CELLS IMMOBILIZATION OF SOME MICROORGANISMS AS A TOOL FOR BIOREMEDIATION: B- ASPERGILLUS NIGER

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Abstract: Current study included the use of immobilization technique (using sodium alginate and calcium chloride) for Fungi (Aspergillus niger) and studying their efficiency in treating some heavy metals such as Lead and cadmium. Standard solutions of lead and cadmium at concentrations (10, 20 and 30) ppm were used. Heavy metals concentrations were measured by atomic absorption device and the correlation between the heavy metals and the immobilized cells was measured by FTIR &SEM techniques. The results of current study show the lead concentrations after treatment by immobilized Aspergillus niger were (6.75, 11.6 and 13.9) ppm respectively, and the removal efficiency was (32.4,42and 54)% . Cadmium Concentrations after treatment were (3.7 , 6.16 and 8.62 ) ppm respectively, whereas removal efficiency in was (63,69 &71)% respectively. Current study showed a correlation between cells & heavy metals which principally included with bioadsorption surface for it and that morphological alterations in cells were occur because of the bioadsorption for heavy metals and the porous that found on cells surface that lead to enter the heavy metals to inner surface and increase the removal efficiency, all results of SEM were appear alteration in the surface cells shape by binding the lead and cadmium at the surfaces.

Introduction:

Large-scale production of wastewater is an inevitable consequence of all contemporary societies. Most wastewaters are usually hazardous to human populations and the environment and must be treated prior to disposal into streams, lakes, seas, and land surfaces\textsuperscript{1,2}. Immobilization is one of the biotechnological method for treatment that define as the physical confinement or
localization of intact cells to a certain region of space; without loss of desired biological activity\(^3\). Immobilized cells exhibit many advantages over free cells, such as relative ease of product separation, reuse of biocatalysts, high volumetric productivity, improved process control and reduced susceptibility of cells to contamination\(^4\).

Heavy metals are naturally occurring elements, and are present in varying concentrations in all ecosystems. There is a huge number of heavy metals are found in elemental form and in a variety of other chemical compounds.\(^5,6\) Yeasts and molds are easy to cultivate, can be genetically and morphologically manipulated, and can produce a high biomass yield\(^7\). They are extensively used as biosorbents for the removal of toxic metals from polluted wastewaters, with excellent abilities for metal uptake and recovery\(^8,9,10\). *Aspergillus niger* is a filamentous fungi and resemble the structure of a plant. When viewed under the microscope, *A. niger* consists of a smooth and colorless conidiophores and spores. A closer look will reveal the conidial heads of the organism to be globose and dark brown in color that have been shown to divide into a number of columns as the *A. niger* continues to age. In microscopy, the carbon black/dark brown color of the spores (as well as the conidia) is used to distinguish *A. niger* from other species in the same genus\(^11\) have been exploited for remediation procedures\(^12,13\).

**Material and Methods**

**1-Fungal Isolation:**

*A. niger* was selected for immobilization experiments as it was isolated through 1 g of Biology Department soil was taken and suspended by adding 10 ml of distilled water and then make a series of a decimal dilution reaching to \(10^{-5}\). PDA medium was prepared for the cultivation of fungi then the
sample was incubated for 7 days. After incubation period, different types of fungus were observed, so the required culture was purified for several times in the same medium until a pure culture of the required fungi was obtained.

After obtaining a pure culture of the required fungus, it was identified to confirm the fungus isolates that returned to the *Aspergillus niger*¹⁴. It was transferred to the 100 ml PDB liquid medium and incubated for 14 days and then used in the immobilization experiment.

**2- Beads Formation:**

100 ml from studied organisms culture in the stationary phase had been taken and concentrated by filtering by Millipore filter paper 0.45µm, then the concentrated organisms were mixed with an equal volume of the 2% sodium alginate soluble solution and well shaked to homogenize this ingredients and put the mixture in a medical syringe. At this time, calcium chloride solution (1M.0) was prepared in a separate beaker and the contents of the syringe are gradually dropped in the calcium chloride solution. A drop of solution (organisms and sodium alginate) is solidify and become immobilized in the form of beads in the beaker, and left for 5-10min. Then the beads (immobilized organisms) are separated from calcium chloride solution by the tea strainer and wash gently with tap water and rinse thoroughly with distilled water¹⁵.

**3-Heavy Metals Treatment:**

**3-1-Mesurement by Atomic absorption:**

Lead and cadmium were selected to test the removal efficiency of immobilized organisms with sodium alginate in their treatment. Three
standard concentrations of each metal (10, 20, 30) mg/l (Sigma-Aldrich) and from each concentration of 10 ml for use in the experiment.

