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Bioleaching of some Rare Earth Elements from Egyptian Monazite using *Aspergillus ficuum* and *Pseudomonas aeruginosa*

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Abstract

Aspergillus ficuum and *Pseudomonas aeruginosa* exhibit good potential in generating varieties of organic acids effective for bioleaching some rare earth elements (REEs) from Egyptian monazite (purity 97 %) and (thorium-uranium) concentrate. Batch experiments are performed to compare the bioleaching efficiencies of the one and 2-step bioleaching processes. The highest percentages of bioleached REEs from monazite and (Th-U) concentrate directly by *A. ficuum* are found to be 75.4, 63.8 % at a pulp density 0.6, 1.2 % (w/v), respectively, after 9 days of incubation at 30 °C and 63.5, 52.6 % by *P. aeruginosa* after 8 days of incubation at 35 °C using a shaking incubator at 175 rpm. It is also found that 14.3 and 1.4 g/l of citric and oxalic acid, respectively, are produced by *A. ficuum*, while 6.3 g/l of 2-ketogluconic acid is produced by *P. aeruginosa*. The highest percentages of chemical leaching of REEs from 0.6 % monazite using citric acid 14.3 g/l, oxalic acid 1.4 g/l, citric/oxalic acids 15.7 g/l and 2-ketogluconic acid 6.3 g/l after 24 h are 55.7, 26.0, 58.8 and 45.6 %, respectively. This work addresses the area of beneficiation of the used mineral to solubilize REEs through the biotechnological route in Egypt, where the bioleaching method is more effective than the chemical one using organic acids.

Keywords: Monazite, rare earth elements, bioleaching, *Aspergillus ficuum*, *Pesudomonas aeruginosa*, chemical leaching

Introduction

Bioleaching is an emerging technology with significant potential to add value to the mining industry so as to deliver attractive environmental and social benefits to all associated parties [1]. The bioprocessing of ores and concentrates to recover rare earth elements (lanthanides) and other metals, is an established technique, as well as an evolving area of biotechnology [2]. The bioleaching process presents 2 advantages, as compared to the conventional chemical leaching processes: (a) the very low concentrations of organic compounds present in such a situation represent a lower ecological risk; and (b) even with a lower final yield, the economical cost of a such process is lower. Both characteristics could facilitate its industrial application [3].

The most important microorganisms used in metal bioleaching reactions were fungi related to *Aspergillis* and *Penicillium*, and bacteria belonging to *Bacillus* and *Pseudomonas* genera. The bioleaching potentiality is based on their bio-acid production (citric, oxalic and 2-ketogluconic acids) and the reducing properties when the samples were directly subjected with the microorganisms in their growth media [4].

Monazite, bastnesite and xenotime are considered the most accessible sources of REEs. Egyptian monazite chemically processes both acid and alkaline breakdowns. But alkaline leaching of monazite is the most preferable and is widely used due to economic reasons. The by-product tri-sodium phosphate

may be used in the fertilizer industry, and the alkaline method could be less complicated. However, increasing the number of environmental regulations has heightened the need for new, green methods. One possible solution is to develop other leaching processes, such as bioleaching. Lanthanide elements are now used in a greater variety of applications. One such application is as a catalyst for petroleum cracking**,** as phosphors in color television sets, as polishing powder, and in flint stones, ignition devices, super conductors, hydrogen storage, secondary batteries, optical lenses, streetlights, and searchlights [5].

The main objective of this study is the use of *A. ficuum* and *P. aeruginosa* for REEs solubilization from monazite and (Th-U) concentrate, as until now there has been no published work about the microbial leaching of REEs from monazite.

Materials and methods

Tested samples

Egyptian monazite mineral was physically upgraded from black beach sand from Abou-Khashaba by the Nuclear Materials Authority of Egypt, while (Th-U) concentrate was obtained from treatment of Egyptian monazite mineral after chemical breakdown by sodium hydroxide 50 %. Monazite (97 %) was ground and then dried, and its particles size was found to be 44 micron (325 Mesh). Total REEs was determined spectrophotometerically using the colorimetric determination spectrometer (Metertech Inc, model SP-8001, UV-Visible); in all measurements, 2 matched $5cm^3$ quartz cells with a path length of 1cm were used for sample and blank measurements using arsenazoI. All measurements were carried out at laboratory temperature [6].

