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Environmental parameters affecting biosorption of heavy metals by mixed culture of *Aspergillus niger* and *Aspergillus flavus* in industrial wastewater

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Abstract

This work was conducted to study the effect of some environmental conditions on bioremoval of heavy metals from industrial wastewater by consortium cultures of *Aspergillus niger and A. flavus*. Many procedures on the effect of incubation period, temperature, pH and concentration of metals were carried out in the laboratory. Results proved that the high culture growth and maximum heavy metals removal were after 21 days incubation at 30 ^{o}C, pH of 5.4 and metal concentration at 10 ppm. While the lowest culture growth and minimum heavy metals removal were after 7 days incubation at 5 ^{o}C , pH of 10.8 and metal concentration at 100 ppm.

Keywords: *Aspergillus niger*, *Aspergillus flavus*, Heavy Metals, Industrial waste water, Bioremoval.

Introduction

Increased use of metals and chemicals in industries has resulted in generation of large quantities of effluent that contain high level of toxic heavy metals **(Malik, 2004, Chuah *et al.*, 2005).** Unlike organic chemicals, metals persist in environment indefinitely posing threats to all the organisms which are exposed to them. Wastewater may be of simple composition if derived from single industry, e.g., electroplating wastewater, or in other cases could be a heterogeneous mix (coming from different industries) of many dissolved metal ions at various pH with salts, colloidal and particulate matters present as well. Using microorganisms as biosorbents for heavy metals is an attractive alternative to existing methods such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment filtration, ion exchange and membrane technologies for toxicity reduction and recovery of valuable metals from industrial effluents, because of good performance and low cost of biosorbent material **(Brierley *et al.*, 1986)** . **Asha *et al.* (2013)** stated that bioremediation is the use of biological interventions of biodiversity for mitigation of the environmental pollutants. The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment .Bioremediation uses biological agents, mainly microorganisms i.e. yeast, fungi or bacteria to clean up

contaminated soil and water **(Strong and Burgess, 2008).** The investigation of the efficacy of metal uptake by microbial biomass is essential for the industrial application of biosorption, as it yields information about the equilibrium of the process that is necessary to design the equipment to be employed.

Biosorption process was found to be affected by temperature, pH of the medium, biomass concentration and metal interference. biosorption process Temperature does not seem to influence biosorption performance in the 20-35 °C range **(Aksu *et. al.*, 1992)**. PH seems to be the most important parameter in the biosorption process: it affects the solution chemistry of the metals, the activity of the functional groups in the biomass and competition between metallic ions **(Machado *et al.*, 2010). However,** Biomass concentration in solution seems to influence specific uptake: for lower values of biomass concentrations, there is an increase in the specific uptake **(Fourest and Roux, 1992)**. Furthermore, biosorption is may be influenced by the presence of other metal ions **(Sakaguchi and Nakajima, 1991).**

Materials and methods

1- Antagonistic study of fungal isolates

The two fungal isolates were isolated from industrial waste water for antagonistic effect on Czapek-Dox agar medium. Disks of 1ml were taken from 10 days old fungal cultures and placed on Czapek- Dox agar plates where the separated distance of them is 1.5Cm. The plates were incubated at 30^0C for 10 days .the results were recorded.

2-Microbial growth under different culture conditions

2-1-Effect of incubation period:

Twelve Erlenmeyer 100 ml conical flasks each containing 50ml industrial water supplemented with constituents of Czapek DOX medium were sterilized and inoculated with equal volume (1ml) of fungal spore suspension where 1ml contains ($4x10^6$ CFU).Flasks were incubated at 30 ^{0}C for 7, 14, 21and 30 days. Three non-inoculated flasks were used as control. The biosorbents were then removed from the solution by filtration and the supernatant liquid was used to determine the residual metal ions concentration. The results were expressed as (mg) fungaldry weight.

