FUNGAL LEACHING OF RARE EARTH ELEMENTS FROM LOWER CARBONIFEROUS CARBONACEOUS SHALES, SOUTHWESTERN SINAI, EGYPT

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Abstract. The main concern of this study is to test the ability of some fungi isolated from Sinai Peninsula carbonaceous shales to perform microbial leaching of rare earth elements (REEs) from these rocks. Two methods of REEs bioleaching were tested using both direct (active) and indirect (passive) processes. Direct bioleaching process of carbonaceous shale samples was found to be more effective than indirect process for all tested microorganisms. Bioleaching efficiency of REEs using different fungal strains increases with decreasing REEs concentrations when their growth rate decreases. Strains of *Aspergillus* (*A. niger* and *A. flavus*) were found to be the most efficient organisms. The maximum extraction yield of total REEs was 86% at 7days incubation time, 1% pulp density, and 30 ºC incubation temperature. *A. niger* and *A. flavus* exhibit good potential in generating a variety of organic acids (citric and oxalic) effective for REEs solubilization. Citric and oxalic acids contents produced by *A. niger* was higher than by *A. flavus* interpreting higher REEs bioleaching efficiency of *A. niger* than *A. flavus*. From properly prepared pregnant bio-leach liquor, the leached REEs were recovered in the form of REEs oxalate product using classical chemical technique.

Key words, Bioleaching, REEs, *Aspergillus niger*, *Aspergillus flavus*.

INTRODUCTION

Rare earth elements are a collection of seventeen chemical elements in the periodic table, specifically the fifteen lanthanoids plus scandium and yttrium [3]. Scandium and yttrium are considered rare earth elements since they tend to occur in the same ore deposits as the lanthanides and exhibit similar chemical properties. Geochemical properties of rare earth elements are typically dispersed and not plenty. REEs are widely used in industry [11], agriculture [18], and medicine [4].

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Both radioactive and REEs can enter the food chain, resulting in their intake by human. Thus, it has become increasingly important to know the behavior of these elements in the environment. The environmental behavior of REEs is affected by biotic and abiotic factors. The former include interaction with microorganisms and plants, and their originated substances, such as citric acid and humic substance [25].

The conventional chemical techniques for dissolving REEs are very expensive due to high energy required and are associated with environmental hazards, as production of harmful gases therefore the potential use of microorganisms in the bioleaching of these elements has been investigated [17].

Bioleaching is an emerging technology with significant potentials to add value to the mining industries so as to deliver attractive environmental and social benefits to all the associates [20].

Bioleaching process includes four main mechanisms, i.e. acidolysis, complexolysis, redoxolysis and bioaccumulation. Acidolysis is the main principal mechanism in metals bioleaching in which the fungus and bacterium produce varieties of organic acids such as citric, oxalic and gluconic acids [13].

The microorganisms involved in industrial mineral leaching processes are numerous, and they grow in mixed communities. These microorganisms are either chemolithoautotrophic bacteria, such as *Thiobacillus thiooxidans, Thiobacillus ferrooxidans, Leptospirillum ferrooxidans* and thermophilic bacteria or a number of heterotrophic organisms. However, the information available on the bioleaching of rare metals is limited.

Accumulation of La, Ce and Nd in the ectomycorrhizal fungus *Russula pectinatoides* in forest sites has been reported [1]. Among REEs, La^{3+} has an ionic radius similar to that of calcium (Ca^{2+}) and is, therefore, able to competitively replace Ca ions in biological systems [8]. Nevertheless, a biological role for lanthanides is not known so far. Although REEs are not thus far considered essential to the cellular life cycle and beneficial effects on crops have not been clearly demonstrated [5]. The accumulation of La, Ce and Nd in Agaricales fungi has been reported [1], but little information is available about effects of REEs on fungal microorganisms. Differential effects associated with REEs enrichment of the growth media on beneficial and detrimental soil-borne fungi have been reported [6], but the consequence of REEs treatment on fungal structures such as plasma membrane, cell wall and external matrix has not been investigated yet. D'Aquino *et al.* [5] indicated that all *Trichoderma* strains showed a good tolerance to the presence of REEs in the culture media and some growth enhancing effects were observed in liquid cultures of *T. harzianum* strains, but not in *T. atroviride*. Accumulation of REEs in fungal biomass, both at intracellular level and in the extracellular matrix, was observed.

The filamentous, acid-producing fungal strains *Penicillium tricolor RM-10* isolated from red mud and used in one-step bioleaching experiments at a total concentration of 2% (w/v) red mud was generally found to give the maximum leaching ratios of the REEs and radioactive elements. However, the highest extraction yields were achieved under two-step bioleaching process at 10% (w/v) pulp density, but only densities of 2% and 5% (w/v) red mud processed under both one- and two-step bioleaching can meet the radioactivity regulations in China [21].

The aim of the present study is to determine whether various fungi isolated from Sinai Peninsula carbonaceous shales can be effectively used for the extraction of REEs from the same rock material. The metabolic activities of tested organisms were also studied to interpret the mechanism of REEs solubilization by isolated microorganisms and potentiality of extrapolating the obtained results to pilot-scale flowsheet.

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 previously classified by many authors. This section was classified by Ball [2] to lower sandstone and Carboniferous limestone and then classified to Um Bogma group (lower sandstone and fossiliferous dolomite) and Ataqa group [15]. Ataqa group was classified into El Hashash Formation (old), Magharet El Meyiah Formation and Abu Zarab Formation (young) [24]. In this work the last classification of our concern is performed on samples from Magharet El Meyiah Formation (Table 1). El Aassy *et al.* [7] pointed to the low-grade REEs resource in Magharet El Meyiah Formation. This formation in Budaa locality was the main target of this study.

