BIOLEACHING OF URANIUM BY *ASPERGILLUS NIGER* AND *ASPERGILLUS TERREUS* ISOLATED FROM URANIFEROUS SEDIMENTARY ROCKS, SOUTHWESTERN SINAI, EGYPT

MAISA M. AMIN, I.E. ELAASSY*, M.G. EL-FEKY*, A.M. SALLAM**, MONA S. TALAAT**, NILLY A. KAWADY**

*Nuclear Material Authority, Cairo, Egypt
**Physics Department, Biophysics group, Faculty of Science, Ain Shams University, Cairo, Egypt
E-mail: ab_m_sallam@sci.asu.edu.eg

Abstract. The strategic situation of Sinai made it an urgent national target for the sustainable development. One of the important factors in such development is the exploration and the processing of uraniferous rock materials. Consequently, the Lower Carboniferous sedimentary rocks were chosen for the present study to test the uranium bioleaching capacity of fungal strains isolated from exposed sedimentary rocks in southwestern Sinai. Eight fungal species were isolated from three grades of uraniferous sedimentary rock samples in southwestern Sinai, Egypt and tested for their bioleaching activity. *Aspergillus niger* (*A. niger*) and *Aspergillus terreus* (*A. terreus*) were the only isolates which gave a high grade leaching efficiency of uranium from the studied uraniferous rocks. The most favorable factors for solubilization of uranium were 7 days incubation time, 3% ore concentration, solid/liquid ratio 1/3 and 30 °C incubation temperature. Both fungi produced organic acids (oxalic, citric, formic and ascorbic) in the culture filtrate which are the key compounds of bioleaching processes. Applying these conditions on one kilogram of Ag-3 sample (the lowest U grade), the *A. niger* strain gave high uranium leaching efficiency of 71.4%. The recovery test of U has been performed by proper precipitation to obtain a high quality uranium concentrate.

Key words: Bioleaching of uranium, uraniferous sedimentary rock, *Aspergillus niger*, *Aspergillus terreus*, solubilization of uranium, uranium bioleaching efficiency.

INTRODUCTION

In the light of the expected shortage of nonrenewable resources, increased efforts are absolutely necessary to find new sources of raw materials with the aid of new or improved technologies. A possibility to obtain metals from low grade mineral resources is offered by the use of microbiological leaching processes [13].

Biohydrometallurgy or bioleaching is the interaction between metals and microbes with the specific aim of converting insoluble metal sulfides to soluble

Received October 2013;
in final form November 2013.

ROMANIAN J. BIOPHYS., Vol. 23, No. 4, P. 231–247, BUCHAREST, 2013

metal sulfates. Bioleaching has been defined as the dissolution of metals from their mineral sources by certain naturally occurring microorganisms or the use of collection microorganisms to transform solid compounds to soluble and extractable elements through the production of organic acids, further recovered by water filtration [2, 11].

Several studies have been published about the bioleaching of uranium by different fungal strains [1] found that the bioleaching process using *A. niger* was successfully applied for leaching of Cu and U from representative samples of Abu Thor area, west central Sinai, Egypt. The maximum leaching efficiencies were 97.41% and 79%, respectively [7] used *A. niger* for attaining a high uranium leaching efficiency from processed carbonate-rich latosol which reached up to 81.83%.

GEOLOGICAL OUTLINE

The lower Carboniferous (upper Visean) Um Bogma Formation is located in the southern extreme of Wadi (Vally) Naseib and consisted of three members named lower member sandy dolostone, middle member siltstone and marl intercalations and upper member dolostone [12] karstification process affected the lower (sub-soil) and middle (top soil) to form soil profile hosting several metallic forms [6]. [5] pointed to lateritization processes that affected the three members. The three members are the main target of this study.

The aim of this work was to isolate different fungal strains from three sedimentary samples and to investigate their uranium bioleaching capacity.

MATERIALS AND METHODS

ORE SAMPLES

Three radioactive geological samples were collected in sterile polyethylene bags from the prospective area in southwestern Sinai, Egypt. These samples (Table 1) were chosen to represent three different grades of uranium concentrations.

CHEMICAL ANALYSES OF ORE SAMPLES

The pulverized samples were analyzed by conventional wet chemical techniques [18]. 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 iron 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 was not higher than ±1 %. Uranium was analyzed by titration against NH_4VO_3 [4]. All the chemical analyses were carried out in the laboratories of the Nuclear Materials Authority, Egypt.

Table 1

Samples collected from the three members of Um Bogma Formation

Ag-1	Sandy dolostone, grey to brown, medium hard, with Cu mineralization (lower member).
Ag-2	Grey shale with sulfur patches (upper member).
Ag-3	Sandy dolostone with shale interlayer, grey to brown shale (middle member).

MICROBIOLOGICAL STUDIES

The microbiological studies included the fungal strains isolation from the studied rock samples and then the bioleaching ability assay, as follows:

FUNGAL ISOLATION

The isolation medium used in this work was Dox agar medium of composition (g/l): $NaNO_3$, 2; $KH_2\ PO_4$, 1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.5H_2O$ trace; sucrose, 30; agar, 15.5g, yeast extract was added to initiate the fungal growth. The pH value of the media was adjusted to be 6.5 before autoclaving at 120°C and 1.5 atm. for 20 minutes. Two techniques were used for the fungal strains isolation from the ore; the first one was the direct-plating technique, in which fine ore-powder was spread directly on the surface of Dox agar plates under aseptic conditions. The agar plates were incubated at 28°C ±2 until the fungal colonies grew. The second technique was the dilution plating in which 1g of the ore powder was taken under aseptic conditions and mixed well with 9 mL of sterile distilled water, and 0.1 mL of this mixture was spread under aseptic conditions by sterile glass rod on the surface of an agar plate. The plates were incubated at 28°C ±2 until colony development was obtained. 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 [9, 15].

