

Biosorption of Rare Earth Elements by Two Fungal Genera from Lower Carboniferous Carbonaceous Shales, Southwestern Sinai, Egypt

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ABSTRACT

This work aims to test the ability of two fungal genera A. niger and A. flavus, for sorption of rare earth elements (REEs) during bioleaching process, from Sinai carbonaceous shales. The sorption of heavy metals and REEs was found to be influenced by initial ore concentration, contact time and temperature. The maximum biosorption efficiency by using A. flavus and A. niger was observed at 5% ore concentration, 9 days of contact time and 40 °C. It is noticed that the A. flavus has higher biosorption efficiency than A. niger. The Environmental Scanning electron microscope and EDX analysis indicate the selectivity of both fungi for Cerium (Ce) from other REEs. Transmission electron microscope analysis of A. niger showed that elements were accumulated and precipitated around its cell wall and form black dense area around it. By increasing ore concentration the cell wall starts to hydrolysis and metal ions inter to the resulting cell large vacuole.

KEYWORDS: Biosorption; Rare Earth Elements (REEs); A. niger; A. flavus

INTRODUCTION

Rare earth elements (REEs) or rare earth metals are a collection of seventeen chemical elements in the periodic table, specifically the fifteen lanthanoids plus scandium and yttrium [1]. Investigation of environmental and biological effects of lanthanides or rare earth elements has recently attracted much attention because lanthanides have been widely applied in many fields of industry, pharmacy, and agriculture [2]. Recent evidence indicates that a great many types of human activity, such as fossil-fuel combustion, waste incineration, metallurgic processes, apply of mineral fertilizers etc., cause REEs emissions to soil, air and water.

The toxic characteristics of REEs heavy metals are displayed as follows: (1) the toxicity can last for a long time in nature; (2) some heavy metals even could be transformed from relevant low toxic species into more toxic forms in a certain environment, mercury is such a case; (3) the bioaccumulation and bioaugmentation of heavy metal by food chain could damage normal physiological activity and endanger human life finally; (4) metals can only be transformed and changed in valence and species, but cannot be degraded by any methods including biotreatment; (5) the toxicity of heavy metals occurs even in low concentration of about 1.0–10 mg/L. Some strong toxic metal ions, such as Hg and Cd, are very toxic even in lower concentration of 0.001–0.1 mg/L [3, 4, 5].

Conventional methods for removing metal from aqueous solution include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies, evaporation recovery and adsorption on activated carbon etc. These processes are extremely expensive and ineffective, especially when metal ion concentration in aqueous solution is as low as one to 100 mg/L, they also produce large amount of sludge to be treated with great difficulties. It is therefore important to develop new methods for metal removal.

The alternative process is biosorption, which utilizes various natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. These biosorbents possess metal sequestering properties and can decrease the concentration of heavy metal ions in solution from ppt to ppb level. They can effectively sequester dissolved metal ions out of dilute complex solutions with high efficiency and quickly.

Biosorption of metals is the removal of metal ions by live or dead biomass due to high attractive forces present between them. These attractive forces were due to the presence of various functional groups on the cell walls of biomass [6].

Biosorption suggests many advantages than other techniques for the removal of metal ions as: (1) rich sources and chemically stable; (2) a high surface area (porous); (3) high efficiency to treat low concentrations of heavy metals in wastewater; (4) easy to produce high concentrations of metal ion adsorption active groups; and (5) easy to make chemical modification. In addition, it is reversible process where metal ions can be desorbed easily, thus recycling the adsorbent material and minimize the chemical biological sludge [7].

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This work aims to investigate the ability of *A. niger* and *A. flavus* for biosorption of REEs and other elements (during bioleaching of Sinai carbonaceous shales sample). The study involved the role of environmental factors on the biosorption of REEs and the changes that happened to cells of *A. niger* using environmental scanning electron microscope (ESEM-EDX) and transmission electron microscope.