EXPERIMENTATION

SAMPLES PREPARATION

A total of three representative samples (Table 1) were collected from the study area B.1, B.2 and B.3. Each sample was ground to (60 mesh) and mixed well to avoid heterogeneous distribution of mineral constituents.

CHEMICAL ANALYSES OF ORE SAMPLES

The pulverized samples were analyzed by conventional wet chemical techniques [23]. Whereas SiO_2, Al_2O_3, TiO_2 and P_2O_5 were determined using a spectrophotometric method, the contents of Na and K were determined by a flame photometric technique. Total Fe as Fe_2O_3, 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 analyses were carried out in the laboratories of the Nuclear Materials Authority, Cairo, Egypt.

Table 1

Samples collected from Magharet El Meyiah Formation, Budaa locality

Location	***Sample No.***	***Description***
Budaa. I Station at the northern part of the cliff to the north from G-Um Rinna.	**B. 3**	From the top it is calcareous black shale, dark, friable, 20 cm thickness.
Budaa. II In the entrance of the old pit.	**B. 1**	Calcareous and evaporitic shale from the middle part of the bed, grey and brown, 1m thickness.
Budaa. IV At El Naqb, El Hashash Fm. At the base, thick and the overlying Magharet EL Meyiah Fm. shows highly carbonaceous clays.	**B. 2**	Fosiliferous and sheeted carbonaceous, shaley coal.

FUNGAL ISOLATION

The Dox agar medium composition (g/L): $NaNO_3$, 2; KH_2PO_4 1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.5H_2O$ 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 before autoclaving at 1.5 atm. for 20 minutes. 1g of the ore powder was taken under aseptic conditions and mixed well with 9 mL of sterile distilled water; 0.1 mL of this mixture was spread under aseptic conditions by sterile glass rod on the surface of agar plate. The plates were incubated at 30 ºC ± 2 until development of the colony. Hyphal tips of each colony were removed and plated on the surface of agar plates. The developed colonies were examined with a microscope to detect contamination. The pure isolated fungi were identified according to [10] and [19].

OPTIMIZATION OF BIOLEACHING PARAMETERS AND RECOVERY OF REEs

Bioleaching process upon REEs bearing minerals was performed by using *A. niger* and *A. flavus* however different bioleaching affecting factors were investigated. These factors were ore concentration 1–7%, incubation period 3–10 days and temperature 20–45 ºC. To study the procedures of recovering REEs from the working samples, proper leach liquor has been prepared using 200 g ore and applied the determined optimum conditions. The recovery of REEs was carried out by direct precipitation using 30% oxalic acid at pH 1. The obtained REEs precipitate was calcinated at 800°C before being analyzed by ESEM-EDAX analysis.

PRODUCTION OF ORGANIC ACIDS BY *A. NIGER* AND *A. FLAVUS*

Both tested organisms were cultivated on Dox liquid media for 7 days at 30 °C. Organic acids were determined in the filtrates by High Performance Liquid Chromatography (HPLC) at the Regional Center for Mycology and Biotechnology, Al Azhar Univ., Cairo, Egypt.

RESULTS AND DISCUSSION

CHEMICAL ANALYSES OF ORE SAMPLES

Chemical analyses of the three carbonaceous shale samples (Table 2) B.1, B.2 and B.3 showed a high content of SiO_2, which was represented by 53.4%, 27.3% and 50.3%, respectively. Also, moderate contents of Al_2O_3 were recorded as 17.8%, 5.1% and 10.7% for samples B.1, B.2 and B.3, respectively (Table 2). The total concentration of rare earth elements of the studied samples B.1, B.2 and B.3 was also determined as 318, 244 and 200 ppm, respectively (Table 2 and Figure 1). The well noticed thing is the high studied loss on ignition especially in sample B.2 which measured 61.1%.

THE EFFECT OF FUNGAL ACTIVITY ON THE BIOLEACHING OF REEs

Eight fungal genera were isolated from the studied samples. The most dominant were *Aspergillus* and *Penicillium* sp.. The *Aspergillus* species were identified as *A. niger, A. flavus, A. terreus* and *A. ficuum*, whereas *Penicillium* species were *P. aeruginosa, P. oxalicum, P. cyclopium* and *P. diversum*. All of the tested fungi could grow in the presence of 1% ore concentration of the studied samples B.1, B.2 and B.3. Two test methods were carried out for bioleaching processes. The first method includes the direct contact between the tested sample and microorganism whereas the second one, indirect, represents the addition of the tested sample to the culture filtrate of the organism.

Table 2

Major oxides and REEs analyses of the tested samples (wt. %)

Major oxides (Wt. %)	**B.1**	**B.2**	**B.3**
SiO_2	53.4	27.3	50.3
Al_2O_3	17.8	5.1	10.7
TiO_2	1.1	0.46	0.84
Fe_2O_3	0.043	0.033	0.023
MnO	0.00145	ULD	ULD
CaO	3.9	1.68	5.04
MgO	3.6	2.41	6.45
Na_2O	1.311	0.263	0.871
K_2O	1.119	0.337	0.627
P_2O_5	0.061	0.04	0.023
L.O.I	**17.6**	**61.1**	**24.09**
Total	**99.92**	**98.723**	**98.9**
REEs (ppm)	**318**	**244**	**200**

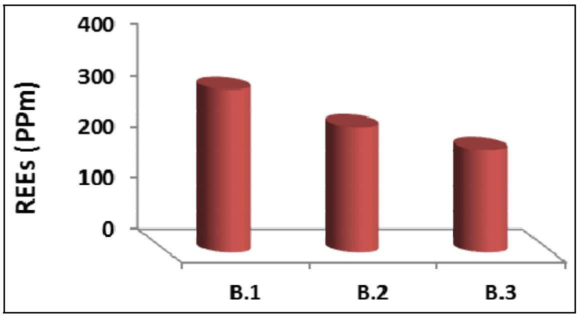


Fig. 1. REEs concentration (ppm) of carbonaceous shale samples (B.1, B.2 and B.3).

