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STUDIES INCLUDING TOLERANCE, AFFINITY AND CAPACITY OF CERTAIN FUNGAL SPECIES FOR SOME TOXIC ELEMENTS IN AQUEOUS SOLUTION

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Abstract

Some fungal species isolated from the soil of the radioactive repository site at Hot Laboratories Center were investigated for their tolerance, affinity and capacity towards different metal ions namely; Cerium Ce(III), Chromium Cr(VI), Cobalt Co(II), and Cadmium Cd(II). They were identified as *Aspergillus tamarii, Aspergillus niger, Penicellium chrysogenum, Cunninghamella elegans and Rhizopus stolonifer*. The obtained results showed that, the lowest concentration of metal ion could be tolerated by fungal species was 50ppm for Co(II) & Cd(II) , while the highest one was 2000ppm for Cr(VI) & Ce(III). Also, tolerance towards mixture of ions was varied among the fungi under study. The affinity of the fungal species of study for metal ions under investigation was found to have the following order; Ce(III)>Cd(II)>Co(II)>Cr(VI). Results showed that *Aspergillus niger* had high capacity for Ce(III) ions uptake, while *Cunninghamella elegans* had high capacity for Co(II) ions uptake. The effects of chemical and physical pretreatments of fungal biomass on the uptake capacity were studied using *Aspergillus niger* & Ce(III). The uptake of Ce(III) by *Aspergillus niger* was studied using different concentrations of Ce(III). It was found that one gram dry weight of *Aspergillus niger* could accumulate 285.7 ± 16.9, 571.43 ± 23.9 and 1142.86 ± 33.8 mg of Ce(III) from 50, 100 and 200 ppm solutions, respectively.

Introduction

The release of large quantities of heavy metals from industries into the environment has resulted in a number of environmental problems. Pollution of the natural environment by heavy metals has become a serious problem in some industrial countries **(Inthorn *et al.*, 1996)**. Low level heavy metal pollution of rivers, lakes and oceans environments is now wide spread , and because heavy metals are concentrated in the food chain, this cause damage to aquatic ecosystems as well as being danger to human health **(Nagase *et al.*, 1997)**.

Biosorption of heavy metals from aqueous solutions is a relatively new technology for the treatment of industrial wastewater **(Volesky, 1990)**. Also, it's defined as a process that utilizes inexpensive dead biomass to sequester toxic heavy metals and to remove contaminants from industrial effluents **(Ting *et al.*, 1991 and**

Singh *et al.*, 1998). The use of biological materials for heavy metal removal and recovery technologies had gained important credibility during recent years, because of the good performance, low cost of the complexing material and the natural affinity of biological sources.

Fungi cell walls contain large quantity of polysaccharides and proteins, which offer many functional groups (such as carboxyl, hydroxyl, sulfate, phosphate and amino groups) for binding metal ions **(Bayramoglu *et al.*, 2003 and Zhou *et al.*, 2004)**. The cell surfaces of microorganisms are negatively charged owing to the presence of various anionic structures. This gives microorganisms an ability to bind metal cations **(Chen & Hao, 1998).** The mode of interaction between metal species and microbial cell components may be simple adsorption, ion exchange, electrostatic interaction, complexation, precipitation, and crystallization **(Crist *et al.*, 1994).** Biological process for removal of metal ions from solution could be divided into three categories: biosorption of metal ions onto the surface of microorganisms **(Ozdemir *et al.*, 2003 and Liu *et al.*, 2004)**, chemical transformation of metal ions and intracellular uptake of metal ions by microorganisms. The later two processes needed viable microorganisms and were called bioaccumulation **(Dursun *et al.*, 2003 and Uslu *et al.*, 2003).**

A number of mechanisms by which microorganisms tolerated and removed heavy metals had been proposed. Microorganisms modulated metal toxicity by maintaining a low intracellular concentration of toxic metals via (i) extracellular accumulation / precipitation; (ii) cell-surface sorption or complexation (adsorption to the cell surface) and (iii) intracellular accumulation (accumulation in the precellular or endocellular regions of the cell) **(Muraleedharan *et al.*, 1991; Gadd, 1993 and Bridge *et al.*, 1999).**