REEs bioefficiency (%) = $100\ (C_i - Cf) / Ci$

where

C_i = initial REEs concentration, Cf = REEs concentration after bioleaching process.

Microorganisms and growth conditions

The fungal strain *A. ficuum* was isolated from the monazite sample using Modified Czapek's- Dox agar (MCDA) [7]. *A. ficuum* was identified as described by [8]. *P. aeruginosa* obtained from previous work [9] was cultured on Nutrient agar (NA) [10]. Organisms were grown in the absence and the presence of different weights of monazite and (Th-U) concentrate. *A. ficuum* cultures were incubated at 28 °C for 7 days and *P. aeruginosa* cultures were incubated at 35 °C for 3 days; then the dry weights were determined as mg/ml [11].

Production of organic acids

The efficiency of the tested strains to produce organic acids was examined by adding 0.5 g/l of $CaCO_3$ [12] to the growth agar media. The tested fungus and bacterium were inoculated on MCDA and NA media, respectively. Diameters of the clear zone were determined.

The organic acids produced in the presence of monazite with the organisms were estimated using high-performance liquid chromatography (HPLC) (PERKIN ELMER, Binary 250, LC Pump, USA) with a Pack column (6.0×150 mm in length) at a flow rate of 0.8 mL/min (at room temperature 30 °C). The mobile phase 0.01N H_2SO_4 was detected with a UV detector at 210 nm for citric and oxalic acid estimation [13]. 0.1 % ortho-phosphoric acid was used as a mobile phase for gluconic acid [14] under the same conditions. The organic acids were identified by comparing the retention times and quantified on the basis of beak areas in HPLC-chromatogram.

Microbial bioleaching techniques

The organic acids can be produced as follows.

Direct bioleaching process [15]: In direct bioleaching, 100 ml (MCDB) Modified Czapek's -Dox broth and (NB) Nutrient broth media containing 1g of monazite or (Th-U) concentrate inoculated with 1 ml (approximately 1×10^7 spores/mL) fungal spore suspension or 1 ml (approximately 5×10^8 cells/mL) of bacterial cells. Cultures were incubated in a rotary shaking incubator at 175 rpm at 30 °C for 5 and 10 days for *A. ficuum* and 35 °C for 4 and 8 days for *P .aeruginosa* [16].

Indirect bioleaching process [17]: The production of organic acids can be performed by an indirect bioleaching process. Organisms were cultivated in their corresponding media under the previous growth conditions. All cultures were incubated in a rotary shaking incubator at 175 rpm at 30 °C for 10 days for *A. ficuum* and 35 °C for 8 days for *P .aeruginosa* [16]. After separation of the microbial biomass, 1g of sterile monazite or (Th-U) concentrate was added to the filtrates (1 g/100 ml) followed by shaking at 150 rpm for 24 h.

For each bioleaching the final pH of filtrates and percentages of liberated REEs in supernatants were determined.

Effect of certain growth factors on *A. ficuum* and *P. aeruginosa* bioleaching activities

The effects of incubation period (from 3 to 12 days), shaking speed (from 120 to 200 rpm), and incubation temperature (from 20 to 40 °C) on one-step bioleaching of REEs were investigated. Tested organisms were cultivated in their corresponding media in the presence of 0.5 g/ 50 ml of monazite or (Th-U) concentrate. Then, the effect of ore weight (from 0.1 to 0.9 g/ 50 ml) was tested. To study the effect of carbon and nitrogen sources on bioleaching of REEs from monazite and (Th-U) concentrate *A. ficuum* was cultivated in an equimolecular amount of nitrogen or carbon sources. Carbon sources (3g/l) and nitrogen sources (1 g/l) were added to the NB medium of *P. aeruginosa*.

Chemical leaching of some REEs using organic acids

Chemical leaching of REEs from monazite and (Th-U) concentrate was carried out as previously mentioned by [17] at concentrations of 0.3 g of monazite or 0.6 g (Th-U) concentrate. The organic acid concentrations used were above and below those detected by HPLC in previous experiment. Also, citric and oxalic acids mixtures were tested. These experiments were carried out under the same conditions of indirect bioleaching.

Results

Mineral characterization

The chemical composition of monazite and (Th-U) concentrate was shown in **Table 1**. The results indicated that monazite and (Th-U) concentrate was reached in RE_2O_3, ThO_2, P_2O_5.