It was found that direct bioleaching process of REEs from the tested samples was more effective than indirect process for all tested microorganisms (Table 3). This may be due to the fact that bio-acids appeared to be more active in the presence of the fungi suggesting that the mechanism is not simply a direct chemical attack on the minerals but the fungi participate in the leaching process [22]. Also, the bioleaching percentages of REEs from the tested samples by *Aspergillus sp*. were more than *Penicilium sp*. These results showed that as the concentrations of REEs in the original samples are decreasing the bioleaching efficiencies in the whole fungi increase (Table 3). The reverse is the case in the growth of fungus which increases with the increase of REEs concentrations except in *A. terreus* and *P. oxalicum*. This may be attributed to solubilization of some metal ions which may affect the growth of isolated organisms. Also mineralogy of the ore has affected the bioleaching efficiency of REEs. Valix *et al.* [27] reported the metal loss through electro-sorption properties of the ore, effective and selective leaching by certain strains for some heavy metals more than for others.

Table 3

Bioleaching efficiency of REEs using the dominant fungal isolates

Fungus sp.	**S. No.**	**Direct (%)**	**Indirect (%)**	**Final pH**	**Growth (mg/100mL)**
A. niger	**B. 1**	23	14	5.52	531
	B. 2	48	36	5.18	476
	B. 3	86	45	4.24	453
A. flavus	**B. 1**	28	20	5.97	511
	B. 2	25	21	6.06	482
	B. 3	62	35	4.64	463
A. terreus	**B. 1**	21	18	5.77	567
	B. 2	27	32	6.36	534
	B. 3	57	33	5.43	616
A. ficuum	**B. 1**	23	11	6.44	581
	B. 2	28	15	5.83	545
	B. 3	55	22	5.56	515
P. aeruginosa	**B. 1**	20	13	5.65	742
	B. 2	22	18	5.68	694
	B. 3	33	21	5.11	675
P. cyclopium	**B. 1**	18	12	6.47	778
	B. 2	22	20	6.64	751
	B. 3	45	25	6.81	732
P. diversum	**B. 1**	21	15	4.87	697
	B. 2	20	21	5.34	678
	B. 3	40	23	5.65	647
P. oxalicum	**B. 1**	19	10	6.66	675
	B. 2	20	15	6.53	659
	B. 3	41	19	6.21	716

The growth of *Penicillium sp.* in the presence of carbonaceous shale samples B.1, B.2 and B.3 was higher than for *Aspergillus sp.*; this may be attributed to the ability of these organisms to tolerate and grow under stress of REEs and carbonaceus materials. The highest leaching efficiency of REEs occurs when the final pH shifted to more acidity.

From Table 3 it could be mentioned that *A. niger* was superior followed by *A. flavus*. Since the bioleaching efficiency of REEs obtained with *A. niger* and *A. flavus* were higher than those obtained with the other fungi, the authors decided to investigate in more detail the process of bioleaching with these two microorganisms.

DETERMINATION OF ORGANIC ACIDS

The recognition of organic acids produced by the tested fungal strains during leaching of the studied samples was carried out using high performance liquid chromatography (HPLC). Data presented in Figure 2 indicated that the extracted filtrate had two peaks, the first for citric acid, while the second for oxalic acid. The charts proved that the acids varied in their quantities according to the fungal strain.

The concentration of citric and oxalic acids in the fermented liquor by *A. niger* were 23.35 and 4.90 mg/mL, respectively whereas being 10.19 and 4.44 mg/mL, respectively for *A. flavus*. So the citric and oxalic acids contents produced by *A. niger* was higher than that produced by *A. flavus* often at a 7 days incubation period. This explains the higher REEs bioleaching efficiency of *A. niger* than of *A. flavus*.

OPTIMIZATION OF *A. NIGER* AND *A. FLAVUS* ON BIOLEACHING EFFICIENCY OF REEs

The optimization of *A. niger* and *A. flavus* on bioleaching efficiency of REEs was affected by ore concentrations, incubation periods and incubation temperatures.

Effect of ore concentrations

This experiment was carried out by adding 1, 2, 3, 5 and 7 grams, respectively, of the studied samples to 100 mL volume of Czapez's-Dox broth media inoculated with 1 mL of *A. niger* and *A. flavus* spore suspension. The cultures were incubated for 7 days at 30 °C. The obtained results were illustrated in Table 4 and Figure 3 which indicate that the amount of released REEs decreased with increasing ore concentrations. This decrease is steady in sample B.3 and not in the other two samples (Fig. 3).

The highest bioleaching efficiency of REEs from the samples was obtained at 1% by *A. niger* followed by *A. flavus*, suggesting that the best leaching efficiency of REEs was obtained with decreasing REEs content. A well noticed point is that at 1%, 5% and 7% ore concentrations and high REEs content, the efficiency of *A. flavus* is higher than that of *A. niger*. This means that *A. flavus* can work better under stress of REEs content than *A. niger*.