Determination of organic acids produced by *A. niger* and *A. terreus*

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

Investigation of factors affecting the uranium bioleaching

Several factors were investigated with the aim of obtaining the optimum conditions for uranium solubilization, i.e. the activity of different fungal-strains, influence of ore concentrations on fungal growth, S/L ratio, incubation periods and incubation temperatures.

To prepare the leach liquor : 100 mL of Dox liquid medium was placed in 250 mL measuring flasks. The flasks were supplemented with different ore concentrations and autoclaved at 1.5 atm. for 20 min. After cooling, the flasks were inoculated with 0.5 mL spore suspension and finally incubated at 30°C for 7days in an orbital shaker at 100 rpm. Triplicate sets of flasks for each ore and organism were prepared. At the end of incubation period, the mycelia mats were harvested and washed several time with distilled water. Each growing fungus was dried at 85°C for 24 hours and dry weights were determined. The culture filtrate of each treatment was filtered, centrifuged and kept for uranium determination.

RESULTS AND DISCUSSION

CHEMICAL ANALYSES OF ORE SAMPLES

The chemical analyses of the studied uraniferous sedimentary rocks, which were used in this study, revealed the presence of high contents of SiO_2 and Al_2O_3 in the sample Ag-2 and high contents of CaO, MgO and loss on ignition (L.O.I) in the samples Ag-1 and Ag-3 (Table 2). The three samples contained different uranium grades, i.e. high grade with 800 ppm (Ag-1), moderate grade with 660 ppm (Ag-2) and (Ag-3) with the lowest grade of uranium concentration (77 ppm).

Table 2

The chemical analyses of the tested samples (wt. %)

Sample	Ag-1	Ag-2	Ag-3
SiO_2	17.7	78.6	11.4
Al_2O_3	4.59	8.67	3.06
Fe_2O_3	0.044	0.032	0.03
MnO	0.481	0.02	0.43
CaO	22.4	2.24	26.9
MgO	15.7	1.6	16.9
Na_2O	0.236	0.18	0.307
K_2O	0.539	1.173	0.337
P_2O_5	0.161	0.615	0.142
TiO_2	0.19	0.53	0.111
L.O.I	37.9	6.32	40.6
Total	99.94	99.99	100.21
U	0.08	0.066	0.0077

EFFECT OF DIFFERENT ORE CONCENTRATIONS ON THE FUNGAL GROWTH

Eight fungal species were isolated from the tested samples (Ag-1, Ag-2, and Ag-3). They were identified as *A. niger*, *A. terreus*, *A. fumigatus*, *A. flavus*, *Penicillium italicum*, *P. diversum*, *P. steckii* and *Mucor* species. All these fungal species were grown in the presence of different ore concentrations (Table 3 and Figs. 1, a, b and c). Growth of all *Aspergillus*, *Penicillium* and *Mucor* species was noticeably decreased with increasing the ore concentrations excepting *A. flavus* at 2% in both Ag-1 and Ag-2 and *P. italicum* for Ag-2. At ore concentrations higher than 4% the growth of *Penicillium* species was slightly decreased with increasing of the ore concentration in the media. This observation suggested that *Penicillium* species have a lower ability to grow under stress of uranium concentration than *Aspergillus* species.

In addition, growth inhibition may be due to the increase in the concentration of heavy metals in the leaching environment leading to increase in toxicity, which may inhibit the growth of the microorganisms [20].

From Table 3, the 1% ore concentration is the best for all fungal strains growth. The highest uranium concentration sample (800 ppm) gave the highest growth at the 1% concentration for *A. terreus*, *A. fumigatus*, *A. flavus*, *P. diversum* and *Mucor* species, while *A. niger*, *P. steckii* and *P. italicum* gave high growth in the moderate uranium concentrations (660 ppm). This means that the uranium concentrations and the lithologic type play a certain role in the growth of the fungal species.

Table 3

Effect of different ore concentrations (Ag-1, Ag-2 and Ag-3) on the growth of isolated fungi. Data are expressed as mycelial dry weight (mg/100ml culture medium)

Fungus sp.	**Sample**	**Ore concentrations**					
		Control	**1%**	**2%**	**4%**	**6%**	**8%**
A. niger	Ag-1	768 ± 11.8	638 ± 2.01	566 ± 3.11	458 ± 4.78	249 ±8.01	176 ±9.13
	Ag-2		744 ± 0.37	667 ± 1.56	458 ± 4.78	306 ±7.13	213 ±8.56
	Ag-3		675 ± 1.44	515 ± 3.90	358 ± 3.55	266 ±7.75	177 ±9.11
A. terreus	Ag-1	754 ± 11.6	677 ± 1.19	568 ± 2.87	545 ±3.22	313 ±6.80	212 ±8.36
	Ag-2		644 ± 1.69	585 ± 2.61	410 ± 5.31	351 ±6.21	160 ±9.17
	Ag-3		564 ± 2.93	538 ± 3.33	420 ± 5.15	318 ±6.73	161 ±9.15
A. fumigatus	Ag-1	783 ± 13.6	772 ± 1.71	655 ± 3.52	637 ± 3.79	354 ±8.16	144 ±11.4
	Ag-2		749 ± 2.07	653 ± 3.55	459 ± 6.54	227 ±10.1	109 ±11.9

Table 3 (continued)