Heat inactivation of biomass could produce additional binding sites via denaturation of proteins on cell wall structures **(Tuzun *et al.*, 2005 and Hafez *et al.*, 1997)**. Also, alkali treatment could cause hydrolysis of protein and deacetylation of chitin into chitosan. It had been reported that the performance of a microbial biomass depended on its surface properties. It was found that, the pretreatment of *Aspergillus. niger* by NaOH resulted in a significant improvement in metal ions removal in comparison with un-pretreated biomass **(Kapoor and Viraraghavan, 1998).**

The Cr(VI) sorption ability of powdered biomass of *Rhizopus nigricans* was investigated by **Bai and Abraham, (2001)**. The influence of solution pH, agitation,

Cr(VI) concentration, biomass dosage, contact time, temperature and biomass particle size was studied. The results cleared that, the optimum pH for biosorption of Cr(VI) was found to be 2.0. Higher adsorption percentage was noted at lower initial concentrations of Cr(VI) ions, while adsorption capacity of the biomass increased with increasing concentration of ions. Moreover the adsorption capacity increased with increase in temperature and agitation speed and the optimum were determined as 45°C at 120 rpm.

The present study was conducted to investigate the tolerance, affinity and capacity of certain fungal species namely *Aspergillus tamarii, Aspergillus niger, penicillium chrysogenum, Cunninghamella elegans and Rhizopus stolonifer* for different metal ions (Ce, Cd, Cr and Co). The effect of pretreatment of fungal biomass on the uptake of metal ions was investigated using *Aspergillus niger* and Ce(III) ions. Also, the uptake of Ce(III) by *Aspergillus niger* using different concentrations of Ce(III) was studied.

Materials and methods:-

Chemicals: All chemicals used were of analytical purity grades. Solutions of Ce(III), Co(II), Cr(VI) and Cd(II) were prepared as stock solutions and other concentrations were obtained by dilution. Solutions of hydrochloric acid and sodium hydroxide with different concentrations were used to adjust the pH to the desired values.

Fungal isolates: Five fungal species were isolated from soils of repository site at Waste Management Facility, Hot Laboratory Center, Atomic Energy Authority, Egypt. The fungal species were isolated using dilution plate method, quadrant type **(Benson, 1985)**. The isolated fungi were identified in The Regional Center for Mycology and Biotechnology - Al-Azhar University *as; Aspergillus tamarii, Aspergillus niger, penicillium chrysogenum, Cunninghamella elegans and Rhizopus stolonife*r according to **(Pit, 1979; Domsch *et al*., 1993 and Samson *et al*., 2000).**

Tolerance experiments: The growth of *Aspergillus tamarii, Aspergillus niger, penicillium chrysogenum, Cunninghamella elegans and Rhizopus stolonife*r on Sabouraud's dextrose agar had been studied in the presence of various concentrations of Ce(III), Cr(VI), Cd(II), Co(II) and mixed ions concentrations. The concentrations ranged from 200 to 2000ppm for Ce(III) and Cr(VI), while the concentrations of Cd(II) or Co(II) were ranged from 50 – 1000 ppm. The mixed ions concentrations ranged from 200-600 ppm with equal ratio. The incubation temperature was adjusted to 28°C and the growth was observed after 3 – 15 days.

Biomass pretreatment: The fungal biomass of *Aspergillus niger* was subjected to chemical and physical pretreatments. Chemical treatments were conducted by washing for 15 min with: a) 0.025, 0.05 & 0.1N NaOH; b) 0.125, 0.25 & 0.5% glutraldehyde; c) 0.25, 0.5 & 1.0% anhydrous $CaCl_2$. On the other hand, physical pretreatments were carried out by: a) boiling in bidistilled water for 15 min; b) autoclaving for 15 min at 121ºC & 1.5 psi; c) washing with bidistilled water. After the pretreatments the biomass was washed with deionized water until the pH of the washing solution was close to pH 6.8-7.2. The biomass had been dried at 60ºC for 24 h to get the dry weight.