The final pHs were increased with increasing concentrations in the growth media, whereas the best leaching efficiency of REEs has occurred only when the final pH of the media became acidic. This agrees with that recorded by Liu *et al.* [16], where they obtain a high leaching capacity of some metals at 2% (w/v) solids concentration by indigenous sulfur-oxidizing bacteria. The highest dissolution of these metals was detected at acidic pH conditions.

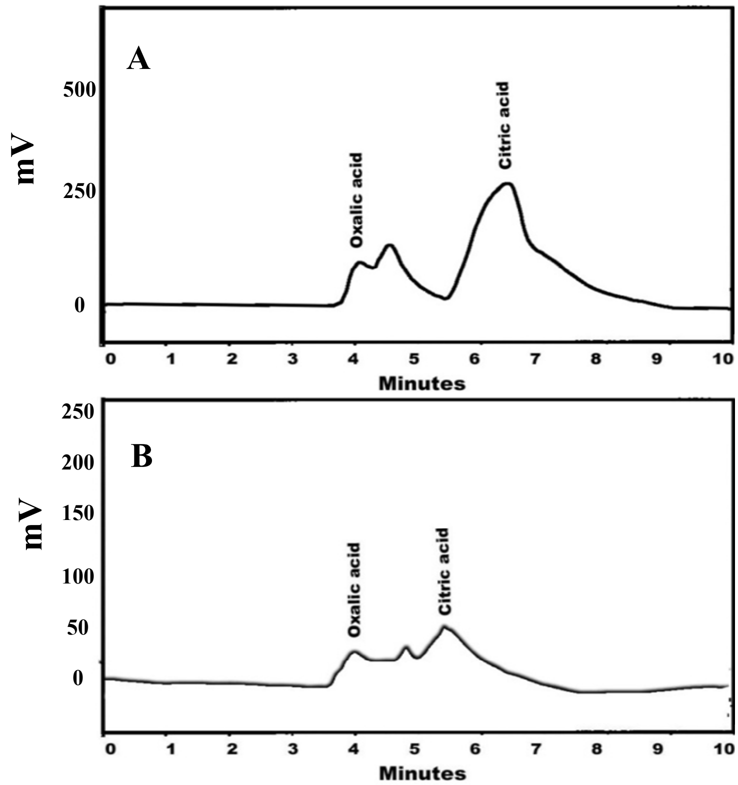


Fig. 2. Determination of organic acids produced by (A) *A. niger* control; (B) *A. flavus* control.

Table 4

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

Fungal sp.		Ore samples	Ore concentrations (%)				
			1	2	3	5	7
A. niger	REEs (%)	B. 1	23	20	13	7	4
		B. 2	48	15	9	7	4
		B. 3	86	40	22	14	11
	Final pH	B. 1	5.52	5.72	6.12	6.54	7.6
		B. 2	5.18	5.57	6.17	6.34	6.8
		B. 3	4.24	4.15	4.33	4.62	5.4
A. flavus	REEs (%)	B. 1	28	18	12	9	5
		B. 2	21	14	6	2	1
		B. 3	60	27	18	9	7
	Final pH	B. 1	5.97	6.08	6.56	7.43	7.7
		B. 2	6.06	6.27	6.76	7.08	8.02
		B. 3	4.64	4.75	5.58	6.6	6.8