GEOLOGIC OUTLINE

Sinai Peninsula has a special interest in natural resources prospecting (petroleum, mineral deposits and groundwater), which could be of great value for developing purposes. The Paleozoic sedimentary rocks in southwestern Sinai were subjected to several attempts for classification since [8] who classified this section to lower sandstone and Carboniferous limestone. While, [9] was classified the section to Um Bogma group (lower sandstone and fossiliferous dolomite) and Ataqa group. The Ataqa group was classified by [10] into El Hashash Formation (old), Magharet El Meyiah Formation and Abu Zarab Formation (young). In this work follow the last classification and stressed on sample collected from Magharet El Meyiah Formation. [11] pointed to the low-grade REEs resource in Magharet El Meyiah Formation. So Budaa locality in southwestern Sinai is the main prospect of this study. Description of the studied ore sample it is calcareous black shale, dark, friable.

MATERIAL AND METHODS

Sample preparation

A representative sample was collected from the study area and grounded to (60 mesh) and then mixed well to avoid heterogeneous distribution of mineral constituents.

Chemical analysis of ore sample

The pulverized sample was analyzed by conventional wet chemical technique [12]. Whereas SiO₂, Al₂O₃, TiO₂ and P₂O₅ were determined using a spectrophotometric method, the contents of Na and K were determined by a flame photometric technique. Total Fe as Fe₂O₃, MgO and CaO were determined by titration methods. The loss on ignition (L.O.I) was determined gravimetrically. The estimated error for major constituents is not more than ±1 %. Total rare earth elements (REEs) were analyzed using spectrophotometer by arsenazo (III). All the chemical analysis was carried out in the laboratories of the Nuclear Materials Authority, Cairo, Egypt.

Preparation of fungal biomass

Fungal strains of *A. niger* and *A. flavus* were isolated from tested ore sample using a culture medium composed of Dox agar medium of the following composition (g/L): NaNO₃, 2; KH₂PO₄ 1; MgSO₄.7H₂O, 0.5; KCl, 0.5; FeSO₄.5H₂O trace; sucrose, 30; agar, 15.5, yeast extract was added to initiate fungal growth. The pH value of the media was adjusted to be 6.5. It is one of the most useful pH for the general cultivation of fungi.

Biosorption controlling factors

To optimize the relevant biosorption factors during bioleaching process, ore concentrations, contact time and temperature were considered. To testify the effect of these factors, 100 ml of Dox liquid medium in 250 ml measuring flask was subjected to autoclave at 1.5 atm. for 20 minutes. After cooling, the flask was incubated with 1 ml of *A. niger* and *A. flavus* spore suspension then they were incubated at 30 °C for 7 days. With variable ore concentrations, contact time and temperature in an orbital shaker at 100 rpm. At the end of contact time the mycelia were harvested and washed several time to remove any sample particles and then examined using (ESEM) and chemically by removing REEs from biomass using HCl, 0.1 N to determine the biosorption efficiency [13].

Preparation of *A. niger* for TEM studies

A. niger was cultivated on Dox agar plates supplemented with different ore sample concentration (control, 3%, 7% and 9%). All the treatments of hyphal tips (fixation, embedding, staining and cutting) and the examination under TEM occurred at Regional Central Mycology and Biotechnology, Al Azhar Univ. Cairo, Egypt.

RESULTS AND DISCUSSION

Chemical analysis of ore Sample

Chemical analysis of the carbonaceous shale sample showed high content of SiO₂, 50.3% and moderate content of Al₂O₃ 10.7% (Table 1). The total rare earth elements concentration of the studied sample was 200 ppm, (Table 1). The well noticed thing is the high loss on ignition (24.09 %).