Screening & Uptake experiments: In batch experiment, 0.5 gram of fungal biomass was immersed in 50 ml of metal ion solution. The pH of the solutions was adjusted to 4.5 – 5.0 ± 0.2 except for Cr(VI) the pH was adjusted to 2.0 ± 0.2. The temperature was adjusted at 25 ± 3°C and stirring was 250 rpm. Samples were taken at different contact times and the amount of the ion accumulated on fungal biomass was estimated. The quantitative determination of chromium, cobalt and cadmium were carried out using atomic absorption spectrophotometer (Buck) model 210 VGP, and measurement of cerium was carried out using UV-spectrophotometer Shimadzu -160.

Calculations: The concentration of metal ion solution at different contact times (t) with fungal biomass (C_t) was given directly by atomic absorption spectrophotometer, while for UV- spectrophotometer $C_t = A_t$ X k; where A_t is absorbance at time (t), k is a constant. The uptake amount in (mg) of metal ion at time (t) by 0.5 g wet weight of fungal biomass (Q_t) = $C_0 - C_t$, where C_0 is the initial concentration. The uptake percent of metal ion at different contact times with fungal biomass = Q_t / C_0X100. The uptake amount in (mg) of studied ion at time (t) by 1g dry weight of fungal biomass (Q) = Qt / dry wt.

Result and discussion:-

1- Tolerance tests

The five isolated fungal species exhibited different resistances (Minimum Inhibitory concentration MIC values) towards the metal ions under test Ce(III), Cr(VI), Cd(II) and Co(II). The highest MIC value was observed for Ce(III) & Cr(VI) (>2000ppm) and the lowest MIC value was for Cd(II) & Co(II) (>50ppm). Also, for mixture of the ions with concentration ranged from (200 – 600ppm) in equal ratio, the tolerances varied. It was found that *Rhizopus stolonifer* failed to tolerate any of

mixture concentration, while *Penicillium chrysogenum and Aspergillus niger* could tolerate all mixture concentrations (i.e. MIC_{mix} > 600 ppm). For *Cunninghamella elegans* and *Aspergillus tamarii* MIC_{mix} > 400 & 200 ppm, respectively, results given in Table (1) & Fig.(1). It was also found that the period needed for growth increased with the increase in the metal ion concentration in growth media.

Table (1): MIC of the studied fungal species toward different metal ions

FUNGI / ELEMENT	*A. tam.*	*A. nig.*	*Pen. chry.*	*Cun. ele.*	*Rhi. sto.*
	MIC (ppm)	MIC (ppm)	MIC (ppm)	MIC (ppm)	MIC (ppm)
Ce(III)	> 600	>2000	>2000	>2000	>2000
Cr(VI)	>2000	>2000	>2000	>2000	>2000
Cd(II)	>50	>50	>1000	>1000	>200
Co(II)	>600	>200	>400	>100	>50
Mixed ions (equal ratio)	>200	>600	>600	>400	0

The high MIC value could be explained by; low toxicity of such metal ions, or addition chelation co-precipitation, or metabolic active uptake mechanisms. Also, this depends on the detoxification mechanism of each fungal species **(Zafar *et al.*, 2007 and Hildebrandt *et al.*, 2007)**. This indicated that metal- resistance phenotypes were well-distributed among the representative fungal genera **(Kapoor *et al.*, 1999 and Say *et al.*, 2003)**.