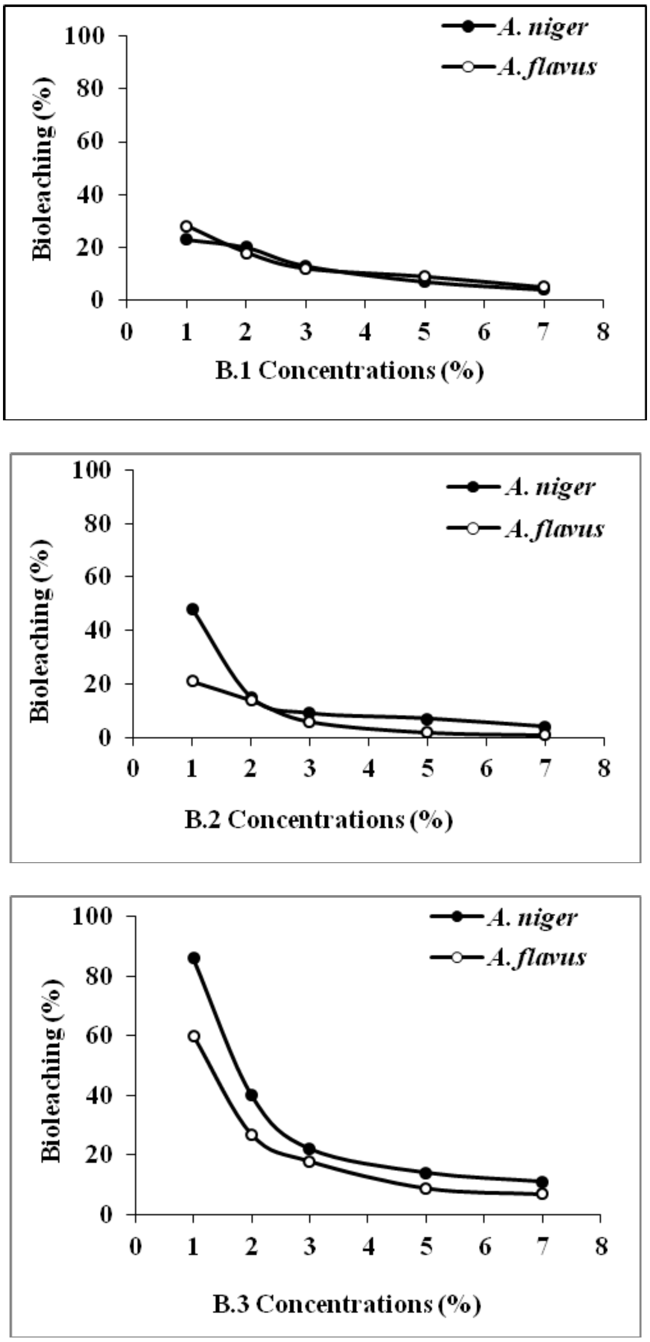


Fig. 3. Effect of different ore concentrations on REEs solubilization by *A. niger* and *A. flavus* grown on Dox liquid medium at 30 °C for 7days.

Effect of incubation periods

This factor was studied by mixing 1 gram of each sample with 100 mL Czapez's-Dox broth inoculated with 1 mL of *A. niger* and *A. flavus* spore suspension at 30 ^{o}C for different incubation periods, 3, 5, 7, 9 and 10 days. The obtained results were illustrated in Table 5 and Figure 4.

The results showed that the maximum bioleaching efficiency of REEs using *A. niger* and *A. flavus* was obtained at 7 days of incubation and rapidly decreased after these days of incubation especially in B.1 and B.2 (Fig. 4).

The bioleaching efficiencies increase with decreasing of REEs content in the original samples (Table 5). It reached in B.3 to 86% and 62% for *A. niger* and *A. flavus*, respectively. The behavior of B.2 is deviated something from this result especially with *A. flavus* which may be attributed to the high content of carbon which plays its role in the leaching efficiency *A. flavus.*

The incubation periods curve in sample B.3 has a plateau in *A. niger*, while it is nearly symmetric in *A. flavus* (Fig. 4).

This phenomenon may be attributed to the effect of some metal ions released to the medium which affect the activity of organism. The maximum antibiotic production was achieved on the 7th to the 8th day of incubation of *Actinomyces lavendulae* culture [14].

Table 5

Effect of different incubation periods on REEs solubilization from 1% of ore concentrations of samples B.1, B.2 and B.3 using *A. niger* and *A. flavus* grown on Dox liquid medium at 30 ^{o}C

Fungal sp.		**Ore samples**	**Incubation periods (Days)**				
			3	**5**	**7**	**9**	**10**
A. niger	**REEs (%)**	**B. 1**	16	20	22	14	5
		B. 2	10	16	39	16	8
		B. 3	30	84	86	77	41
	Final pH	**B. 1**	6.63	5.62	5.52	6.4	7.6
		B. 2	6.47	5.47	5.18	6.58	6.7
		B. 3	6.65	5.41	4.24	6.59	7.4
A. flavus	**REEs (%)**	**B. 1**	14	17	28	17	12
		B. 2	13	18	25	15	6
		B. 3	13	38	62	33	16
	Final pH	**B. 1**	6.28	6.02	5.97	6.26	6.75
		B. 2	6.48	6.12	6.06	6.32	6.89
		B. 3	5.35	5.53	4.64	5.53	7.11

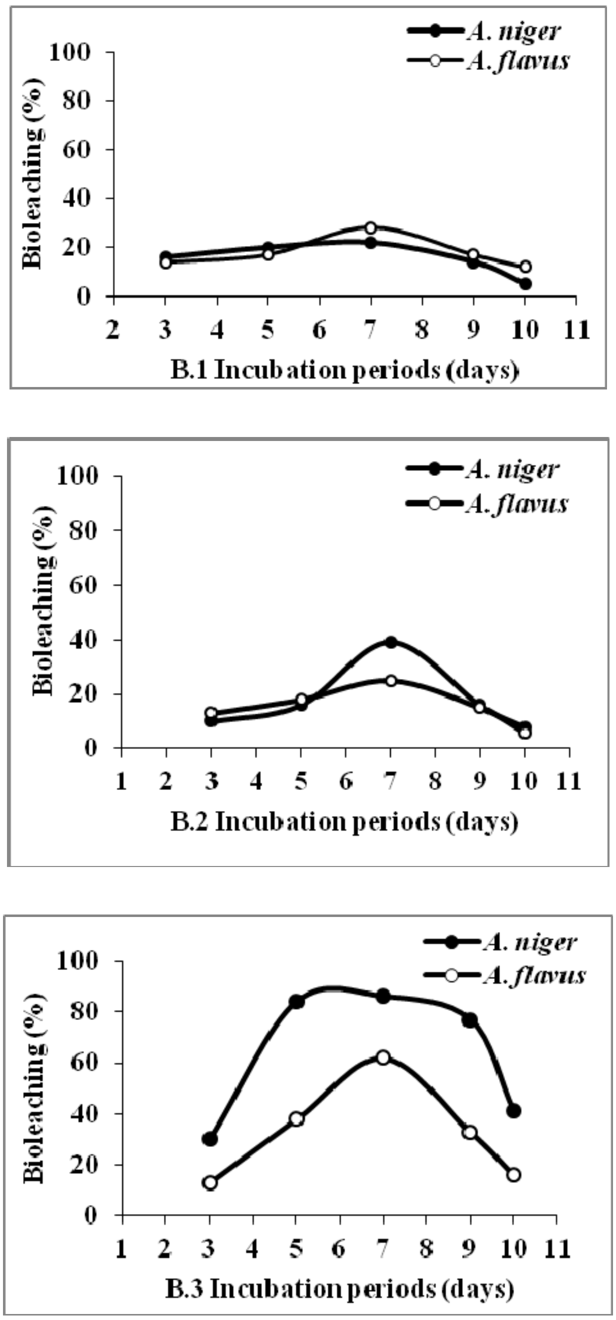


Fig. 4. Effect of different incubation periods on REEs solubilization from 1% of ore concentrations of samples B.1, B.2 and B.3 using *A. niger* and *A. flavus* grown on Dox liquid medium at 30 ^{o}C.