2-2-Effect of Temperature:

Twelve Erlenmeyer 100 ml conical flasks each containing 50ml industrial water supplemented with constituents of Cazpek DOX medium were sterilized and inoculated with equal volume (1ml) of fungal spore suspension where 1ml contains ($4x10^6$ CFU). Flasks were incubated at 5, 20, 30 and 40 ^{0}C for 21days. Three control non inoculated flasks were set up. The biosorbents were then removed from the solution by filtration and the supernatant liquid was used to determine the residual metal ions concentration. The results were expressed as (mg) fungal dry weight

2-3-Effect of pH:

Twelve 100 ml Erlenmeyer conical flasks each containing 50 ml of industrial waste water supplemented with constituents of Cazpek DOX medium were sterilized and inoculated with equal volume (1ml) of fungal spore suspension where 1ml contains ($4x10^6$ CFU). The pH of the medium was adjusted to 3.6, 4.6, 5.4, 6.4, 8, 9.5 and 10.8 by using different concentrations of Citric acid and Na_2HPO4, Na_2HPO_4 and NaH_2PO4, Na_2CO_3 and $NaHCO_3$

Flasks were incubated at 30 ^{0}C for 21 days. Non-inoculated three flasks were used as control. The bio sorbents were then removed from the solution by filtration and the supernatant liquid was used to determine the residual metal ions concentration .The results obtained were recorded as the average of three replications for each pH measurement**.** The results were expressed as mg fungal dry weight**.**

2-4-Effect of heavy metals concentration:

Sterilized solutions of different heavy metals concentrations of 10, 20, 50, 100 Mg^{-1} of each (Fe, Cu, Zn ,Cr and Cd) was added separately to each of fourty100 ml conical flasks each contains 50 ml DOX medium. Flasks then inoculated with with equal volume (1ml) of fungal spore suspension where 1ml contains ($4x10^6$ CFU).Non-inoculated three flasks were used as control. Flasks were incubated at 30 ^{0}C for 21 days. The results were expressed as mg fungal dry weight

3-1-Effect of incubation period:

Twelve Erlenmeyer 100 ml conical flasks each containing 50ml industrial water supplemented with constituents of DOX media were sterilized and inoculated with equal volume (1ml) of fungal spore suspension where 1ml contains ($4x10^6$ CFU). Flasks were incubated at 30 ^{0}C for (7, 14, 21 and 30 days).Three non-inoculated flasks were used as control. The biosorbents were then removed from the solution by filtration and the supernatant liquid was used to determine the residual metal ions concentration. The degradation of heavy metals (Fe, Cu, Zn, Cr and Cd) was estimated using atomic absorption spectrophotometer (ASS), flame type multi wave 3000 . The percentage of metal ions removed by microbial cultures was calculated according to the equation:

% removal= $(C_i-C_f/C_i)*100$ where

C_i: initial metal concentration

C_f: final or residual metal concentration

3-2-Effect of Temperature

Twelve Erlenmeyer 100 ml conical flasks each containing 50ml industrial water supplemented with constituents of DOX media were sterilized and inoculated with equal volume (1ml) of fungal spore suspension where 1ml contains ($4x10^6$ CFU). Flasks were incubated at 5, 20, 30 and 40 ^{0}C for 21days. Three non-inoculated flasks were used as control. The bio sorbents were then removed from the solution by filtration and the supernatant liquid was used to determine the residual metal ions concentration. The concentration of heavy metals was estimated using atomic absorption spectrophotometer**.** The percentage of metal ions removed by microbial cultures were calculated

3-3- Effect of pH

Twelve 100 ml Erlenmeyer conical flasks each containing 50 ml of industrial waste water supplemented with constituents of Cazpek DOX medium were sterilized and inoculated with equal volume (1ml) of fungal spore suspension where 1ml contains ($4x10^6$ CFU). The pH was adjusted to 3.6, 4.6, 5.4, 6.4, 8, 9.5 and 10.8. Flasks were incubated at 30 ^{0}C for 21 days. Non-inoculated three flasks were used as control. The biosorbents were then removed from the solution by filtration and the supernatant liquid was used to determine the residual metal ions concentration. The concentrations of heavy metals (Cu, Fe, Cd, Cr and Zn) were estimated using atomic absorption spectrophotometer. The percentages of metal ions removed by microbial cultures were calculated