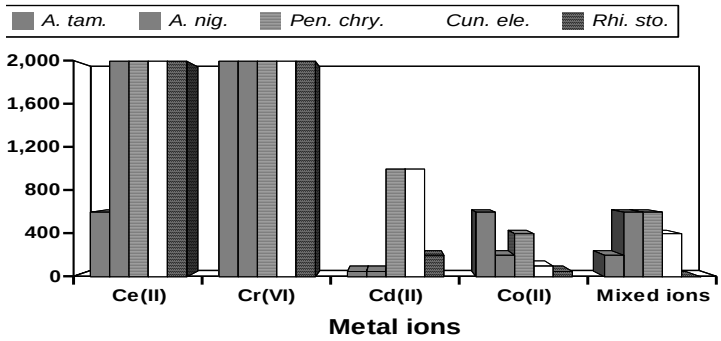

Fig.(1): MIC values of the studied fungal species toward different metal ions

Also, results revealed that fungal biomass obtained from metal-resistant fungi had the same capacity for metal ion uptake as the fungal biomass that wasn't exposed to high metal ion concentrations. This could be explain that the capability of fungal species to live on high metal ion concentrations was depending on detoxification mechanisms, while the absorption capacity depended (mainly) on cell wall composition **(Bayramoglu *et al.*, 2003 and Zhou *et al.*, 2004)**. Fungi could tolerate and detoxify metals by several mechanisms including; valence transformation, extra and intra-cellular precipitation and metabolic active uptake **(Gadd, 1993 and Zafar *et al.*, 2007)**.

2- *Screening test:*

Screening of the studied fungal species for their affinity and capacity towards metal ions under investigation (Ce(III), Cr(VI), Cd(II) and Co(II)) showed differences in the affinity and capacity of the fungal species under study towards metal ions.

Aspergillus niger showed highest accumulation capacity for Ce(III) ions; while *Cunninghamella elegans* exhibited high capacity for most of the metal ions under study Cd(II), Co(II) & Cr(VI). These differences in accumulation capacities could be attributed to wide variation in the chemical composition of their cell walls and the chemical properties of the metal ions **(Gadd, 1990)**.

Results given in Fig.(2) indicated that the metal ions under study could be arranged according to their absorption affinity is Ce(III) > Cd(II) > Co(II) > Cr(VI). Also, results revealed that the capacity of the fungal species differed from one metal ion to another. The difference in absorption capacities depend on many factors such as; cell wall composition of the fungal species, the chemistry of the metal ions and the toxicity.

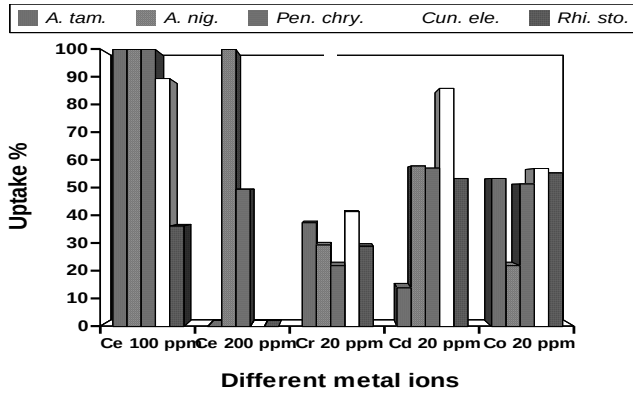

Fig. (2): The uptake percent of different metal ions by the studied fungal species.

Biosorption is relatively rapid and can be reversible. It involves a physicochemical interaction between the metal and functional groups such as ketones, aldehydes and carboxyls present on the microorganism's cell surface. It is often followed by a slower metal binding process in which additional metal ion is bound, often irreversibly. This slow phase of metal uptake can be attributed to a number of mechanisms, including covalent bonding, surface precipitation, redox reactions, crystallization on the cell surface or, most often, diffusion into the cell interior and binding to proteins and other intracellular sites **(Ozer *et al*., 1999).**

3- *Pretreatment test:*

Obtained results in Fig.(3) indicated that biosorption of Ce(III) by pretreated *Aspergillus niger* either increased or decreased depending on the pretreatment method. The order of maximum uptake of Ce(III) by *A. niger* is as follows; pretreated with autoclaving ≥ washing with distilled water > washing with 0.25% glutraldehyde > washing with 0.05N NaOH > washing with 0.5% anhy.CaCl2 > Boiling. An increase in biosorption of cerium ions as a result of pretreatment could be due to an exposure of active metal binding sites embedded in the cell wall or chemical modifications of the cell wall components. The increase in metal biosorption after pretreating the fungal biomass could be attributed to the removal of surface impurities and to the exposure of available binding sites for metal biosorption **(Huang & Huang, 1996)**.