Effect of incubation temperatures

This experiment was carried out by growing of *A. niger* and *A. flavus* in 100 mL Czapez's-Dox broth supplemented with 1gram of each sample B.1, B.2 and B.3. The flasks were incubated at different temperatures (20, 30, 35, 40 and 45 °C) for 7 days. The obtained results were illustrated in Table 6 and Figure 6.

Results in Table 6 showed that the best leaching efficiency of REEs from ore samples by *A. niger* and *A. flavus* occurred at 30 °C. At this temperature *A. niger* solubilized 14%, 36% and 73% from the total amount of REEs found in samples B.1, B.2 and B.3 respectively (Fig. 5). On the other hand, *A. flavus* solubilized 30%, 23% and 61% from the total amount of REEs found in samples B.1, B.2 and B.3 respectively.

A very important notice is that *A. niger* was not affected by the high content of carbon in sample B.2, while *A. flavus* was affected as noticed after roasting the sample at 1000 °C. The result in this case reached 40% leachability (Table 6). Frattini *et al.* [9] stated that high organic matter of the sludge can have inhibitory effect on the bioleaching process, since *At. ferrooxidans* is very much sensitive to various organic compounds such as organic acids, simple sugars and amino acids**.**

Table 6

Effect of different incubation temperatures on REEs solubilization from 1% ore concentrations of samples B.1, B.2 and B.3 by *A. niger* and *A. flavus* grown on Dox liquid medium for 7 days

Fungal sp.		**Ore samples**	**Incubation temperatures (°C)**				
			20	**30**	**37**	**40**	**45**
A. niger	**REEs (%)**	**B. 1**	8	14	11	3	2
		B. 2	14	36	22	10	4
		B. 3	24	73	72	32	20
	Final pH	**B. 1**	3.81	5.52	4.97	5.77	6.35
		B. 2	3.62	5.18	4.69	5.27	6.47
		B. 3	3.74	4.24	3.88	4.92	5.45
A. flavus	**REEs (%)**	**B. 1**	25	30	22	6	3
		B. 2	20	23–40*	11	5	2
		B. 3	22	61	57	47	18
	Final pH	**B. 1**	6.06	5.97	5.4	5.66	6.5
		B. 2	6.25	6.06	5.42	6.25	6.7
		B. 3	5.6	4.64	3.21	4.37	5.22

* This result after roasting at 1000 °C

Both fungi had nearly the same behaviour at the temperatures 30 °C and 37 °C in sample B.3 (Fig. 5). James *et al.* [12] stated that temperature was a regulatory factor in the secondary metabolism of *Streptomyces thermaviolaceus*.

Also we noticed that the final pH was in counteractive relation with the leaching efficiency of REEs; where, the highest leaching efficiency of REEs in both fungi occurred at the lowest pH value. With increasing the pH, the bioleaching efficiency started to decrease.