Table 1. Major oxides and REEs analysis of the tested sample (wt. %)

Major oxides	(Wt. %)
SiO ₂	50.3
Al ₂ O ₃	10.7
TiO ₂	0.84
Fe ₂ O ₃	0.023
MnO	ULD*
CaO	5.04
MgO	6.45
Na ₂ O	0.871
K ₂ O	0.627
P ₂ O ₅	0.023
L.O.I	24.09
Total	98.9
REEs (ppm)	200

*ULD: Under limit of detection

Factors influencing biosorption during bioleaching process

Effect of ore concentrations

REEs biosorption by *A. niger* and *A. flavus*, grown on Dox liquid medium at 30 °C for 7 days, from different ore concentrations were found. The obtained results, as illustrated in table (2) and fig. (1), indicate that, the ore concentration plays an important role in the amount of REEs biosorption either by *A. niger* or *A. flavus*. It is noticed that the biosorption increases with the increase in ore concentrations reaching its highest biosorption efficiency of REEs from the sample obtained at ore concentration 5% by both *A. flavus* and *A. niger*. The obtained results showed that the biosorption efficiency of *A. flavus* is more than that of *A. niger*. This means that *A. flavus* can work under stress of REEs content than *A. niger*. The amount of REEs accumulated on biomass of both fungi was found to be increased with increasing the sample concentrations in the growth media. The present work obtained results are supported with many other previous works as that [5] who mentioned that the uptake rate of the metal ion increases with the increase in the initial concentration if the amount of biomass is kept unchanged.

Table 2. Effect of different ore concentrations on REEs biosorption by *A. niger* and *A. flavus* grown on Dox liquid medium at 30 °C for 7days

Fungal sp.	REEs (%)		Ore Concentration (%)				
			1	2	3	5	7
<i>A. niger</i>	REEs (%)	Biosorption	10	10	17	18	18
		Final pH	4.24	4.15	4.33	4.62	5.4
<i>A. flavus</i>	REEs (%)	Biosorption	10	11	20	21	21
		Final pH	4.64	4.75	5.58	6.6	6.8

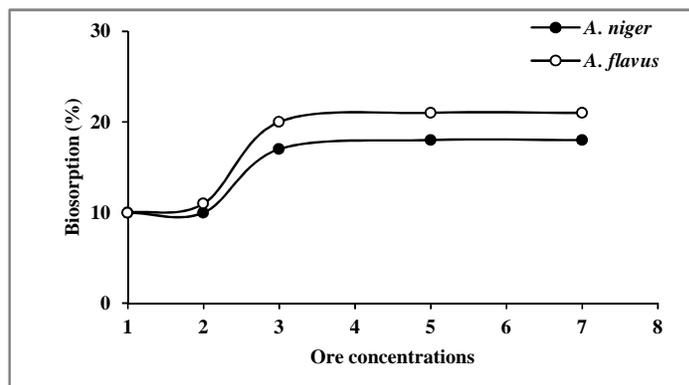


Fig. 1. Effect of different ore concentrations on REEs biosorption from studied samples using *A. niger* and *A. flavus* grown on Dox liquid medium at 30 °C for 7days

[14] Found that the equilibrium uptake for cadmium (II) ion by deactivated protonated yeast was directly proportional to the ratio of the initial metal ion concentration to the sorbent mass. Therefore, both

aspects cannot be neglected when assessing the influence of concentration of the metal ion and biomass on biosorption, otherwise, error would occur [15].

[16] Showed that the adsorption amount of La^{3+} increased with the enhancement of the initial concentration of REEs ions from 5 to 15 mg/L. When it is beyond 15 mg/L, the amount of adsorption did not increase, but dropped in a small scale.

Effect of contact time

As it is clear from the obtained results, illustrated in table (3) and fig. (2), showed a duplicate the biosorption efficiency of REEs at seven and nine days. A rapid increase of REEs biosorption efficiency, with respect to time for both *A. niger* and *A. flavus*. It reached a maximum value at 9 days of incubation. The biosorption efficiency of *A. flavus* is more than that of *A. niger* at 9 days of incubation and slightly decrease after these days of incubation, especially in *A. flavus* (Fig. 2).