Effect of monazite and (Th-U) concentrate on the growth of *A. ficu*um and *P. aeruginosa*

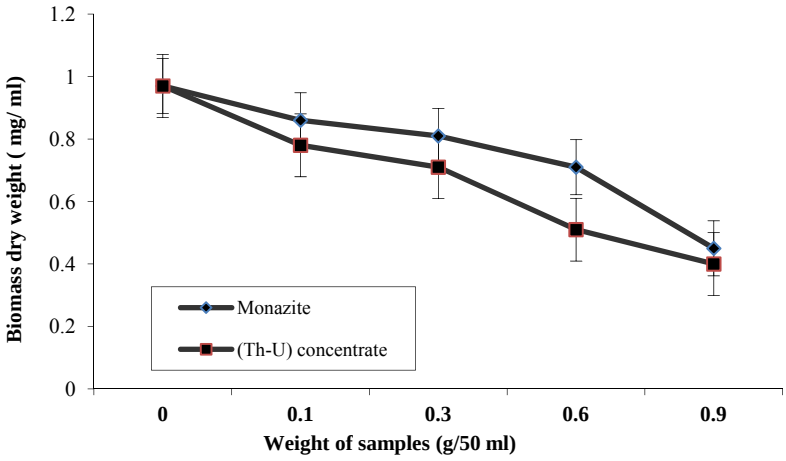
This experiment was carried out to study the effect of sample concentrations on the fungal or bacterial biomass. At the end of the incubation periods (7 days for *A. ficuum* and 3 days for *P. aeruginosa*) results were obtained and are shown in **Figures 1a, 1b**, The dry weights of the tested organisms were clearly decreased as the concentrations of monazite and (Th-U) concentrate was increased, compared to control.

Production of organic acids by *A. ficuum* and *P. aeruginosa*

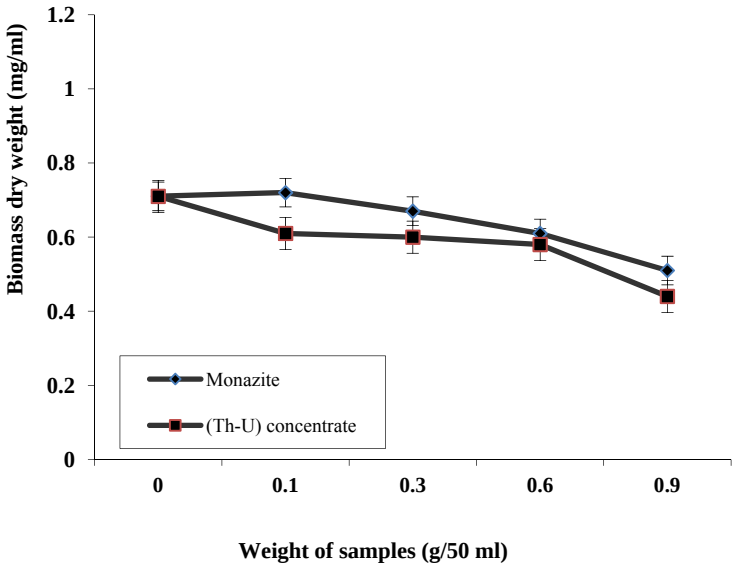
Table 2 illustrates the total acid produced by microorganisms using 2 testing methods: the first one indicated the diameter of the clear zones formed in the growth medium MCDA and NA media containing calcium carbonate as an indicator for acid production after 7 and 3 days for *A. ficuum* and *P. aeruginosa*, respectively.

In the second method, organic acids produced in the fungal and bacterial filtrates were analyzed using HPLC in presence of monazite after 9 and 8 days for *A. ficuum* and *P. aeruginosa*, respectively. It

was found that the main acids produced by *A. ficuum* were citric acid (14.3 g/l) followed by oxalic acid (1.4 g/l), while the main acid produced by *P. aeruginosa* was 2-ketogluconic acid (6.2 g/l).



(a)



(b)

Figure 1 Growth of *A. ficuum* (a) and *P. aeruginosa* (b) in the presence of different weights of monazite and (Th-U) concentrate, where *A. ficuum* cultures were incubated at 28 °C for 7 days and *P. aeruginosa* cultures were incubated at 35 °C for 3 days.

Table 1 Chemical analysis of monazite and (Th-U) concentrate.