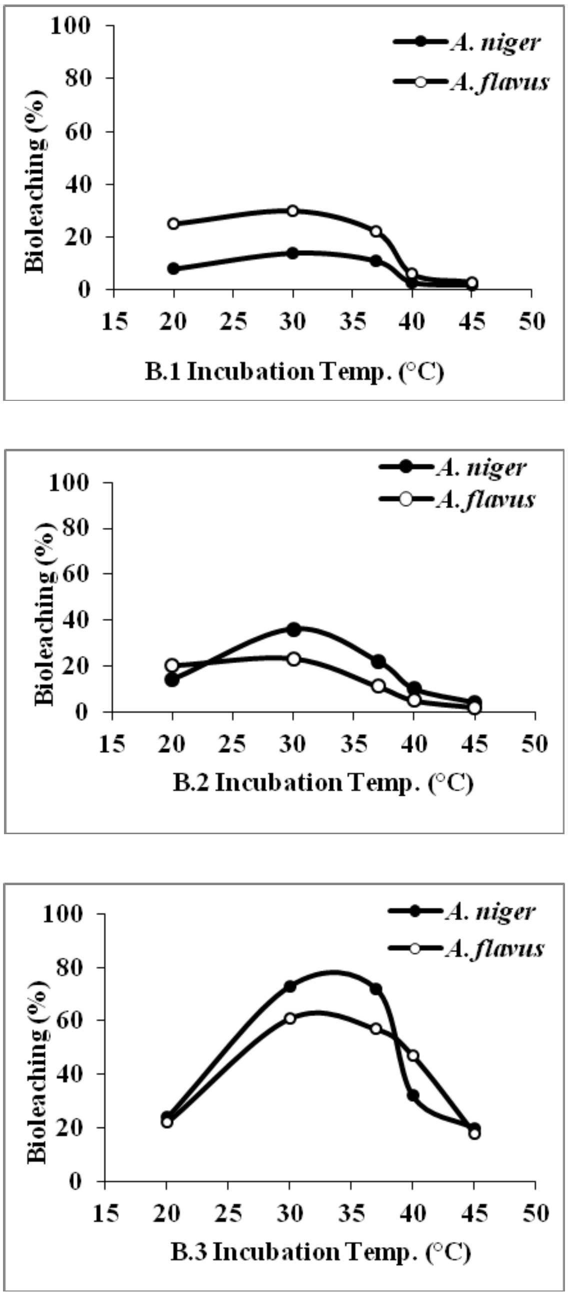


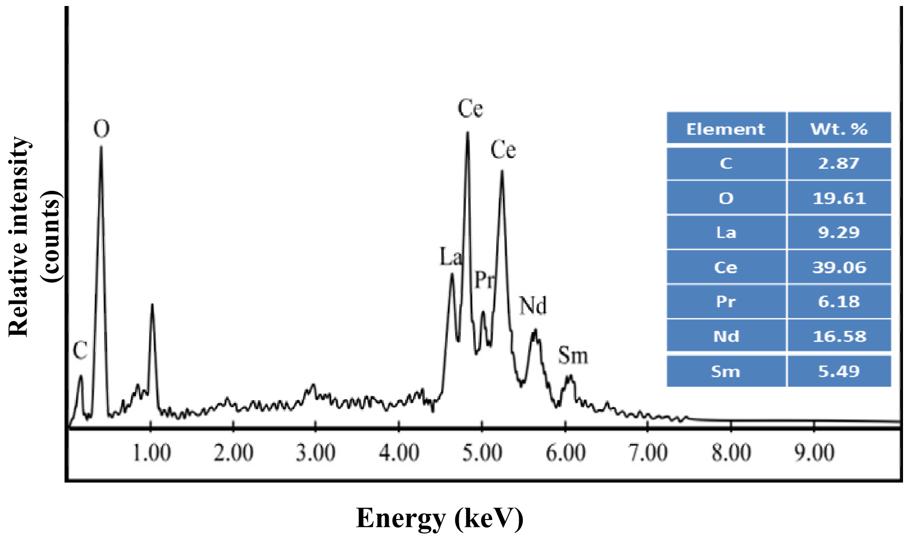
Fig. 5. Effect of different incubation temperatures on REEs solubilization from 1% of ore concentration of samples (B.1, B.2 and B.3) by *A. niger* and *A. flavus* grown on Dox liquid medium for 7 days.

OPTIMIZATION OF BIOLEACHING PARAMETERS AND RECOVERY OF REEs

From the previously mentioned experiments, it can be concluded that the optimum conditions of solubilization are 1% ore concentration, and incubation for 7 days at 30 °C.

APPLICATION OF OPTIMUM CONDITIONS ON SAMPLE B.3

By applying the optimum conditions upon a solid sample (B.3) using *A. niger* the leaching efficiency was 67.5%. The REEs oxalate was precipitated using 30% oxalic acid at pH 1. The precipitated REEs oxalate was then calcined at 800 °C before being analyzed by Environmental Scanning Electron Microscope – Energy Depressive X-ray (ESEM-EDAX) analysis. The result of EDAX analysis is shown in Figure 6.



Element	Wt. %
C	2.87
O	19.61
La	9.29
Ce	39.06
Pr	6.18
Nd	16.58
Sm	5.49

Fig. 6. ESEM-EDAX. Spectrum of the prepared calcined product from (B.3) after precipitation at pH 1 and calcination at 800 °C.

From the latter, it is clearly evident that the total REEs concentrate includes assays of La, Ce and Nd about 9.29, 39.06 and 16.58 % while of Pr and Sm assay of about 6.18 and 5.49 %, respectively.

The bioleaching process of REEs by *A. niger* indicates the selective activity of this fungal strain, the precipitated elements containing light rare earth elements (LREEs), but no heavy rare earth elements (HREEs). This agrees with the study of Tsuruta [26], using a solution containing five REEs (Y, La, Sm, Er, and Lu) and *Mucor juvanicus,* that preferentially removed Sm, while *Stirtoniopsis jlavoviridis* was used, Lu was preferentially accumulated. These results indicated that microorganisms are performing a selective leaching and accumulation of separate REEs.

CONCLUSIONS

Eight fungal strains were isolated from the studied carbonaceous shale samples. Various bioleaching methods indicate that the direct REEs bioleaching process performed by the tested samples was more effective than the indirect one for all tested microorganisms and the bioleaching efficiencies in the whole fungi increase with decreasing the REEs content in the original samples. The maximum extraction yield of total REEs was 86% at 7 days incubation time, 1% pulp density, and 30 ºC incubation temperature. *A. niger* and *A. flavus* exhibited a good potential in generating a variety of organic acids (citric and oxalic acids) effective for the light REEs solubilization. The content of citric and oxalic acids produced by *A. niger* was higher than by *A. flavus* interpreting a higher REEs bioleaching efficiency of *A. niger* than of *A. flavus*. For the REEs recovery, the oxalic acid was used for precipitation, and then the precipitate was analyzed by ESEM-EDAX analysis.