	Ag-3		673 ± 3.24	662 ± 3.41	434 ± 6.93	325 ±8.61	251 ±9.75
A. flavus	Ag-1	787 ± 12.1	750 ± 0.60	761 ± 0.43	531 ± 3.95	362 ±6.56	158 ±9.71
	Ag-2		692 ± 1.47	715 ± 1.11	526 ± 4.03	453 ±5.13	213 ±8.86
	Ag-3		580 ± 3.19	563 ± 3.46	318 ± 7.23	305 ±7.50	228 ±8.63
P. steckii	Ag-1	778 ± 12.0	775 ± 0.15	766 ± 0.19	758 ± 0.31	551 ±3.50	487 ±4.50
	Ag-2		779 ± 0.12	762 ± 0.25	455 ± 4.98	423 ±5.48	375 ±6.21
	Ag-3		657 ± 1.87	622 ± 2.41	542 ± 3.64	483 ±4.55	455 ±4.98
P. italicum	Ag-1	775 ± 12.0	765 ± 0.15	698 ± 1.18	688 ± 1.34	623 ±2.35	541 ±3.61
	Ag-2		766 ± 0.14	757 ± 0.28	585 ± 2.93	547 ±3.52	448 ±5.05
	Ag-3		735 ± 0.62	687 ± 1.36	563 ± 3.27	475 ±4.63	454 ±4.95
P. diversum	Ag-1	786 ± 12.1	765 ± 0.33	677 ± 1.68	657 ± 1.99	551 ±3.62	187 ±9.24
	Ag-2		733 ± 0.82	552 ± 3.61	456 ± 5.09	425 ±5.57	276 ±7.87
	Ag-3		656 ± 2.01	621 ± 2.54	432 ± 5.46	384 ±6.20	245 ±8.35
Mucor sp.	Ag-1	875 ± 13.5	770 ± 1.62	665 ± 3.24	535 ± 5.25	354 ±8.04	164 ±11.1
	Ag-2		759 ± 1.79	651 ± 3.45	569 ± 4.72	337 ±8.30	199 ±10.4
	Ag-3		770 ± 1.62	582 ± 4.52	484 ± 6.03	263 ±9.44	232 ±9.92

THE EFFECT OF FUNGAL ACTIVITY ON THE URANIUM BIOLEACHING

There was variation in the leaching efficiencies of the different investigated fungal strains (Table 4) in the following order: *Aspergillus* species > *Penicillium* species > *Mucor* species. Within Aspergillus species, *A. niger* gave the highest uranium leachability, as well as *P. diversum* among *Penicillium*.

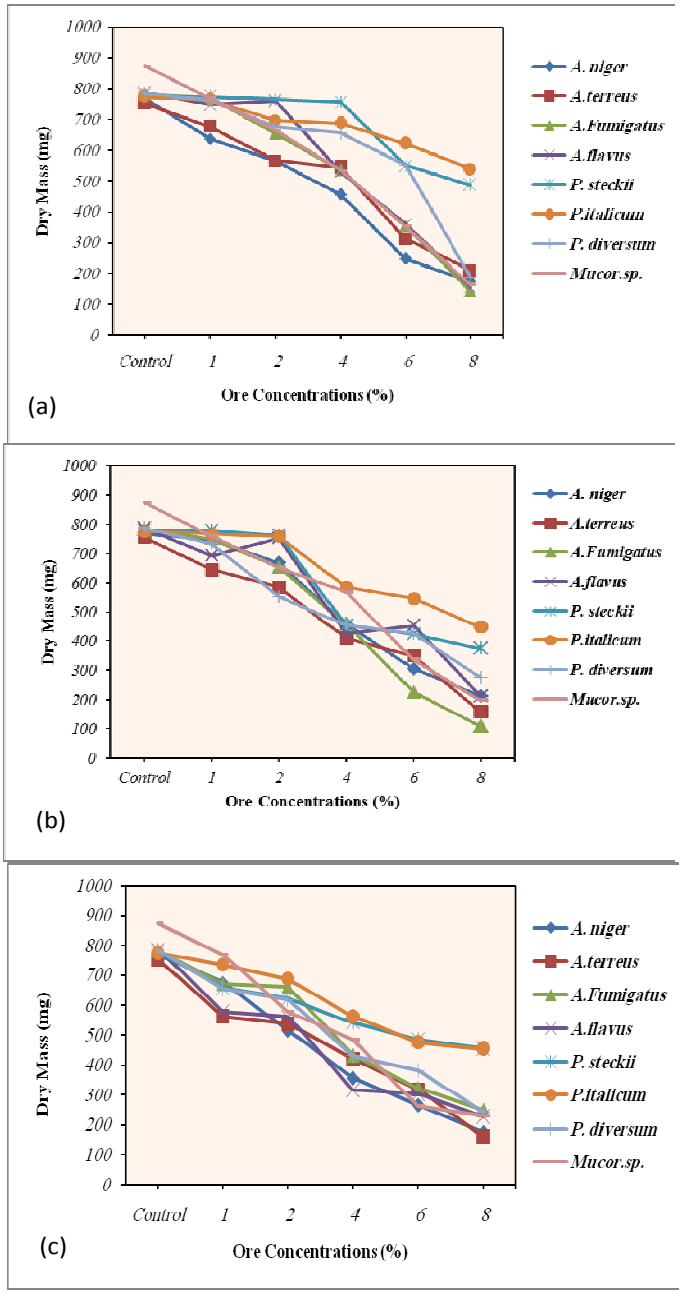

Fig. 1. Relationship between different ore concentrations and growth of isolated fungi: (a): Ag-1, (b): Ag-2 and (c): Ag-3.

Variations in bioleaching efficiencies of uranium are strongly influenced by the ore mineralogy, 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 [19].