Results

1-Antagonistic effect:

The antagonistic effect between *A. niger* and *A.flavus* showed that there is no antagonistic relation between them

2-Effect of different environmental factors on growth of A. niger and A. flavus in mixed culture

2-1-Effect of incubation period:

Results in table (1) showed that the high culture growth (405mg / 50ml) was reported at incubation period of 21 days while the lowest growth (340mg / 50ml) was recorded at incubation period of 7 days. Moreover, after 14 and 30 days of incubation the growth was 374 mg / 50 ml and 384 mg/50 ml respectively.

2-2-Effect of incubation temperature:

Table 1 illustrated that the high culture growth (417mg /50ml) was detected after 21 days incubation at 30 ^{0}C, while the

lowest one (94mg /50ml) was recorded at 5 ^{0}C of the same incubation period. Furthermore, the cultural growth was (344mg /50ml) and 235mg /50ml at 40 ^{0}C and 20^{0}C respectively.

2-3-Effect of pH on mixed culture growth:

The data in table (1) indicated that the high culture growth (512 mg /50ml) was detected after incubation period of 21 days at incubation PH 5.4 while the lowest growth (211 mg/50ml) was recorded at incubation PH of 10.8.While at PH 3.6 the cultural growth was (372mg/50ml),at PH 4.6 the cultural growth was(425mg/50ml),at PH 6.4 the cultural growth was recorded (456mg/50ml),at PH 8 the cultural growth was(403mg/50ml)and at PH 9.5 the cultural growth was recorded(289 mg/50ml).

Table (1): Effect of incubation period, temperature and PH on growth of *A. niger* and *A. flavus* in mixed culture

factor	Incubation period (day)				Temp (^{0}C)				PH						
	7	14	21	30	5	20	30	40	3.6	4.6	5.4	6.4	8	9.5	10.8
Growth (mg/50ml)	340	374	405	384	94	235	417	344	372	425	215	456	403	289	211

2-4-Effect of different concentrations of heavy metals on mixed culture growth:

The results in table (2) indicated that for Cu^{2+} the highest culture growth (405mg /50ml) was reported after incubation period of 21 days at concentration 10 ppm whereas the lowest growth (308 mg /50ml) was recorded at concentration of 100 ppm. With the increase of copper concentration up to 20 ppm and 50ppm the cultural growth was taken median value of 329mg/50ml and 311mg /50ml respectively.

For Zn^{2+} the high culture growth (442mg/50ml) was reported after incubation period of 21 days at concentration of 10 ppm while the lowest growth (359mg /50ml) was recorded at concentration of 100 ppm. The cultural growth becomes (403mg /50ml) and (326mg/50ml) at concentration of 20 ppm and 50ppm respectively. Likewise, the highest (382mg/50ml) and the lowest (324mg/50ml) culture growth were reported after incubation period of 21 days at Fe^2 concentration of 10 ppm and 100 ppm respectively. By the way, the cultural growth of 342mg /50ml and 326mg /50ml was recorded at Fe^2 concentration of 20 ppm and 50ppm respectively.

For Cd $^{2+}$the high culture growth (403mg/50ml) was reported after incubation period of 21 days at concentration 10 ppm while the lowest growth (332mg/50ml) was recorded at concentration 100 ppm. The cultural growth was recorded (376mg/50ml) at concentration of 20 ppm and (336mg/50ml) at concentration of 50ppm.

And for Cr^{5+} the high culture growth (398mg/50ml) was reported after incubation period of 21 days at concentration 10 ppm while the lowest growth (342mg/50ml) was recorded at concentration 100 ppm. The cultural growth was recorded (396mg/50ml) at concentration of 20 ppm and (348mg/50ml) at concentration of 50ppm.