Samples	Elements (%)											
	RE_2O_3	Fe_2O_3	MnO	CaO	MgO	ThO_2	UO_2	Na_2O	K_2O	SiO_2	Al_2O_3	P_2O_5
Monazite	55.8	0.8	UDL	0.5	0.3	4.5	0.4	0.2	0.1	1.6	UDL	27.7
(Th-U) concentrate	20.1	5.5	0.4	0.1	0.3	19.0	2.4	0.5	0.5	2.9	UDL	6.9

UDL: Under detection limit.

Table 2 Acid production of the microorganisms after 7 and 3 days for clear zone, while after 9 and 8 days for HPLC.

Microorganisms	Diameter of clear zone (mm)	Organic acid produced (g/l)		Final pH filtrate
A. ficuum	36	Citric Oxalic	14.30 1.4	3.0
P. aeruginosa	30	2-ketogluconic	6.2	4.6

Table 3 Bioleaching of some REEs from monazite and (Th-U) concentrate by A. ficuum and *P. aeruginosa*.

Tested samples	Bioleaching methods	Direct				Indirect			
	Incubation Periods	5 day for fungi 4 day for bacteria		10 day for fungi 8 day for bacteria		12 h		24 h	
	Organisms	REEs %	Final pH	REEs %	Final pH	REEs %	Filtrate pH	REEs %	Filtrate pH
Monazite	***A. ficuum***	55.1±0.3	3.9	60.6±0.8	3.0	50.1±1.0	4.1	55.0±0.9	4.4
	P. aeruginosa	44.6±0.9	7.8	52.6±0.6	6.0	43.5±0.6	7.6	47.7±0.6	7.7
(Th-U) Concentrate	***A. ficuum***	35.1±0.6	3.7	50.3±0.9	3.2	36.4±0.7	4.1	44.0±0.8	4.3
	P. aeruginosa	40.2±0.6	7.8	42.1±0.8	6.5	38.4±0.7	7.5	39.0±1.0	7.7

Microbial bioleaching

Table 3 shows a comparison between the results obtained using the direct and the indirect methods for bioleaching of some REEs from monazite or (Th-U) concentrate by *A. ficuum* and *P. aeruginosa*.

Direct bioleaching process means that *A. ficuum* or *P. aeruginosa* were grown in their specific media containing 1 % of the tested samples for certain incubation periods using a shaking incubator. In the direct bioleaching process, the results appeared to show that the bioleaching of some REEs from the tested samples increased as the incubation periods increased in the case of fungal and bacterial bioleaching. The bioleaching of some REEs by the fungus was 60.6 and 50.3 % after 10 days in the case of monazite and (Th-U) concentrate, respectively, which were higher than that obtained by the bacterium 52.6, 42.1 % after 8 days in case of monazite and (Th-U) concentrate, respectively. The final pH of the growth medium decreased as the bioleaching process proceeded in both cases.

Indirect bioleaching process means supernatant liquors of the cultures containing 1 % of the tested samples were shaken for a certain time. This experiment was performed to evaluate the ability of organic acids produced by the organisms to leach REEs from the tested samples. From the results shown in **Table 3**, that a 1 day shaking time induced the highest bioleaching of some REEs, where 55.0, 47.7 % were obtained from monazite and (Th-U) concentrate, respectively, by *A. ficuum*. Also, 44.0, 39.0 % were obtained from monazite and (Th-U) concentrate, respectively, by *P. aeruginosa*. In addition, the final pH of the growth medium increased as the bioleaching process proceeded in both cases.

