8
4.5	1.073	0.07	3.2
5	1.090	0.085	2.7
5.5	1.111	0.105	1

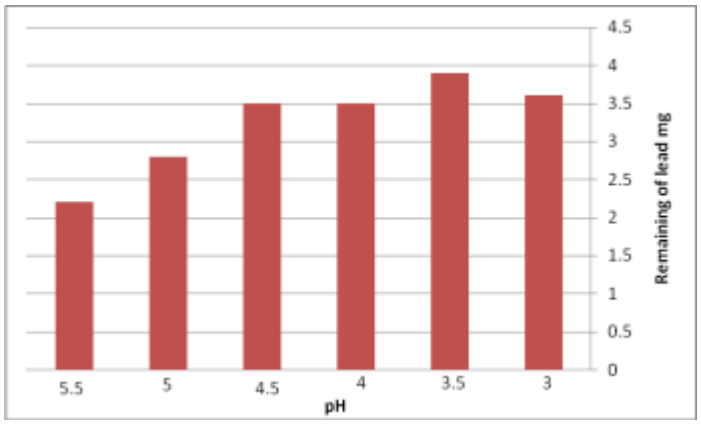

Figure 1: The effect of pH on uptake of Pb by *Aspergillis niger* water isolates

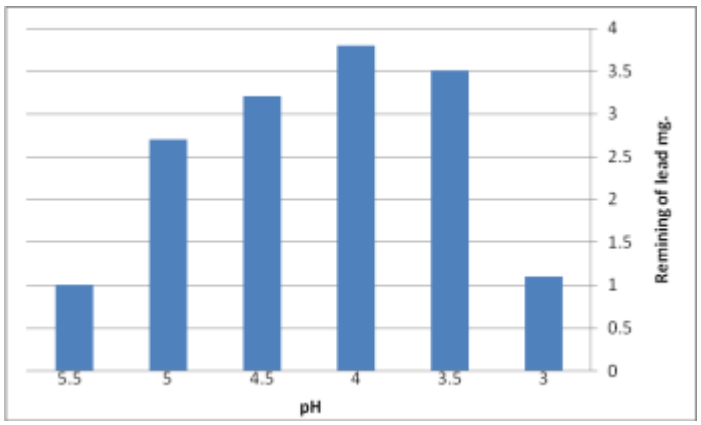

Figure 2: The effect of pH on uptake of Pb by *Aspergillis niger* soil isolates

Result and discussion

Isolates- related t0 lead removal

Table (1) showed that the two local isolates of *A. niger* which have the ability to reduce lead metal ions during growth on a mineral salt medium. The differences between the two isolates in their total biomass (fresh and dry weight) and in removal of metal ions may mainly depend on the efficiency of fungal cells, physiological states, surface properties of cells, pH and other physiochemical parameters of the metal solutions and limited extent on temperature and the presences of various ligands in solution. [11,13, 14].

pH –related to lead removal

The effect of pH as one of the primer factors that influences sorption efficiency of lead metal ions in solution was studied , the results show in table (2,3) and Figure (1 and 2) that the pH effect was clearly on lead ion sorption in both isolates and the highest sorption obtained at pH 5.5 for both isolates, this may be due to the fact the pH effect was in two directions , on the metal solubilities and on the functional groups in the fungal cell walls. At low pH negligible removal of ions may be due to the competition between hydrogen (H^+) and metal ions, and low pH means high acidic environment lead ions to form in soluble salts ,hence it would be unavailable to bind to fungal cell walls. In addition low pH causing decreasing in the negative charge density on cell surface .[15 , 16]. Increasing in the pH up to 5.5 was a remarkable increased in the capacity of metal ions removal. This may be due to the ionizing of functional groups of fungal cell wall such as carboxyl, phosphate and amino groups, the potential sides for binding of heavy metals ions.[17].

References :

1-Hang ,H.; Soha, F.I.; Kamal, K. And Hassan, M. (2004) .Bisorption of heavy metals from waste water using Pseudomonas sp. Enviromental Biotechnology, vol. 7, no.1, page 1of 7.