Table (2): Effect of different concentrations of heavy metals on mixed culture growth

Heavy metals / Conc.	Cu	Zn	Fe	Cd	Cr
10	405	442	382	403	398
20	329	403	342	376	396
50	311	389	326	336	348
100	308	359	324	332	342

3-Effect of different cultural factors on biosorption ofheavy:3-1-Effect of incubation period on heavy metals removal:

Results in figure (1) showed that the highest uptake of heavy metals was detected after incubation period of 21 days Cu^{+2} (20.7 mg/m), Fe^{+2} (28.44 mg/m) Cd^{+2} (14.34 mg/m) and Zn^{+2}(14.15 mg/m) while the lowest uptake of heavy metals was detected after incubation period of 7 days Cu^{+2} (9.58 mg/m), Fe^{2+} (21.34 mg/m) Cd^{+2} (15.2 mg/m) and Zn^{+2} (12.79 mg/m) heavy metals removal was recorded after 7 days.

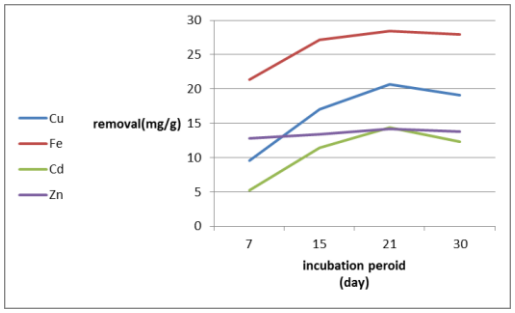

Figure (1): Effect of incubation period on heavy metals removal

3-2-Effect of temperature on heavy metals removal

Results in figure (2) showed that the highest uptake of heavy metals was detected after incubation period of 21 days at incubation temperature of 30 ^{0}C Cu^{+2} (15.76 mg/m), Fe^{+2} (38.63 mg/m) Cd^{+2} (14 mg/m) and Zn^{+2}(17.77 mg/m) while the lowest uptake of heavy metals was detected after incubation temperature of 5 ^{0}C days Cu^{+2} (1.8 mg/m), Fe^{2+} (9.16 mg/m) Cd^{+2} (1.93 mg/m) and Zn^{+2} (3.01 mg/m) heavy.

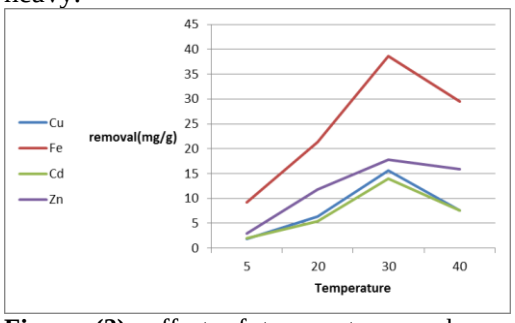

Figure (2): effect of temperature on heavy metals removal

3-3-Effect of pH on heavy metals removal

The data presented in figure (3) show that Cu^{+2} was recorded the highest bio removal (20.19 mg/m) after incubation period of 21 days at incubation pH of (5.4) while the lowest bio removal (6.67 mg/m) at incubation pH of (10.8), where Zn^{+2} was recorded the highest bio removal (20.97 mg/m) after incubation period of 21 days at incubation pH of (5.4) while the lowest bio removal (12.94 mg/m) at incubation pH of (10.8). Also Fe^{+2} was recorded the highest bio removal (76.98 mg/m) after incubation period of 21 days at incubation pH of (5.4) while the lowest bio removal (47.95 mg/m) at incubation pH of (10.8). Cd^{+2} was recorded the highest bio removal (4.36 mg/m) after incubation period of 21 days at incubation pH of (5.4) while the lowest bio removal (3.12 mg/m) at incubation pH of (10.8)