EFFECT OF DIFFERENT SOLID / LIQUID RATIOS ON URANIUM SOLUBILIZATION

From the obtained data (Table 5), the leaching efficiency proportionally increases with the solid / liquid ratio to reach its maximum values at S/L ratio 1/3. Over this ratio the leaching efficiency started to decrease (Figs. 2, a, b and c). At this S/L ratio, *A. niger* solubilized 55.2%, 64.3% and 81.8% uranium from the ores Ag-1, Ag-2 and Ag-3 respectively.; *A. terreus* solubilized 42.5%, 56.2% and 55.8% from the total amount of uranium which was found in the ores Ag-1, Ag-2 and Ag-3 respectively. It is to be noticed that in both *Aspergillus* species, the uranium solubilization efficiency increased with the decrease in the uranium concentration in the ore samples (Ag-3>Ag-2>Ag-1). For *A. terreus* the order was Ag-2> Ag-3>Ag-1, which could indicate that *A. terreus* showed to be more responsive to high uranium concentrations. The decrease of uranium leaching may be due to the toxic effect of uranium concentration on the activity of the fungus and the limitation of the amount of secreted organic acids [3] demonstrated that the leaching efficiency of uranium from phosphate deposits using *Penicillium simplicissium* occurred at 1/4 S/L ratio.

The highest leaching of uranium was detected when the final pH was decreased in the growth media. This may be attributed to the solubilization of other metals that consumed organic acids produced by the tested fungi [10].

EFFECT OF DIFFERENT INCUBATION PERIODS ON URANIUM SOLUBILITY

Uranium solubilization by *A. niger* was highly affected by the incubation periods. The best solubilization activity occurred at 7 days of incubation, where the amounts of uranium solubilized from the uraniferous samples Ag-1, Ag-2 and Ag-3 were 45.6%, 56.4% and 59%, respectively (Table 6 and Figs. 3, a, b and c). However, after 7 days of incubation the amounts of uranium solubilized were slightly decreased with increasing incubation periods up to 10 days. The same pattern of solubility of uranium by *A. niger* was followed also by *A. terreus* resulting in 27.5%, 35.5% and 42.9% leaching activity for Ag-1, Ag-2 and Ag-3, respectively. The amount of uranium solubilized in the growth media was slightly decreased with increasing the incubation period above 7 days. This phenomenon may be attributed to: (1) the effect of some metal ions released in the medium which consumed some of the organic acids produced by tested fungi [16], (2) the final pH was shifted towards acidity up to 7 days of growth, after this period the pH value was found to increase [17].

Table 4

Bioleaching efficiency of uranium by using the dominant fungal isolates

Fungus sp.	Sample	Uranium leaching efficiency (%)	Final pH
A. niger	Ag-1	34	5.64
	Ag-2	51	5.43
	Ag-3	57	5.38
A. terreus	Ag-1	28	5.52
	Ag-2	36	5.18
	Ag-3	42	4.74
A. fumigatus	Ag-1	20	5.97
	Ag-2	23	6.06
	Ag-3	33	5.63
A. flavus	Ag-1	18	5.77
	Ag-2	25	6.36
	Ag-3	28	5.43
P. steckii	Ag-1	13	5.65
	Ag-2	18	5.68
	Ag-3	21	5.11
P. italicum	Ag-1	12	6.47
	Ag-2	20	6.64
	Ag-3	25	6.81
P. diversum	Ag-1	15	4.87
	Ag-2	27	5.34
	Ag-3	33	5.65
Mucor sp.	Ag-1	8	6.66
	Ag-2	14	6.53
	Ag-3	22	6.21

EFFECT OF DIFFERENT INCUBATION TEMPERATURES ON URANIUM SOLUBILIZATION

Solubilization of uranium from the tested ores was found to be highly affected by the incubation temperature. The maximum was reached at 30 ºC. At this temperature, *A. niger* solubilized 43%, 54% and 64% of uranium found in ores Ag-1, Ag-2 and Ag-3, respectively (Table 7 and Figs. 4, a, b and c).

Table 5

Effect of different solid / liquid ratio (Ag-1, Ag-2 and Ag-3) on uranium solubilization by *A. niger* and *A. terreus* grown on Dox liquid medium at 30 °C for 7days

Fungus sp.		**Ore samples**	**Solid / Liquid ratio**				
			1/1	**1/2**	**1/3**	**1/5**	**1/7**
A. niger	U (%)	Ag-1	42.2 ±1.17	44.5 ±1.17	55.2 ±1.15	46.5 ±1.16	44.1 ±1.17
		Ag-2	54 ±1.15	55.1 ±1.15	64.3 ±1.14	61.3 ±1.14	50 ±1.15
		Ag-3	57 ±1.15	72.7 ±1.12	81.8 1.11	49.3 ±1.15	27.2 ±1.19
A. terreus	U (%)	Ag-1	27.1 ±1.19	39.5 ±1.74	42.5 ±1.70	33.5 ±1.18	19.7 ±1.20
		Ag-2	36.8 ±1.18	40 ±1.17	56.2 ±1.15	36 ±1.18	19.3 ±1.20
		Ag-3	44.1 ±1.17	50.6 ±1.16	55.8 ±1.15	42.8 ±1.17	33.7 ±1.18

Table 6

Effect of different incubation periods on uranium solubilization from uraniferous samples (Ag-1, Ag-2 and Ag-3) by *A. niger* and *A. terreus* grown on Dox liquid medium at 30 °C

Fungus sp.		**Ore samples**	**Incubation periods (days)**				
			3	**5**	**7**	**9**	**10**
A. niger	U (%)	Ag-1	17.5 ±1.21	37.5 ±1.18	45.6 ±1.16	33.3 ±1.18	27.8 ±1.19
		Ag-2	16.67 ±1.21	41 ±1.17	56.4 ±1.47	31.8 ±1.19	24 ±1.20
		Ag-3	43 ±1.17	52 ±1.15	59 ±1.14	49.4 ±1.16	43 ±1.17
A. terreus	U (%)	Ag-1	15 ±1.21	26.3 ±1.19	27.5 1.19	20.8 ±1.20	17.9 ±1.21
		Ag-2	15.5 ±1.21	28.2 ±1.19	35.5 ±1.18	18.2 ±1.21	17.8 ±1.21
		Ag-3	28.6 ±1.19	31.2 ±1.19	42.9 ±1.17	26 ±1.19	18.2 ±1.21