Table 3. Effect of contact time on REEs biosorption by *A. niger* and *A. flavus* grown on Dox liquid medium

Fungal sp.	REEs (%)		Contact time (days)				
			3	5	7	9	10
<i>A. niger</i>		Biosorption	5	6	11	20	20
		Final pH	6.65	5.41	4.24	6.59	7.4
<i>A. flavus</i>		Biosorption	7	8	14	24	23
		Final pH	5.35	5.53	4.64	5.53	7.11

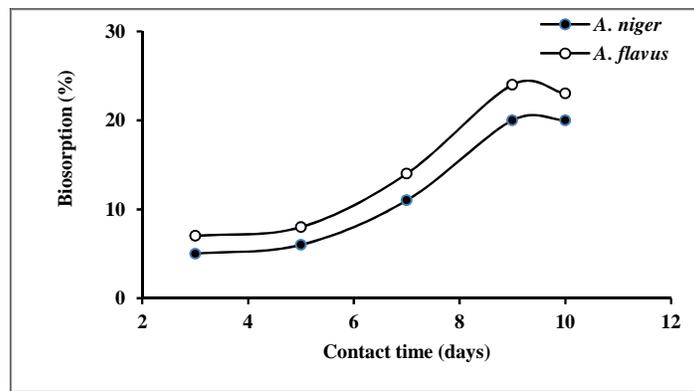


Fig. 2. Effect of contact time on REEs biosorption by *A. niger* and *A. flavus* grown on Dox liquid medium

The highest biosorption efficiency of REEs from the sample was obtained by *A. flavus* followed by *A. niger*. After 7 days of growth, the pH value increased with increasing contact time and consequently the increase in REEs biosorption. The explanation of the pH shift in biosorption process and the pH effect is helpful to identify the mechanism of metal biosorption [17].

[18] Mentioned the biosorption of La and Ce (III) on powdered leaf of *P. orientalis* was studied at pH 4 and 30°C. He found that at the start, the ions adsorbed and occupied selectively at the active sites on the biosorbent. As the contact time increased the active sites on the adsorbents were filled. The rate of adsorption became gradually slower and reached a plateau.

Effect of incubation temperatures

The obtained results, in table (4), showed that the best biosorption efficiency of REEs from ore sample by *A. niger* and *A. flavus* was occurred at 40 °C. At this temperature *A. niger* and *A. flavus* biosorbed 20% and 24%, respectively from the total amount of REEs found in the studied sample (Fig. 3).

Since the sorption process is an energy (thermal) dependent mechanism [19] which changed with different temperature. The obtained results suggested a weak interaction between REEs ions and binding sites of biomass. This could be the reason, that at a high temperature of 45 °C, hydrogen bonding is broken [20].

It is also, noticed that the final pH affects the biosorption efficiency of REEs in both fungi.

Table 4. Effect of different incubation temperatures on REEs biosorption by *A. niger* and *A. flavus* grown on Dox liquid medium

Fungal sp.	REEs (%)		incubation temperatures (°C)				
			20	30	37	40	45
<i>A. niger</i>		Biosorption	9	13	17	20	19
		Final pH	3.74	4.24	3.88	4.92	5.45
<i>A. flavus</i>		Biosorption	12	16	20	24	24
		Final pH	5.6	4.64	3.21	4.37	5.22

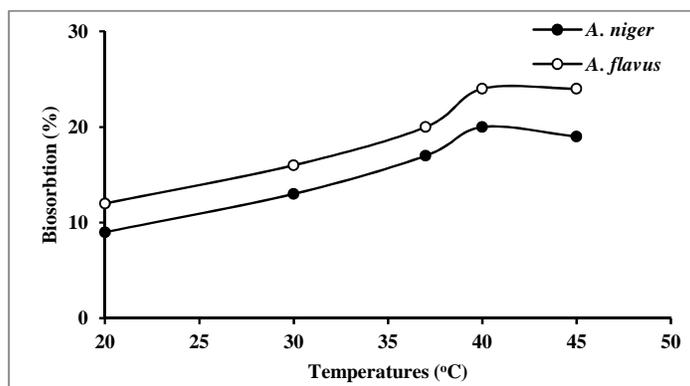


Fig. 3. Effect of different incubation temperatures on REEs biosorption by *A. niger* and *A. flavus* grown on Dox liquid medium

Biosorption elements in the biomass by *A. niger* and *A. flavus* at different ore sample concentrations.