2-Omotayo .R.A.; Jonathan, O. O.; Renate, R.V.; D.M; Jackie, B. and Erik, J.(2006).Anew approach to chemical modification protocols of *Aspergillis niger* and sorption of lead ion by fungal species. Electronic-J of Biotechnology, vol.9, no.4 ,page 340-348

3-Volesky, B. (1990). Biosorption and biosorbents. In: Volesky, B.(ed) Biosorption of heavy metals CRC press , Boca Raton, FL, pp:36.

4- Volesky, B.(1990) Biosorption of heavy metals CRC press, Boca Raton.

5-Todd, R.S. and Raina, M.M. (2003). Impact of metal on the Biodegradation of organic pollutants. Environmental Health perspective, vol.III, no.8, page 1 of 14.

6- Baath .E. (1989).Effect of heavy metals in soil on microbial process and populations. Water Ail Soil poll,47: 335-379.

7- Kapoor, A.; Viraraghavan, T. (1995). Fungal biosorption: an alternative treatment option for heavy metal bearing waste waters. Biores Technol. 53:195-206.

8- Andres, M.Y.J.H. and Hubert, C.J.(1992).Bacterial biosorption and retention of thorium and uranyl cations by Mycobacterium smegmatis J. of Radio analyses letter, vol.166, p: 431-440.

9- Fourest, E. and Roux , J.C. (1992). Heavy metal biosorption by fungal mycelial by –products: mechanisms and influence of pH . Applied microbiology and Biotechnology, vol.37, no.3, p: 399-403.

10- Costa, A.C.A. and Leite, S.G.C. (1991). Metal biosorption by the inactivated cells of Pseudomonas aerugenosa Pu21.

11- Macaskie, L.E. and Dean, A.C.R. (1990). Metal sequestering biochemicals.n: Volesky. B. (ed) Biosorption of heavy metals CRC press, Raton, Fl, pp: 199-248.

12- Clark, H.;E. P. Bordner; E. F. Geidrich; P.W. Kabler and C.B. Huff. (1958). Applied microbiology. IBC pub. N.Y., pp:27-53.

13-A. Kapoor; T. Viraraghavan. (1995). Fungal biosorption-an alternative treatment option for heavy metal bearing waste waters: a review, Bioresour. Technol., 53:195-206.

14- O. Muter; I. Lubinya; D. Millers; L. Grigorjeva; E.Ventinya and A. Rapoport.(2002). Cr (VI) sorption by intact and dehydrated *Candida utilis* cells in the presence of other metals, Process Biochem., 38: 123-131.

15- B.Prasenjit and S.Sumathi (2005). Up take of chromium by Aspergillus foetidus. J. Mater cycles waste Mang,7:88-92 .

16- M. Wei and J.M. Tobin (2004). Determination and modeling of effects of pH on peat biosorption of chromium, copper and cadmium. Biochemical Engineering Journal,18: 1: 33-40.

17- A. Plette; M. Benedditti and V. Riemsdijk. (1996). Competitive binding protons calcium, kadmium and zinc to isolated cell walls of agram - possitive soil bacterium, Environ. Sci. Technol., 30:1902-1910.

18-Sandell ,E.B. (1961). Colormetric determination of traces of metals 3rd edi. Interscience, New York , p 392 .

اختبار كفاءة عزلتين محليتين للفطر *Aspergillus niger* في المعالجة البايولوجية

خليل إبراهيم بندر و قناة محمود عطية و شيماء فاتح علي

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

(تاريخ الاستلام: ١٥ / ١٠ /٢٠٠٨ ، تاريخ القبول: ١ / ٣ / ٢٠٠٩)

الملخص

تم دراسة قابلية عزلتين للفطر *Aspergillus niger* عزلت من موقعين ملوثين في التقاط عنصر الرصاص من الوسط المنماة عليه. وأظهرت النتائج بأن العزلتين كانت لهما القدرة على إزالة عنصر الرصاص من الوسط الغذائي ولا يوجد اختلاف بين العزلتين في قابليتها على إزالة عنصر الرصاص من الوسط الغذائي وبينت النتائج بان درجة الحموضة تلعب دور مهم في التقاط العنصر بواسطة العزلتين من الوسط الغذائي .