A bioreactor was constructed as shown in Figure (1). The heavy metals sample was placed in the reactor and left for 15 minutes for each concentration / per metal. The sample was then collected and measured by atomic absorption.

Removal efficiency (R.E.) was calculated as below:

\[ R.E\% = \frac{C_1 - C_2}{C_1} \times 100 \]

Were:

R.E\%: Removal efficiency, C1: Heavy metal concentration before treatment, C2: Heavy metal concentration before treatment.
Fig. 1. Bioreactor used in this study: a: Scheme for bioreactor, b: Photo
3-2- **Detection of treatment by FT-IR**: Determination of treatment According to the functional group that shift/absent/appear was carried out by FT-IR that depend on mixed the KBr(200mg) with dried samples(1mg), the result were read by X-axis and the Y-axis of the spectrum. The X-axis of an IR spectrum is labeled as "Wavenumber" and ranges in number from 400 on the far right to 4,000 on the far left. The X-axis provides the absorption number. The Y-axis is labeled as "Percent Transmittance" and ranges in number from 0 on the bottom and 100 at the top, the characteristic peaks in the IR spectrum. All IR spectra contain many peaks. However, determine the large peaks on the spectrum because they will provide the data necessary to read the spectrum.and the work done in the laboratories of the Ministry spectrum because they will provide the data necessary to read the spectrum.and the work done in the laboratories of the Ministry of Science and Technology - Department of Environment and Water.

3-3- **Detection of treatment by FE-SEM**: Surface binding between immobilized algae and heavy metals was examined by FESEM that carried out at the University of Tehran / Iran after the use of eppendorf tubes to place the samples and keep them tightly until arrival at the university and the examination.
Results and Discussion:

Trace elements represent the significant aqueous toxins that existence in factories sewage\(^{16}\) and most part kept in liver, muscles, kidneys, spleen, skin, bone, and delicate tissues of individuals\(^{17}\). Take-up of trace elements particles by fungi can show modified strategy for their elimination from sewage. All microorganisms forms can eliminate trace elements from polluted systems\(^{18,19}\).

The results of current study that used the immobilized fungi for heavy metals (Lead and Cadmium) treatment from aqueous solutions these results show that the lead concentration before treatment was 10 ppm and after treatment was 6.75 ppm whereas when the initial concentration before treatment was 20 ppm and final concentration after treatment was 11.6 ppm finally the concentration of lead was 30 ppm and after treatment decreased to 13.9 ppm. (Fig. 2).

(Fig. 3) explain the efficiency removal of \textit{A. niger} for the lead, was (32.4, 42, and 54)\% because the ability of this fungi to biodegradation that represents a hopeful technique for ecosystems cleaning, among the many of organisms that present naturally in the gushing water, \textit{A. niger} can chosen for the current investigation, because of the facility they show for elimination of some trace elements from aqueous solution. The survival of lead on the strain of fungi was recorded, justifying them to be fruitful possibility for metal detoxification\(^{20}\).
Our study proposed that lead bioadsorption by some types of fungi includes both passive and active uptake. At minimum level of trace elements focus, lead particles are acceptable average by the organism and lead take-up
happens because of straightforward ion exchange among metal and practical gatherings present on cell divider surface of cells. While, at maximum level of trace elements, quantity of these elements have been sorbed more than at minimum levels focuses, where all the bonds positions were liberate for reaction. In addition, the raise of lead bioadsorption in some types of fungi were required to add to intracellular take-up of lead happening in metabolically dynamic cells in mix with extracellular bioadsorption.

FT-IR peaks of untreated bioadsorbent demonstrated amany particular and sharp adsorption groups of various wavelengths. The FT-IR spectrums of treated *Aspergillus niger* demonstrated a few moves in some of distinct wavelengths. Variation in spectra portrays alterate in active gatherings of fungi after lead adsorption, make us suggested the likelihood that bioadsorption could be occurred over ion-exchange operation instead of collection.