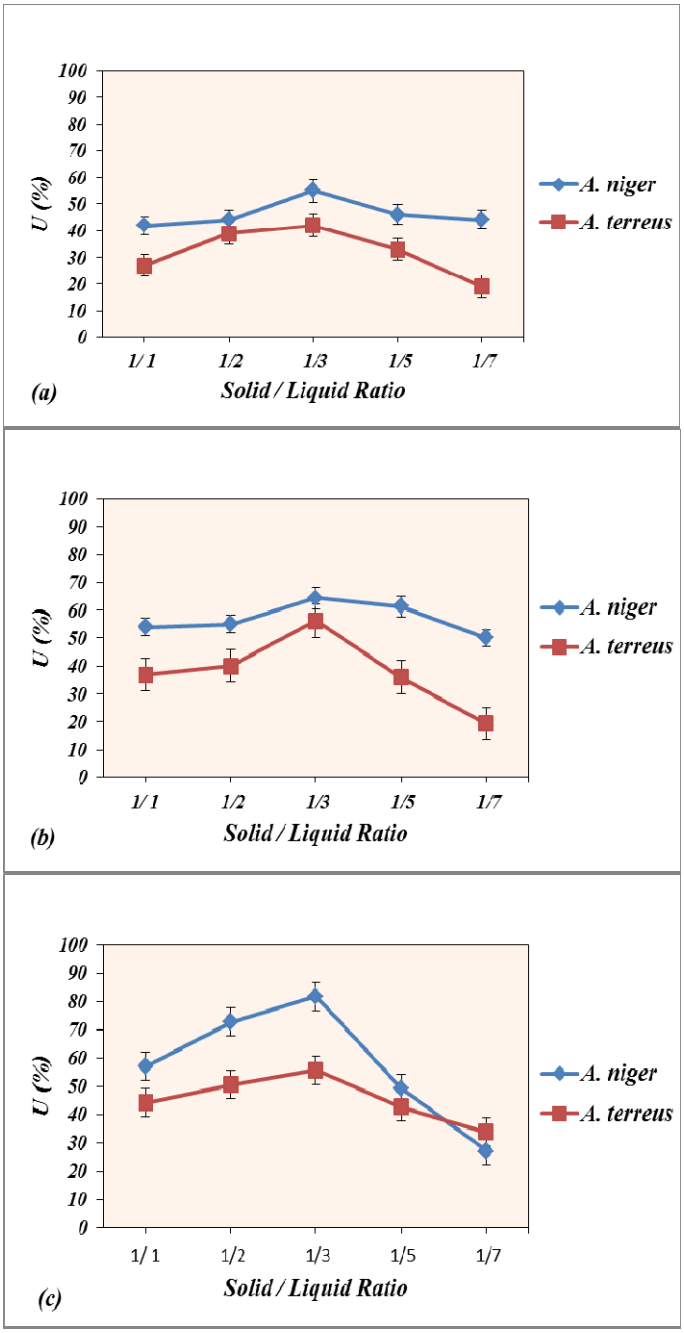

Fig. 2. Relationship between different solid/liquid ratio and uranium solubilization by *A. niger* and *A. terreus* at 30 °C for 7 days: (a): Ag-1, (b): Ag-2 and (c): Ag-3.

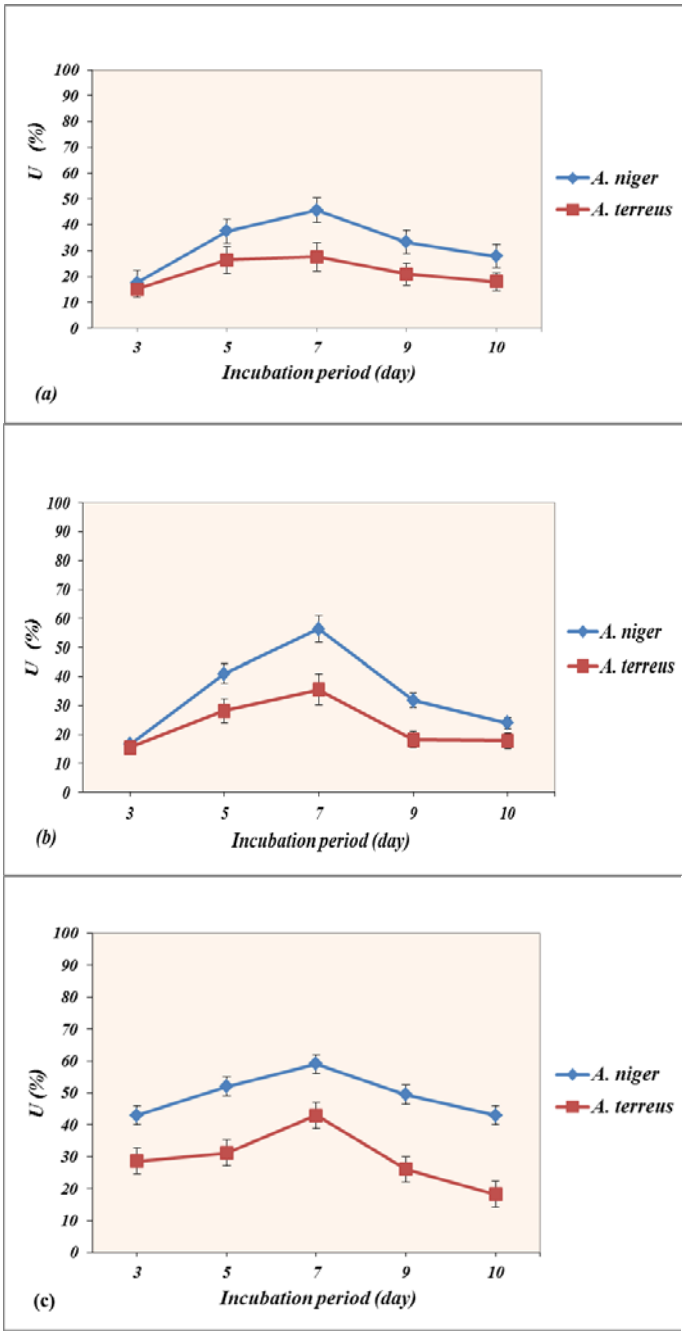

Fig. 3. Relationship between different incubation periods and uranium solubilization by *A. niger* and *A. terreus* at 30 °C: (a): Ag-1, (b): Ag-2 and (c): Ag-3.