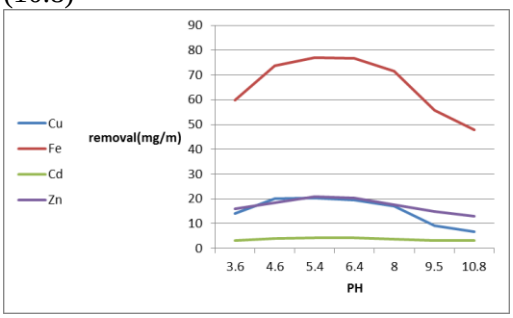

Figure (3): Effect of incubation pH on heavy metals removal

Discussion

Cultural age is considered as an important microbial factor that affects metals uptake. In the present study maximum heavy metal removal by fungal consortium occurred after three weeks incubation. At this incubation period the fungal growth was (405 mg/l) and heavy metals removal were in the following order Cu^{2+}20.7 mg/g, Fe^{2+}28.44 mg/g, Cd^{2+}14.34 mg/g, and Zn^{2+} 14.15 mg/g. This is possibly due to the presence of many highly active enzymes at this growth phase, during which cells are at their most metabolically active stage).A similar result was reported by **(Mondal, *et al.*, 2008).**

The temperature influence on metal uptake was pronounced where with their increases from 5, 20, 30 and40^{0}C, the uptake level was increased gradually up to 30^{0}C where the maximum uptake of Cu^{2+},Fe^{2+},Cd^{2+}, and Zn^{2+} was15.76 mg/g, 38.63 mg/g, 14 mg/g, and 17.77mg/g respectively. Conversely, at temperature of 40^{o}C the uptake level was declined in the following order (Cu^{2+}7.61 mg/g, Fe^{2+}29.5 mg/g, Cd^{2+}7.53 mg/m, and Zn^{2+}15.94 mg/m. By the way the fungal growth was reduced from, 417 mg/50ml at 30^{0}C to 344mg/50ml at 40 ^{0}C. Higher temperatures usually enhance sorption due to increased surface activity and kinetic energy of the solute **(Sag and Kutsal, 2000). Conversely,** high temperature was reported to reduce biosorption capacity of the biomass by causing a Physical damage to the biosorbent and exothermic nature of some adsorption processes in **(Srivastava**

and Thakur, 2006). It is always desirable to conduct biosorption at room temperature, as this condition is easy to replicate **(Vijayaraghavan and Yeoung, 2008)**

The pH considered as one of the most effective parameters in biosorption of heavy metals, the mixed culture was grown in different pH values, it was found that best pH used for broth culture growth (512 mg/l) and heavy metals uptake was pH 5.4 where the uptake level was in the following order; Zn^{2+} (20.97 mg/g), Fe^{2+} (76.98 mg/g), Cu^{2+} (20.19 mg/g) and Cd^{2+} (4.36 mg/g). In fact, the dependence of metal uptake on pH is related to both surface functional groups present on biomass and metal chemistry in solution. Generally, at low pH of 2.0-3.0, the surface ligands are closely associated with the hydronium ions ($H3O^{+}$) and restricted the approach of metal cations. As a result of repulsive force causes decreased metal sorption takes place at low pH. Correspondingly, the pH dependency of metals uptake by *A. niger* and *A. flavus* is due to association and dissociation of certain functional groups like carboxyl and hydroxyl groups, where at low pH, most of the carboxylic groups is not dissociated and cannot bind the metal ions to fungal cell wall **(Choudhary and Sar, 2009).** On the contrary, at high pH value metals get precipitate, where cadmium ions precipitate as $Cd(OH)_2$ and trivalent chromium ions precipitate as $Cr(OH)_3$. This causes very low biosorption of metal ions in high pH value **(Pinoa *et al*., 2006).** The metal binding sites on cell surface and availability of metal ions in solution are affected by pH. Alike, **Johncy *et al*., 2010** stated that, at low pH, the cell surface sites are closely linked to the H^{+} ions, thereby making these unavailable for metal cations. Consequently, with increase in the pH, there is an increase in ligand with negative charges which results in increased binding of cations. The increase in pH causes increased negative charge on cell surface which favoured electrochemical attraction and adsorption of metals 0**.** Therefore, the optimal pH for biosorption of heavy metals by fungal biomass is between 5.0–5.2 **(Shankar *et al*., 2007).**