These experiments were carried out to study the accumulation of different elements at 3% and 7% sample concentration on the biomass of *A. niger* and *A. flavus*. The amount of elements accumulated on the biomass of both fungi was found to increase with increasing ore concentration in the growth medium.

Fig. (4) illustrates ESEM spectrum and the corresponding EDX analysis of the accumulated metal ions from bioleached liquor on biomass of *A. niger* at sample concentration 3%. The adsorption ion efficiency of elements can be arranged in descending order as follow Al > Fe > Si > Ca > Mn > P > U > Co > Cu > S > Ce > K > Ni > Cl > Zn whereas at 7% ore concentration (Fig. 5) the adsorption efficiency arranged as follow Mn > Fe > K > Co > Ce > P > Ni > Si > Al > Zn > Na > Ca > Cu > U. Cu > U.

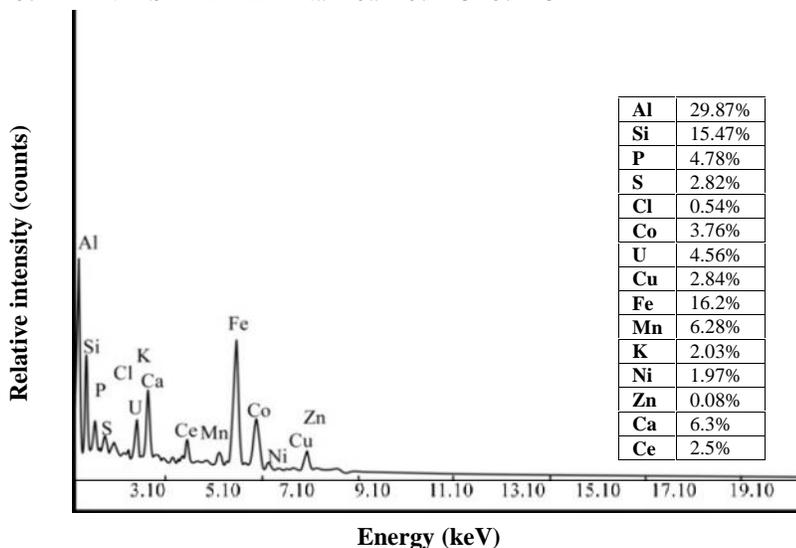


Fig. 4. ESEM with EDX of the biosorbed elements by *A. niger* at 3% sample concentration

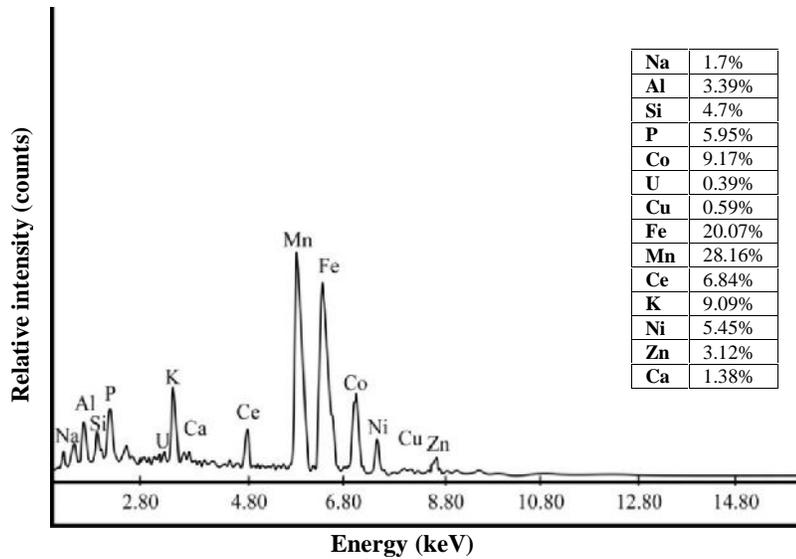


Fig. 5 ESEM with EDX of the biosorbed elements by *A. niger* at 7% sample concentration