Whereas, uranium leached by *A .terreus* at 30ºC was 26%, 34.8% and 45% respectively. At this incubation temperature, the final pH of the medium shifted toward acidity. [8] reported that the maximum bioleaching of heavy metals was 30ºC for *A. niger* and 35ºC for *A. fumigatus*. At higher temperatures (40ºC and 45ºC), the metabolic rate is gradually decreasing till microbial death occurs [14].

Table 7

Effect of different incubation temperatures on uranium solubilization from 1% of uraniferous samples (Ag-1, Ag-2 and Ag-3) by *A. niger* and *A. terreus* grown on Dox liquid medium for 7days

Fungus sp.		**Ore samples**	**Incubation temperatures (°C)**				
			20	**30**	**37**	**40**	**45**
A. niger	U (%)	Ag-1	13 ±1.21	43 ±1.17	45 ±1.16	25 ±1.20	5 ±1.23
		Ag-2	7.5 ±1.22	54 ±1.15	36.4 ±1.18	26 ±1.19	19.6 ±1.20
		Ag-3	43 ±1.17	64 ±1.14	67.5 ±1.13	44 ±1.17	40 ±1.17
A. terreus	U (%)	Ag-1	12 ±1.22	26 ±1.94	28 ±1.19	15 ±1.21	14.6 ±1.21
		Ag-2	7 ±1.22	34.8 ±1.18	32.4 ±1.18	24.6 ±1.20	19.6 ±1,20
		Ag-3	32 ±1.19	45 ±1.16	48.1 ±1.16	35 ±1.18	25 ±1.20

ORGANIC ACIDS PRODUCED BY *A. NIGER* AND *A. TERREUS*

Metabolites of both fungi were subjected to proper chromatographic analysis (Figs. 5, a and b). The analysis proved that *A. niger* secreted in the growth media, formic, acetic, citric and oxalic acids, whereas *A. terreus* secreted citric, oxalic and ascorbic acids, which were effective in uranium solubilization. These acids are essential for the leaching of heavy metals from ore materials and solid wastes [16].

RECOVERY OF URANIUM

From the previously mentioned results concerning the relevant factors influencing uranium bioleaching by different fungal strains and from an economic point of view, the following conditions for uranium bioleaching were selected: 7 days incubation time, 1/3 solid/liquid ratio (w/v), and 30 ºC incubation temperature on one kg of the ore sample Ag-3 using *A. niger*. In these conditions, the uranium leaching efficiency reached 71.4%. Classical chemical techniques were followed to obtain uranium in highly purified form.

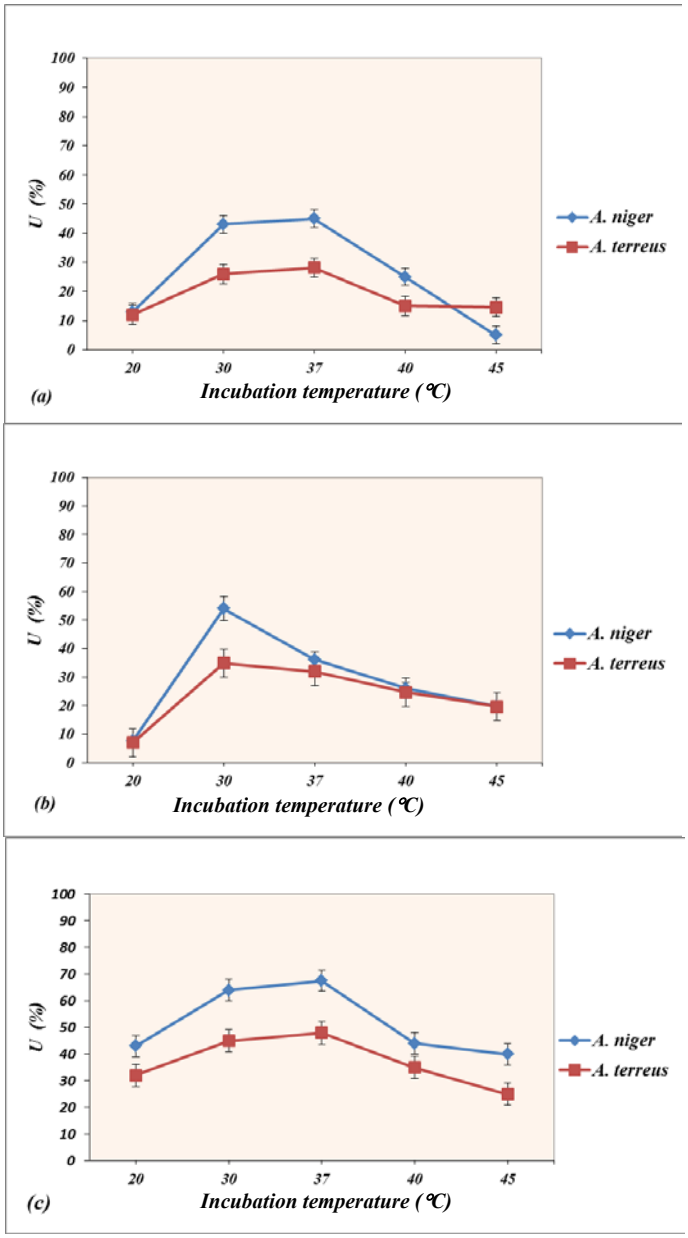

Fig. 4. Relationship between different incubation temperatures and uranium solubilization by *A. niger* and *A. terreus* for 7 days: (a): Ag-1, (b): Ag-2 and (c): Ag-3.