Factors affecting the bioleaching studies

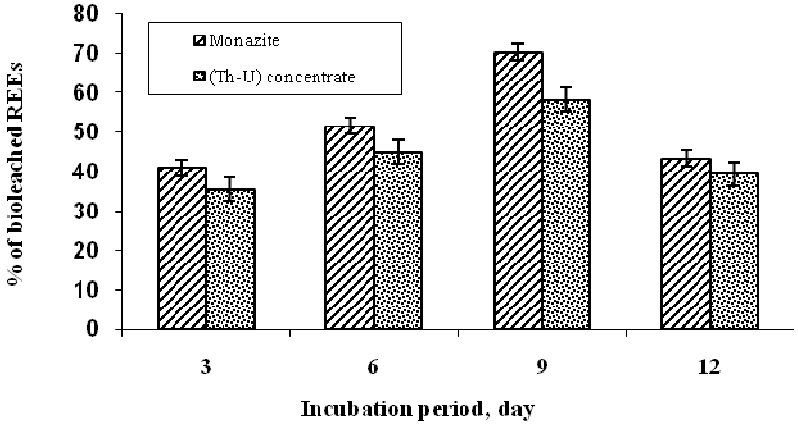
That the maximum bioleaching percentages of some REEs were from 0.6 % monazite and 1.2 % (Th-U) concentrate (**Figure 2a**) by *A. ficuum* after 9 days incubation at 30 °C in a shaking incubator at 175 rpm (data not shown). Also, sodium nitrate and sucrose were found to be the best nitrogen and carbon source, respectively, as appeared in **Figures 2b, 2c**. The bioleaching percentages of some REEs under the previous optimum conditions were 75.6 and 63.9 % for monazite and (Th-U) concentrate, respectively.

In the case of *P. aeruginosa*, the maximum bioleaching percentages of some REEs were from 0.6 % monazite and 1.2 % (Th-U) concentrate (**Figure 3a**) after 8 days incubation at 35 °C in a shaking incubator 175 rpm (data not shown). Also, nutrient broth with additional glucose was found to be the best leaching medium for REEs from tested samples as shown in **Figures 3b, 3c**. The bioleaching percentages of some REEs under the previous optimum conditions were 63.5 and 57.5 % from monazite and (Th-U) concentrate, respectively.

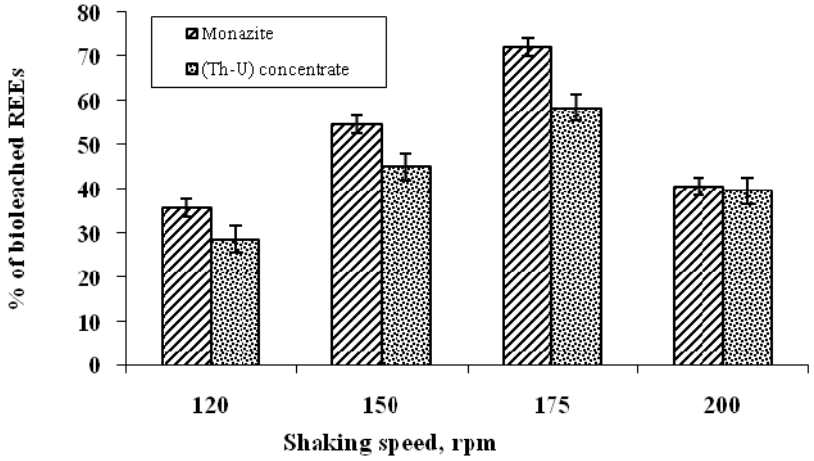
Leaching of REEs using chemical organic acids

Leaching of REEs from monazite (0.6 g/ 50 ml) and (Th-U) concentrate (1.2 g/ 50 ml) by chemical organic acids citric or oxalic were tested. Acids were added in concentrations around that detected from tested organisms by HPLC.

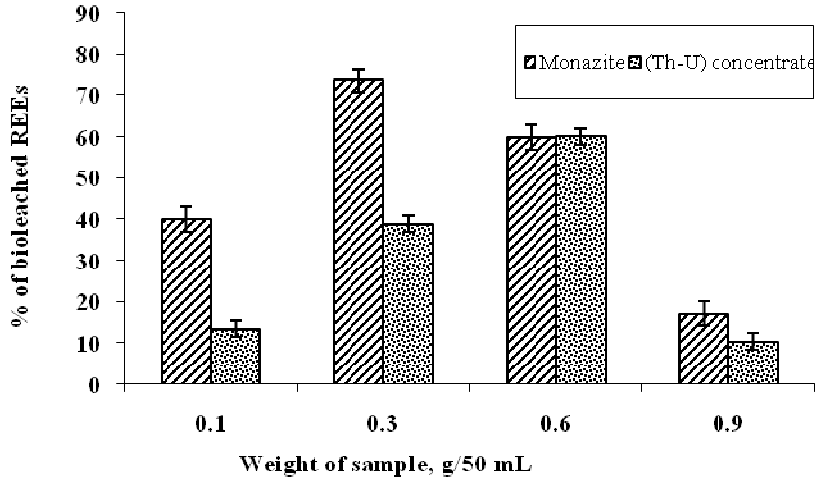
The results in **Table 4** show that the maximum percentages of some REEs leached from monazite and (Th-U) concentrate were 55.7, 39.4 %, respectively, using (14.3 g/l) citric acid, 26.0, 15.1 % respectively, using (1.4 g/l) oxalic acid, and 45.6, 35.3 % respectively, using (6.2 g/l) 2-keto-gluconic acid. Also, when using a mixture of citric/oxalic acids (14.3 / 1.4 g/l) the maximum percentages of some REEs leached were 58.8, 45.4 % from monazite and (Th-U) concentrate, respectively.