The current study showed the bonds of functional groups, for example, Ion-exchange between H of carboxyl (-COOH), hydroxyl (-OH) and amine (-NH₂) gathering of *Aspergillus niger* & lead particles are principally included with bioadsorption surface for it(Fig.4-7).
Fig. 4. FT-IR for Immobilized *A. niger* that treated Pb-10ppm

Fig. 5. FT-IR for Immobilized *A. niger* that treated Pb-20ppm
Fig. 6. FT-IR for Immobilized *A. niger* that treated Pb-30ppm

Fig. 7. FT-IR analysis for Immobilized *A. niger* only without any treatment with
From Figures (8-11) SEM results in the current study for *A. niger* demonstrated that the surface of immobilized *A. niger* was the primary piece of the cell associated with the aggregation of lead from the aqueous solutions, consistently adsorbed to the surface of *A. niger* and increased the adsorption at this surface. By increasing the lead level in aqueous solution, the adsorption process was increased sequestered on the cell membrane and furthermore in the cell inside, showing its infiltration into the cell.

Fig. 8. SEM micrograph of immobilized *A. niger* surface with treat Pb-10ppm
Fig. 9. SEM micrograph of immobilized *A. niger* surface with treat Pb-20ppm

Fig. 10. SEM micrograph of immobilized *A. niger* surface with treat Pb-30ppm
Existence of trace elements in water resources are to make extreme harm for ecosystems, and the poisonous trace elements such as mercury, lead and cadmium called the huge three are in the limelight because of their real sway on the ecosystems. Releases that comprise Cd$^{2+}$, specifically, are carefully planned because of the exceptionally lethal quality of this component and it is propensity to aggregate in the tissues of living life forms. Cadmium is brought into the waterways from purifying, elements, cadmium nickel batteries, PO$_4$ compost,...etc). Elimination of lethal trace elements from sewage is fundamental from the point of view of ecological contamination control.

The initial concentration of cadmium that used in the current study was 10ppm as the first concentration and 3.7 ppm was the final concentration after
treatment by immobilized *A. niger*, and the second concentration was 20ppm before treatment and 6.16 ppm after treatment and the third concentration was 30ppm and 8.62 ppm for before and after treatment respectively. (Fig.12; Table 2).

Fig.12. Cadmium Concentration before and after treatment by *A.niger*

![Cadmium Concentration before and after treatment by *A.niger*](image)

Fig.13. Removal Efficiency of Immobilized *A. niger* for Cadmium

![Removal Efficiency of Immobilized *A. niger* for Cadmium](image)
New efficient handling to eliminate trace elements in watery medium include the utilization of microorganisms. Consideration has been centered of late around the use of fungi organisms for elements elimination from watery medium. Fungal species can aggregate elements by physical, chemical and biological methods. Fungi species for example, Aspergillus, Penicillium, Rhizopus, and Saccharomyces have been used to elements expulsion from water resources.

Removal efficiency in the current study for cadmium by immobilized A. niger was (63,69 &71)% (Fig.13).

The high removal efficiency may return to the ability of functional groups in the surface of beads and the increase the concentration of lead as a result to increase the removal efficiency as a result to increase the cadmium ions that lead to produce pressure on the surface of beads and increase the active transport within the beads, current study is identical to.

The distinctions in bioadsorption ability might be because of the natural capacity of fungi in addition to its cell membrane synthesis prompting variation in reaction of elements with organisms. Many researchers have revealed the bioadsorption capacity of A. sp. in removing the trace elements from aqueous solution.

FTIR spectrum of immobilized fungi for treated cadmium with concentrations (10,20 and 30) ppm (Fig.14-17) show the bands of wavelength that shifted to another spectra after treatment of cadmium, this means the cadmium is binding with the functional groups that record the spectra before treatment such as (O-H,N-H,C=O=C,C-O-H…etc). Each functional group has spectra of wavelength produced from their vibrate ( bonds vibration) and therefore after binding with cadmium
vibrate is altered and finally shifting of spectra from sites to another. Current study identical to\textsuperscript{43,44}.

Fig.(14). FT-IR for Immobilized \textit{A. niger} that treated Cd-10ppm

Fig.15. FT-IR for Immobilized \textit{A. niger} that treated Cd-20ppm
SEM investigation uncovers changes that happened in the surface of the loaded and unloaded Fungi against Cadmium particles, loaded biosorbent indicates alterations contrasted with unloaded fungi, with few structural changes and little porous with infrequent states on the loaded fungi (Fig.17-20). The current study bolsters the prior perceptions of 45 who have likewise 46 who have explored the bioadsorption capacity of Cd(II) utilizing Aspergillus sp.
Fig. 17. SEM micrograph of immobilized *A. niger* surface with treat Cd-10ppm

Fig. 18. SEM micrograph of immobilized *A. niger* surface with treat Cd-20ppm
Fig. 19. SEM micrograph of immobilized *A. niger* surface with treat Cd-30ppm

Fig. 20. SEM micrograph of immobilized *A. niger* surface without treated of cadmium
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