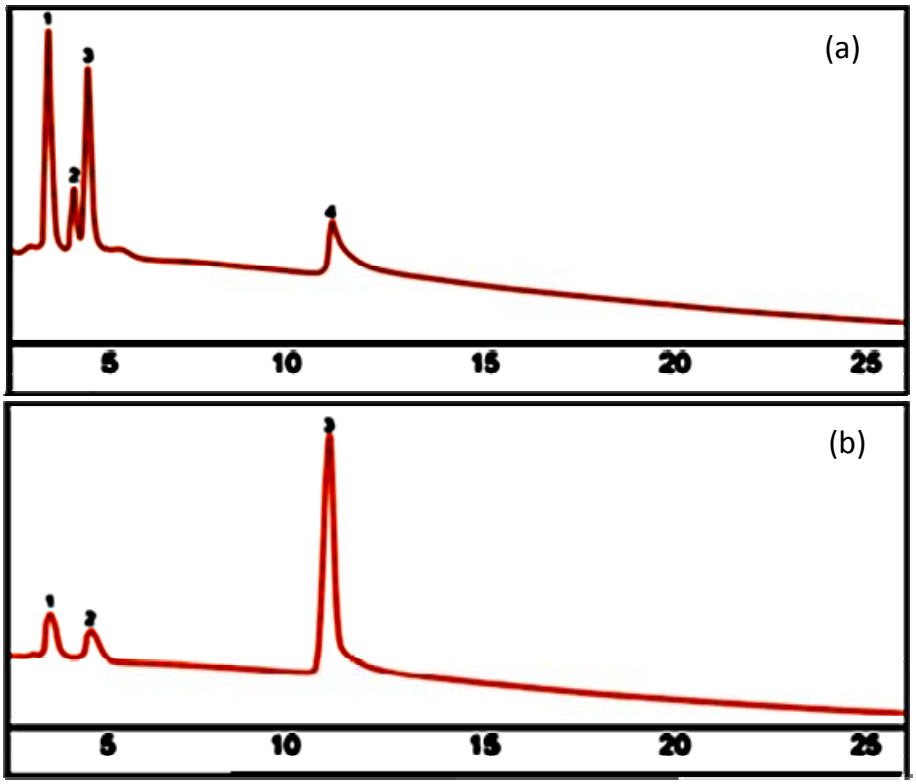

Fig. 5. Typical organic acids chromatograph in the absence of uraniferous samples. (a): *A. niger* grown on Dox liquid medium, 1: formic acid; 2: acetic acid; 3: citric acid and 4: oxalic acid. (b): *A. terreus* grown on Dox liquid medium, 1: ascorbic acid; 2: citric acid; and 3: oxalic acid.

For this purpose, uranium was precipitated from the obtained bioleach liquor after increasing the pH to 7.5 using 10% NaOH and the obtained product was associated with several impurities, mostly iron. So the obtained concentrate was exposed to alkali leaching using a mixed solution of 15% a Na_2CO_3 and 5% $NaHCO_3$ in a S/l ratio of 1/3 for 15 minutes stirring time at 80°C, where uranium would be selectively leached (equation 1). The obtained uranyl concentrate filtrate was adjusted to a pH value of about 5.5 using 1molar H_2SO_4 solution and the resultant precipitate was calcinated at 800°C to U_3O_8 product. The latter was then analyzed by the electron microscope ESEM-EDX analysis and the obtained results indicate that U assay has increased up to about 98% as shown in Fig. 6.

$$UO_3 + 3Na_2CO_3 + H_2O \longrightarrow Na_4UO_2(CO_3)_3 + 2NaOH \quad (1)$$

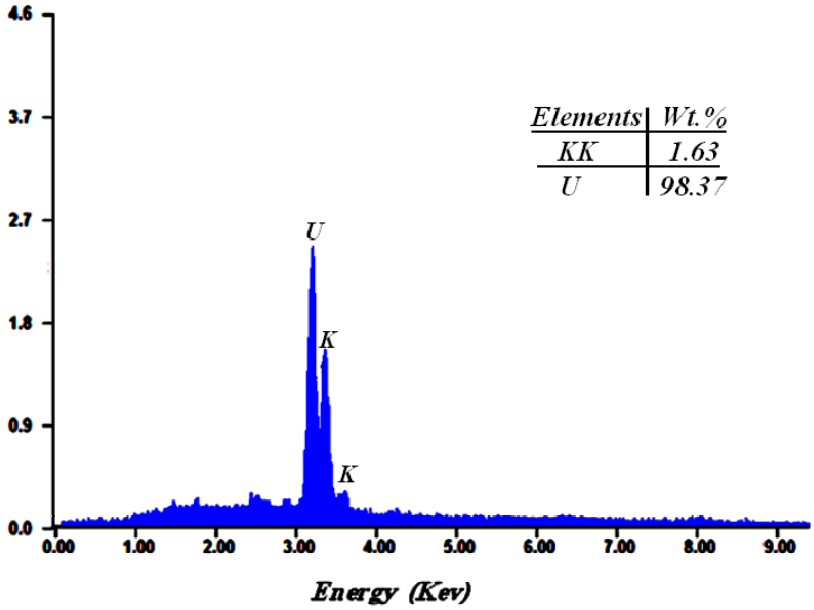

Fig. 6. EDAX chart for the identification of uranium.