Monazite
(Th-U) concentrate
% of bioleached REEs
0
10
20
30
40
50
60
70
80
3
6
9
12
Incubation period, day

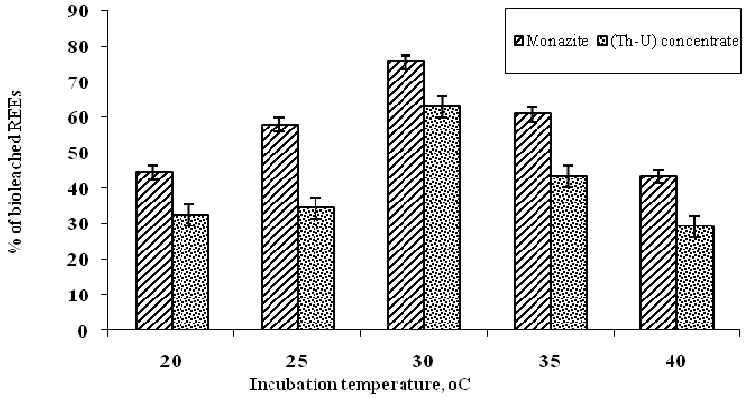
(a)


Monazite
(Th-U) concentrate
% of bioleached REEs
0
10
20
30
40
50
60
70
80
120
150
175
200
Shaking speed, rpm

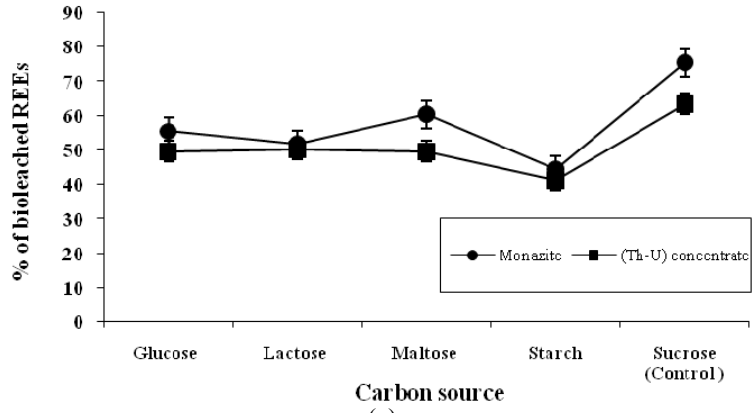
(b)


Monazite
(Th-U) concentrate
% of bioleached REEs
0
10
20
30
40
50
60
70
80
90
0.1
0.3
0.6
0.9
Weight of sample, g/50 mL

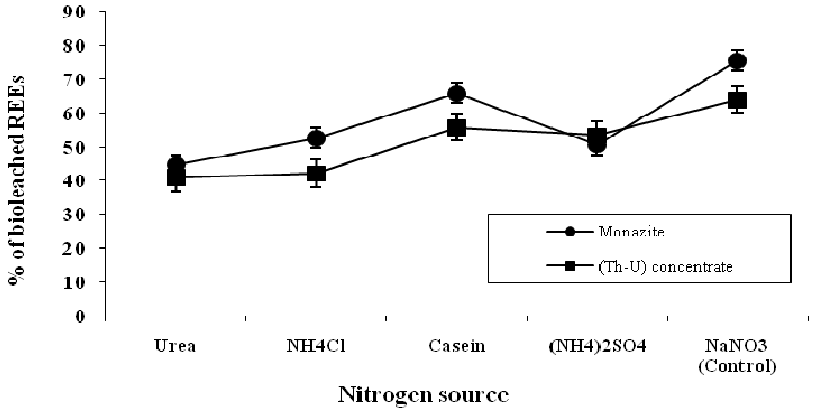
(c)