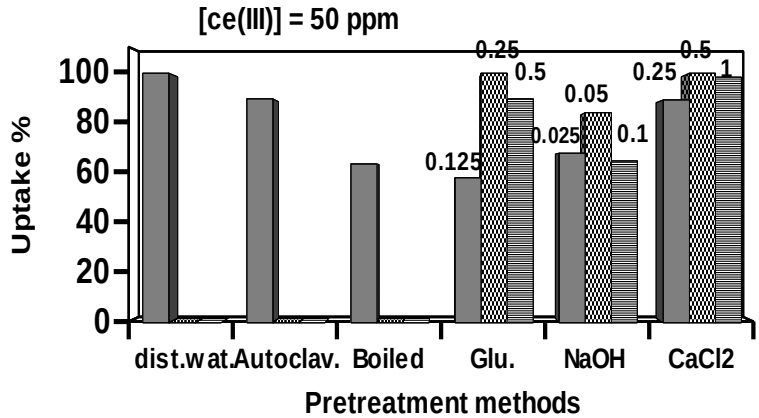

Fig. (3): The uptake percent of Ce (III) by physical and chemical pretreated *Aspergillus niger* after two hours of contact.

Pretreatment with autoclaving resulted in an improvement in Ce(III) biosorption. Glutraldehyde pretreatment reduced biosorption of Ce(III) to a certain extent while, sodium hydroxide, calcium chloride and boiling pretreatment significantly reduced biosorption of Ce(III). The reduction of biosorption capacity in glutraldehyde, sodium hydroxide, calcium chloride and boiling pretreatment may be attributed to the loss of some active groups.

It was found that *Mucor rouxii* biomass pretreated by autoclaving reduced the biosorption of heavy metals **(Yan and Viraraghavan, 2000)**. In the same way **Kapoor & Viraraghavan, (1998)** reported that *A. versicolor* pretreated by autoclaving reduced the biosorption of cadmium, copper, and nickel. Heat and autoclaving pretreatment increased the biosorption capacity of microbial biomass due to the exposure of latent binding sites after pretreatment **(Galun *et al.*, 1987)**.

It was found that Pb^{2+} biosorption by *Saccharomyces uvarum* was more efficient after sodium hydroxide treatment due to unmasking of some cellular groups which can't participate in the sorption process without treatment with alkali **(Ashkenazy *et al.* 1997, Yan and Viraraghavan, 2000)**. In contrast, **(Kapoor & Viraraghavan, 1998**) reported that in nickel biosorption by sodium hydroxide pretreatment there was an approximately 45% reduction in comparison with live cells. As a result of sodium hydroxide treatment, the number of protein amino groups that can be engaged in metallic ion binding markedly decreased. Deproteination should, theoretically, reduce metal retention. Since the cell wall composition can be characteristic of the fungal species, we could say that this conflict is normal.

Gluteraldehyde is a cross-linking reagent with multifunctional groups. According to **(Jianlong, 2002)** gluteraldehyde pretreated *Saccharomyces cerevisiae* biomass retains almost all its original biosorption capacity. In contrast in our study, gluteraldehyde pretreatment reduce Ce(III) biosorption of fungal biomass, according to **(Yan and Viraraghavan, 2000)** the difference in results after a specific pretreatment may be attributed to the different strains of fungi.

4- *Ce(III) uptake:*

Aspergillus niger had the highest biosorption capacity for cerium ions among the fungal species under study. The increase in Ce(III) (metal ion) concentration results in an increase in the amount of metal ion accumulation. At low metal ion concentrations of 50 – 100 ppm, the total amount (100%) of metal ion were found to be accumulated within 2 min while, at high metal ion concentration of 200 ppm,

50% of metal ion was accumulated after 5 min of contact and the remaining amount was accumulated within the rest of the two hours and the results are given in Fig.(4). This could be explained that with a fixed adsorbent weight, the metal ion accumulation increased with the increase of metal ion concentration until reach saturation of active absorption sites on the fungal cell walls **(Ilhan *et al.*, 2004)**.