The data revealed that there was no significant decrease in fungal biomass, with increasing the heavy metals concentration. Biomasses recorded at Cu concentration of 10, 20, 50 and 100 mg/l were 405, 329, 311, 308 mg /50ml respectively. For Zn concentration of 10, 20, 50 and 100 mg/l the biomasses recorde were 442, 403, 389, 359 mg /50ml respectively. Likewise, the biomasses at Fe concentration of 10, 20, 50 and 100 mg/l were 382, 342, 326, 324 mg /50ml respectively. For Cd concentration of 10, 20, 50 and 100 mg/l the biomasses were 403, 376, 336, 332 mg /50ml respectively, whereas for Cr concentration of 10, 20, 50 and 100 mg/l the biomasses were 398, 396, 348 and 342 mg /50ml respectively. By the way, Atuanya **and Oseghu (2006)** found that as the concentration of lead increased from 10 mg L–1 to 1000 mg L–1, a decrease in biomass of fungi was recorded. A like, **Siham (2007)** stated that a decrease in biomass was observed as a result of increases in the concentration of metal ions. She isolated *Alternaria clamydospore, Aspergillus niger, A. oryzae, A. parasiticus, A. tamari, A. ustus, Fusarium sp., Penicillium glabarum* from metal contaminated sites which were found tolerant to lead.

(Shivakumar , 2014) stated that *A. niger* and *A. flavus* showed almost similar uptake ability of metals. *A. niger* showed high Pb (75%) accumulation followed by Zn (49%)> Cu (45%)> Cr (41%) >Ni (25%) and *A. flavus* showed high Pb (82%) accumulation followed by Zn (40%)>Cu (34%)>Ni (20%).Bioaccumulation efficiency of some metals decreased at high metal concentrations due to saturation of the biosorbent **(Rao *et al*., 2005)**.

Conclusion and recommendation

The fungal consortium of *A. flavus* and *A. niger* was able to remove Fe, Cu, Cr and Cd from solution. Therefore, it is proposed to use them as an effective biosorbent agent for removal of heavy metals

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الملخص العربي

عنوان البحث: العوامل البيئية التي تؤثر على الازاله البيولوجية للمعادن الثقيلة من مياه الصرف الصناعى باستخدام مزرعه مختلطه من فطرى *Aspergillus niger* and *Aspergillus flavus*

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قسم علوم البيئه - كلية العلوم - جامعة بورسعيد

أجرى هذا العمل لدراسه تأثير بعض العوامل البيئيه على الازاله البيولوجيه للمعادن الثقيله (النحاس- الزنك- الحديد- الكادميوم- الكروم) من مياه الصرف الصناعى باستخدام مزارع مختلطه من *فطرى* *Aspergillus niger* and *Aspergillus flavus*. وقد أجريت العديد من الاجراءات على تاثير فتره التحضين ودرجه الحراره ودرجه الحموضه وتركيز المعادن فى المختبر .أثبتت النتائج ان أعلى نمو للمزرعه المختلطه وا قصى ازاله للمعادن الثقيله كان عند 21 يوم من فتره التحضين ودرجه حراره 30 م o و 5.4 درجه حموضه وعند تركيز10 جزء من المليون بينما كان أقل نمو للمزرعه المختلطه وا قل ازاله للمعادن الثقيله كان عند 7 يوم من فتره التحضين ودرجه حراره 5 م o و 10.8 درجه حموضه وعند تركيز100 جزء من المليون