REFERENCES

1. ARUGUETE, D.M., J.H. ALDSTADT, G.M. MUELLER, Accumulation of several heavy metals and lanthanides in mushrooms (*Agaricales*) from the Chicago region, *Sci. Total Environ.*, 1998, **224**, 43–56.
2. BALL, J., *Geography and Geology of west-central Sinai,* Ministry of Finance (Egypt). Survey Department, 1916.
3. CONNELLY, N.G., T. DAMHUS, R.M. HARTSHORN, A.T. HUTTON, *Nomenclature of Inorganic Chemistry: IUPAC Recommendations 2005*, RSC Publ., Cambridge, 2005.
4. CUTLER, C.S., C.J. SMITH, G. EHRHARDT, T.T. TYLER, S.S. JURISSON, E.DEUTSCH, *Cancer Biothe., Radiopharm*., 2000, **5**, 531–545.
5. D'AQUINO, L., M. MASSIMO MORGANA, M.A. CARBONI, M. STAIANO, M.A. VITTORI, M. RE, M. LORITO, F. VINALE, K.M. ABADI, S.L. WOO, Effect of some rare earth elements on the growth and lanthanide accumulation in different *Trichoderma* strains, *Soil Biol. Biochem*., 2009, **41**, 2406–2413.
6. D'AQUINO, L., M.A. CARBONI, S.L. WOO, M. MORGANA, L. NARDI, M. LORITO, Effect of rare earth application on the growth of *Trichoderma* spp. and several plant pathogenic fungi, *J. Zhej. Univ*., 2004, **30**, 424.
7. EL AASSY, I.E., F.Y. AHMED, A.E. SHATA, G.A. MOHMOUD, A.S. ASHAMI, M.M. GABR, A.A. RABBOH, New resource of rare earth element in Sinai, Egypt, 7^{th} *International Conference on Geochemistry*, Alexandria, Egypt, 6–7 September, 2006.
8. EVANS, C.H., Interesting and useful biochemical properties of lanthanides, *Trends Biochem. Sci.*, 1983, **8 (12)**, 445–449.

9. FRATTINI, C.J., L.G. LEDUC, G.D. FERRONI, Strain variability and the effects of organic compounds on the growth of the chemolithotrophic bacterium *Thiobacillus ferrooxidans*, *Antonie Van Leeuwenhoek*., 2000, **77**(1), 57–64.
10. GILMAN, J.C., *A manual of soil fungi*, The Iowa State Press, Ames, Iowa, USA, 1957.
11. HUNG, C.M., M. LOU, C.H. LIN, Removal of ammonia solutions used in catalytic wet oxidation processes, *Chemosphere*, 2003, **52**, 989–995.
12. JAMES, P.D.A., C. EDWARDS, M. DAWSON, The effect of temperature and growth rate on secondary metabolism in *Streptomyces thermoviolaceus* growth in a chemostat, *J. of Gen. Microbiol.*, 1991, **137**, 1715–1720.
13. JOHNSON, D.B., Biohydrometallurgy and the environment: intimate and important interplay, *Hydrometallurgy*, 2006, **83**, 153–166.
14. JUSLEN, C.K., H.D. KING, M. KUHN, H.R, LOOSLI, A.V. WARTBURG, Noboritomycins A and B, new Polyether antibiotics, *J. Antibiot*., 1978, **31**, 820–828.
15. KOSTANDI, A.B., Facies maps for the study of the Palaeozoic and Mesozoic sedimentary basins of the Egyptian region, UAR, *Proceedings 1st Arab Petroleum Congress*,1959, **2**, 54–62.
16. LIU, Y.G., M. ZHOU, G.M. ZENG, X. WANG, X. LI, F. TING, W.H. XU, Bioleaching of heavy metals from mine tailings by indigenous sulfur-oxidizing bacteria: Effects of substrate concentration, *Bioresource Technology*, 2008, **99**, 4124–4129
17. MISHRA, A., N. PRADHAN, R.N. KAR, L.B. SUKLA, B.K. MISHRA, Microbial recovery of uranium using native fungal strains, *Hydrometallurgy*, 2009, **95**, 175–177.
18. PANG, X., D. LI, A. PENG, Application of rare earth elements in the agriculture of China and its environmental behavior in soil, *Environ. Sci. Pollut. R*., 2002, **9(2)**, 143–148.
19. PITT, J., *The genus Penicillium and Teleromorphic state. Eupenicillium and Talaromyces,* Academic Press, London, New York, Toronto, Sydney, 1979.
20. PRADHAN, N., K.C. NATHASARMA, K. SRINIVASA RAO, L.B. SUKLA, B.K., MISHRA, Heap bioleaching of chalopyrite: A review, *Min. Engin*., 2008, **21**, 355–365.
21. QU, Y., B. LIAN, Bioleaching of rare earth and radioactive elements from red mud using *Penicillium tricolor* RM-10, *Bioresource Technology*, 2013, **136**, 16–23.
22. SEDIEK, H.A.E., *Fungal leaching of some heavy metals from soil sediments, South Western Sinai, Egypt*, M.SC. Thesis. Botany Department, Faculty of Science, Zagazig University, 2012.
23. SHAPIRO, L., W.W. BRONNOCK, Rapid analysis of silicate, carbonate and phosphate rocks, *U.S.G.S. Bull.*, 1962, **114**, 1–8.
24. SOLIMAN, S.M., M.N. EI-FETOUH, Lithostratigraphy of the Carboniferous Nubian type sandstone in westcentral Sinai, *Egyptian J. Geology*, 1969, **13**, 961.
25. TAKUO, O., Y. SUZUKI, T. NANKAWA, T. YOSHIDA, T. OHNUKI, T. KIMURA, A. J. FRANCIS, Interactions of rare earth elements with bacteria and organic ligands, *J. Alloy. Compd.*, 2006, **408**, 1334–1338.
26. TSURUTA, T., Accumulation of rare earth elements in various microorganisms, *J. Rare Earth.*, 2007, **25 (5)**: 526–532.
27. VALIX, M., F. USAI, R. MALIK, Fungal bio-leaching of low grade laterite carbonaceous rock samples, *Min. Engin*., 2001, **14**, 197–203.