From Fig. (5), it can be observed the low biosorption for some metals as U, Cu, and Ca, which may be attributed to the competition that metal ions face from hydrogen ions or other metal ions for the available biosorption sites [21]. Also the biosorption was higher at 7% sample concentration, this may be due to that at high sample concentration, the pH was increased which reflected on the biosorption. Variable-charge surfaces (including microorganisms and iron minerals) become more negatively charged with increasing pH, and surface groups which have lost protons may be able to adsorb metals more readily from solutions than those which have not [22].

Fig. (6) illustrate ESEM spectrum and the corresponding EDX analysis of the accumulated metal ions from bioleached liquor on biomass of *A. flavus* at sample concentration 3%. The adsorption ion efficiency of elements can be arranged in descending order as follow Fe > Al > Si > Mn > Na > Zn > Co > Ni > K > U > P > Ca > Ce > Cu whereas at 7% (**Fig. 7**), the adsorption efficiency could be arranged as follow Fe > Mn > Al > Ce > Si > P > Zn > K > Co > Nb > Ca > Ni > S > U > Cu > Cl.

The main notification was the selectivity of Ce from REEs being in both fungi. Whereas *A. niger* and *A. flavus* at 7% ore concentration adsorbed 6.84% and 7.01% of Ce, respectively which is more than adsorbed with 3% ore sample concentration.

Previous investigation agree with the results described by [23, 24, 7] who mentioned that, the accumulation of (REEs) from solution by various microorganisms. Heavy metals biosorption was reported by [25] who indicated that the biosorption of *Aspergillus* sp. was 60.94% of Cu and 97.21% of Cd for *Penicillium* sp from industrial waste water. Also, [26] indicated that *A. niger* appears to be potential adsorbent for Pb removal from aqueous solution.

Most heavy metals are abiotic factors in the cell, and there are indications that some living microorganism processes may inhibit the uptake of heavy metals. This may be dependent on the genetic constitution of the cells that can uptake heavy metals [27]. Most heavy metals are abiotic factors in the cell, and there are indications that some living microorganism processes may inhibit the uptake of heavy metals. This may be dependent on the genetic constitution of the cells that can uptake heavy metals [27].

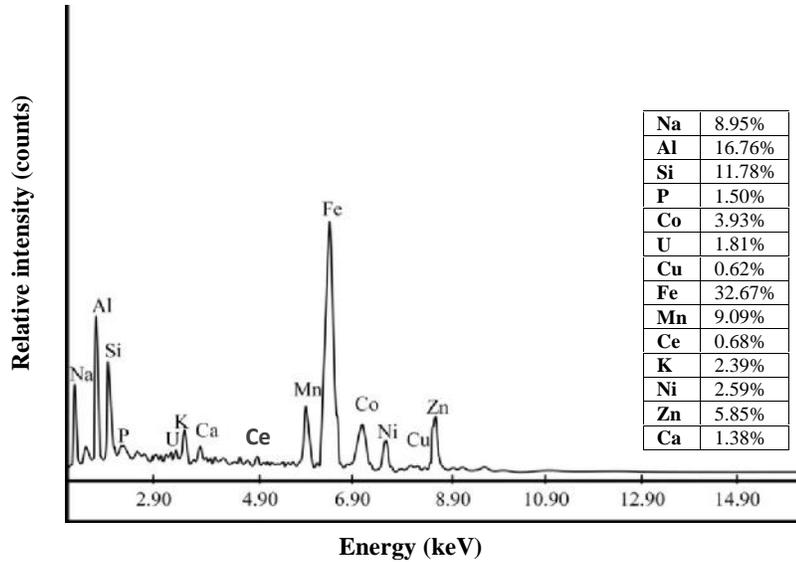


Fig. 6. ESEM with EDX of the biosorbed elements by *A. flavus* at 3% sample concentration