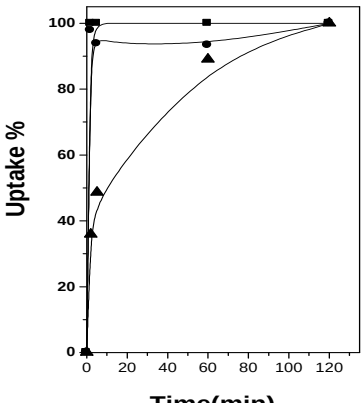

Fig.(4): The uptake percent of Ce(III) by *Aspergillus niger* from different Ce(III) concentrations at different contact times.

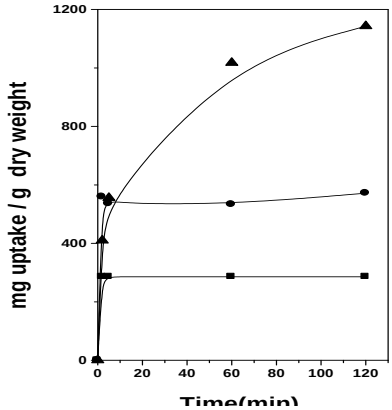

Fig.(5): The amount absorbed of Ce(III) in(mg)/g dry weight of *Aspergillus niger*, from different Ce(III) concentrations, at different contact times.

The uptake of Ce(III) by *Aspergillus niger* was studied using different concentrations of Ce(III). It was found that one gram dry weight of *Aspergillus niger* could accumulate 285.7 ± 16.9, 571.43 ± 23.9 and 1142.86 ± 33.8 mg of Ce(III) from 50, 100 and 200 ppm solutions, respectively, results given in Fig.(5).

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الملخص العربي

تناولت تلك الدراسة عزل عدد من الفطريات من التربة الموجودة بمقابر النفايات المشعة بمركز المعامل الحارة وقد تم تعريفها كالأتي؛ ***Aspergillus tamarii, Aspergillus niger, Penicellium chrysogenum, Cunninghamella elegans and Rhizopus stolonifer.*** تمت دراسة قدرة هذه الفطريات على النمو في وجود تركيزات مختلفة من العناصر تحت الدراسة (سيريوم – كروم –كوبالت وكادميوم) وأيضاً القدرة على النمو فى وجود خليط من هذه العناصر. وجد أن أقل تركيز يمكن للفطريات تحمله كان لعنصر كوبالت وكادميوم (50 جزء في المليون) وأعلى تركيز يمكن للفطريات تحمله كان لعنصر كروم وسيريوم(2000 جزء في المليون). وأيضاً قدرة هذه الفطريات على تحمل خليط العناصر تختلف فيما بينها. وجد أن قابلية الفطريات لامتصاص العناصر تحت الدراسة تأخذ الترتيب الأتي؛ سيريوم > كادميوم > كوبالت > كروم . وأوضحت النتائج أن فطر ***Aspergillus niger,*** له قابلية عالية لامتصاص عنصر سيريوم و أن فطر ***Cunninghamella elegans*** له قابلية عالية لامتصاص عنصر كوبالت. وتمت دراسة تأثير المعالجة الكيميائية والفيزيقية علي كفاءة الأخذ لعنصر سيريوم باستخدام فطر ***Aspergillus niger,*** . ثم دراسة أخذ عنصر سيريوم باستخدام تركيزات مختلفة. وجد أن جرام واحد جاف من خلايا الفطر يستطيع أخذ 285.7 ± 16.9 و 571.43 ± 23.9 1142.86 ± 33.8 ملليجم من 50 و 100 و 200 جزء في المليون من محلول العنصر على الترتيب.