CONCLUSIONS

Eight fungal strains identified as *A. niger*, *A. terreus*, *A. fumigatus*, *A. flavus*, *P. italicum P. diversum*, *P. steckii* and *Mucor* species were isolated from three tested uraniferous samples (Ag-1, Ag-2 and Ag-3). Bioleaching of uranium varied with the content of uranium in the ore and with the organisms used in the bioleaching process. *A. niger* and *A. terreus* gave the highest leaching of uranium. The optimum conditions of uranium leaching from its ores using *A. niger* and *A. terreus* were 7 days incubation time, 1/3 S/L ratio and 30°C incubation temperature leading to a maximum uranium leaching efficiency of 71.4% /one kg of the ore Ag-3 by using *A. niger*.

A. niger and *A. terreus* produced oxalic, citric, ascorbic, formic and acetic acids effective for uranium solubilization.

Pure uranium concentrate was obtained after precipitation and alkaline leaching of the impure precipitate.

REFERENCES

1. ABDEL WAHAB, G.M., M.M. AMIN, S.K. AITA, Bioleaching of Uranium – bearing material from Abu Thor area, West Central Sinai, Egypt for recovering uranium, *Arab J. of Sci. Appl.,* 2012, **45**, 169–178.

2. AKCIL, A., Potential bioleaching developments towards commercial reality: Turkish metal mining's future, *Miner. Eng.*, 2004, **17**, 477–480.
3. AMIN, M. M., Factors controlling uranium bioleaching from different Egyptian phosphates using *Penicillium simplicissimum*, *J. Environ. Sci., Mansoura Univ.,* 2012, **42**, 165–180.
4. DAVEIS, W., W. GRAY, A Rapid and specific titrimetric method for the precise determination of uranium using iron II sulphate as reductant, *Talanta* , 1964, **11**, 1203–1211.
5. EL AASSY, I.E., F.Y. AHMED, S.Y. AFIFI, A.S. ASHAMI, Uranium in Laterites, Southwestern Sinai, Egypt, *1st Seminar on Nuclear Raw Materials and their Technology,* Helwan University, Cairo, Egypt, Nov., 1–3, 2000, pp. 1–20.
6. EL SHARKAWI, M.A., M.M. EL AREF, A. ABDEL MOTTELIB, Syngenetic and paleokarstic copper mineralization in the paleozoic platform sediments of West Central Sinai, *Egypt. Spec. Publ. Int. Ass. Sediment*, 1990, **2**, 159–171.
7. EL SHEIKH, E.M., M.M. AMIN, S.K. AITA, A.A. REZK, Successive recovery of copper and uranium from carbonate-rich latosol, Um Bogma Formation, Abu Thor locality, Southwestern Sinai, Egypt, *Nucl. Sci. Scient. J.*, 2013, **2**, *in press*.
8. ELSAYED, H., *Fungal Leaching of Some Heavy Metals from Soil Sediments, South Western Sinai, Egypt*, M.Sc. Thesis, Faculty of Science, Botany Department, Zagazig University, Egypt, 2012.
9. GILMAN, J.C., *A manual of soil fungi*, The Lowa State Univ. Press, Ames, Lowa, USA, 1957.
10. HEFNAWY, M.A., A.M. HASHAD, M.M. AMIN, Optimization of Uranium leaching parameters by *A. terres* and *P. spinulosum*, *Afr. J. Mycol. Biotechnol*. 2003, **1**, 17–34.
11. HOQUE, M.E., O.J. PHILIP, Biotechnological recovery of heavy metals from secondary sources – An overview, *Mater. Sci. Eng.,* C, 2011, **31**, 57–66.
12. KORA, M. *The Paleozoic outcrops of Um Bogma area, Sinai*, PhD Thesis, Mansoura University, Mansoura, Egypt, 1984.
13. KREBS, W., C. BROMBACHER, P.P. BOSSHARD, R. BACHOFEN, H. BRANDL, Microbial recovery of metals from solids, *FEMS Microbiol. Rev.*, 1997, **20**, 605–617.
14. MOHAPATRA, S., S. BOHIDAR, N. PRADHAN, R.N. KAR,. L.B. SUKLA, Microbial extraction of nickel from *Sukinda* chromite overburden by *Acidithiobacillus ferrooxidans* and *Aspergillus* strains, *Hydrometallurgy*, 2007, **85**, 1–8.
15. PITT, J., The genus *Penicillium* and *Teleromorphic* state. *Eupenicillium* and *Talaromyces*, Academic Press, London, New York, Toronto, Sydney, 1979.
16. REN W.X., P.J. LI, G. YONG, X.J. LI, Biological leaching of heavy metals from a contaminated soil by *Aspergillus niger*, *J. Hazard. Mater.*, 2009, **167**, 164–169.
17. SAMOLIOV, P.M., E.B. KRUGLYAK, L.M. APENYANSKAYA, Effect of conditions of *Actinomyces lavendulae* culturing on the composition of phytobacteriomycin, *Appl. Biochem. Microbiol.* (Russian), 1977, **13**, 388–390.
18. SHAPIRO, L., W.W. BRANNOCK, Rapid analysis of silicate, carbonate and phosphate rocks, *U.S. Geol. Surv. Bull.,* 1962, **114**, 56–63.
19. VALIX, M., F. USAI, R. MALIK, Fungal bioleaching of low grade laterite ores, *Miner. Eng.*, 2001, **14**, 197–203.
20. XU, T.J., Y.P. TING, Optimization on bioleaching of incinerator fly ash by *Aspergillus niger* – use of central composite design, *Enzyme Microb. Tech.*, 2004, 35, 444–454.