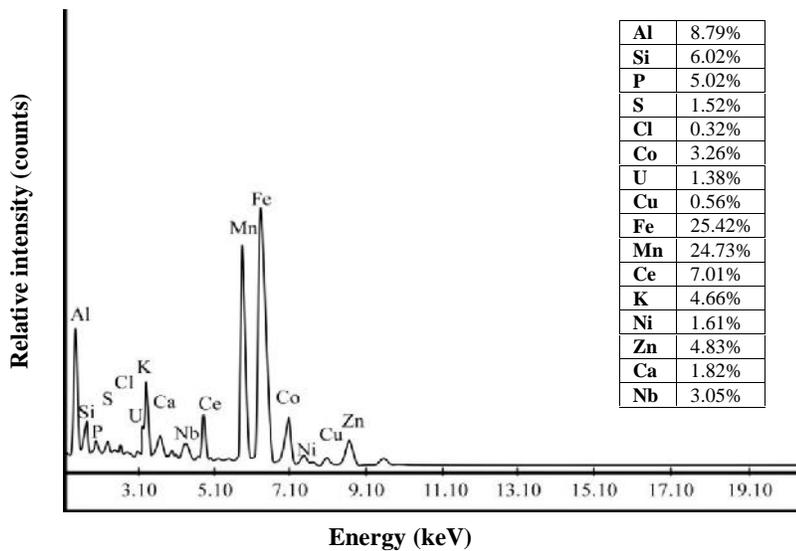


Fig. 7. ESEM with EDX of the biosorbed elements by *A. flavus* at 7% sample concentration

Effect of biosorbed elements on ultrastructure of *A. niger*

Transmission electron microscope (TEM) analysis of *A. niger* grown on Dox agar media contains (0, 3, 7 and 9 %) ore concentration sample showed that elements were accumulated and precipitated around cell wall and form a black dense area around it (Fig. 8). This dense area was increased with increasing ore concentration in the growth media. The thickness of cell walls was markedly increased at 7% ore concentration when compared to control. At 9 % ore concentration cell wall starts to hydrolysis and metal ions inter to the resulting cell large vacuole. [13] suggested that black dense areas were precipitated on the cell wall and large vacuole was formed at 600 gl⁻¹ uranium concentration in *A. niger* and at 200 gl⁻¹ U in *P. cyclospium*, [28] examined Ultra-thin section of the 0.5 mm copper, 0.1 mm selenite of *Fusarium oxysporum* were with (TEM) and deduced that cells seemed to include thicker wall and numerous globules, probably fats. Additionally, numerous vacuoles contained precipitated dense granules of copper containing compounds or elemental selenium.

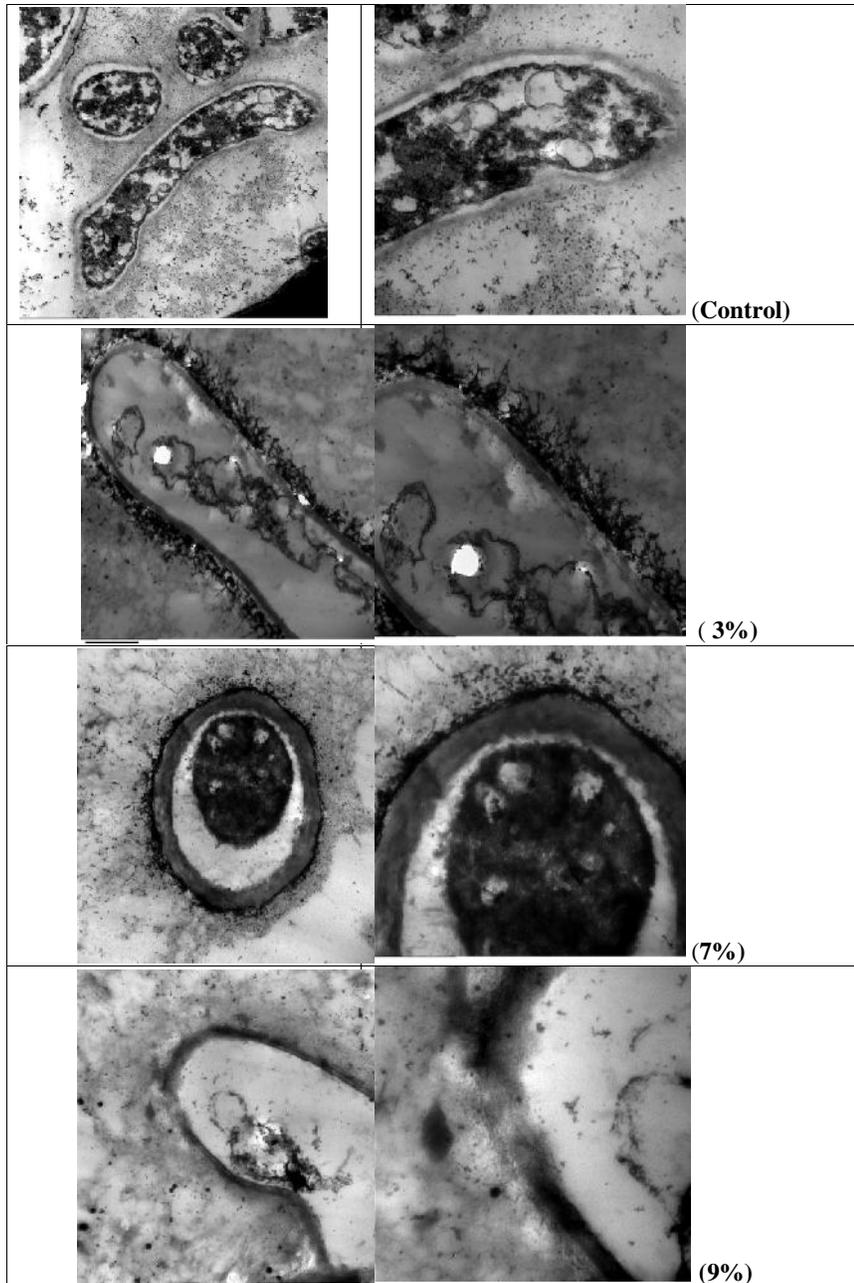


Fig. 8 Ultrastructure of *A.niger* cultivated on Dox medium supplemented with. 1) Control; 2) 3%, 3) 7% and 4) 9% of ore concentration

CONCLUSIONS

Tow fungal genera were isolated (*A. flavus* and *A. niger*) from the studied Sinai carbonaceous shale sample. The maximum biosorption efficiency of REEs obtained from 5% ore concentration of Budaa sample by *A. flavus* followed by *A. niger*. This means that *A. flavus* can work under stress of REEs content than *A. niger*. The best accumulation of REEs occurred at nine days of incubation for *A. flavus* and *A. niger* and from 37-45 °C incubation temperature for dry biomass of *A. niger* and *A. flavus*. (TEM) analysis of *A. niger* showed that elements were accumulated and precipitated around cell wall and form black dense area around it. By increasing ore concentration cell wall starts to hydrolysis and metal ions inter to the resulting cell large vacuole. The main noticed goal is the selectivity of Ce from REEs being in both fungal, whereas *A. niger* and *A. flavus* in 7% ore concentration adsorbed 6.84% and 7.01% of Ce, respectively.

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