(d)

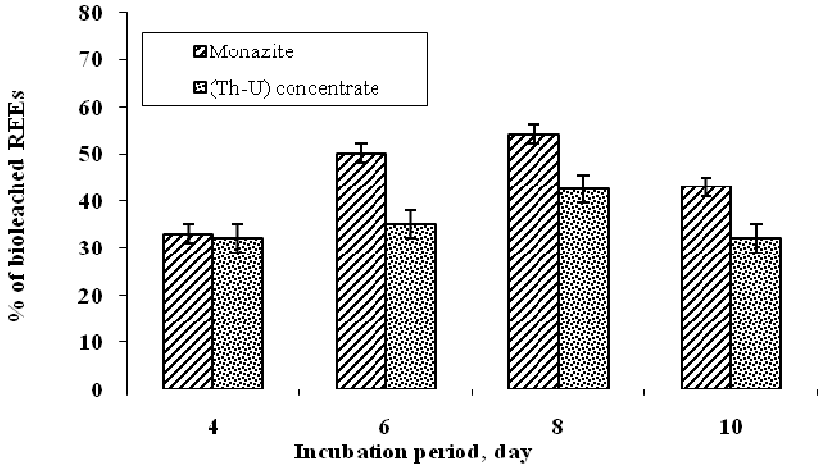


(e)

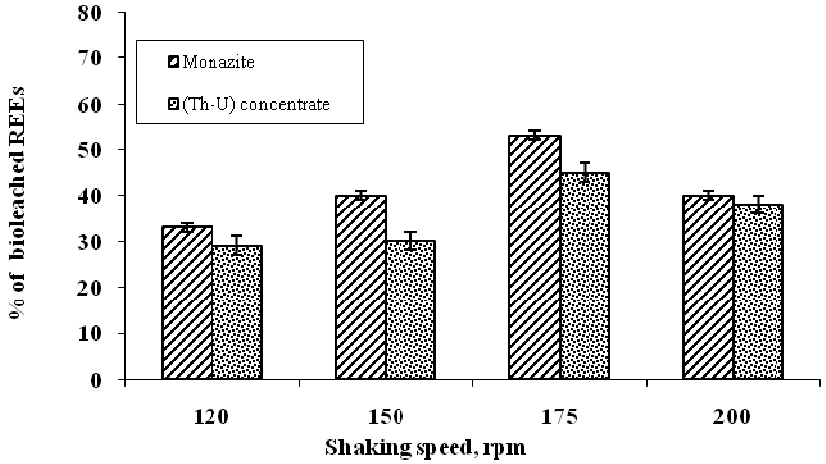


(f)

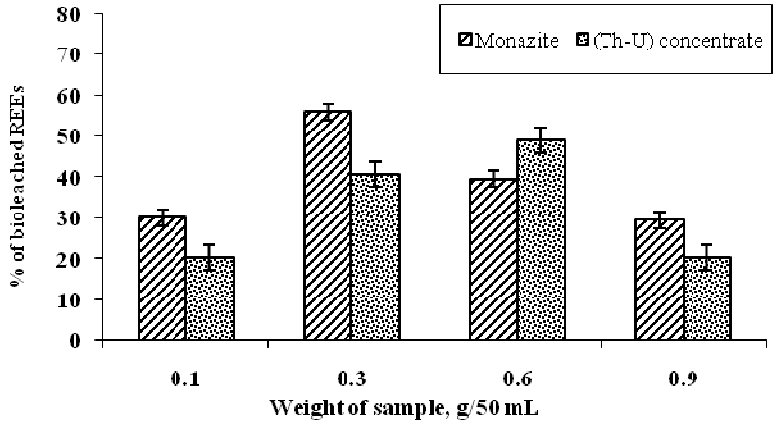
Figure 2 Factors affecting the bioleaching efficiency of some REEs from samples by *A. ficuum* where incubation periods range from 3 - 12 days (a), shaking speed from 120 - 200 rpm (b), incubation temperature from 20 - 40 °C (c), weight of sample from 0.1 - 0.9 g/50 ml (d), carbon sources (e) and nitrogen sources (f).


Monazite
(Th-U) concentrate
% of bioleached REEs
Incubation period, day
0
10
20
30
40
50
60
70
80
4
6
8
10

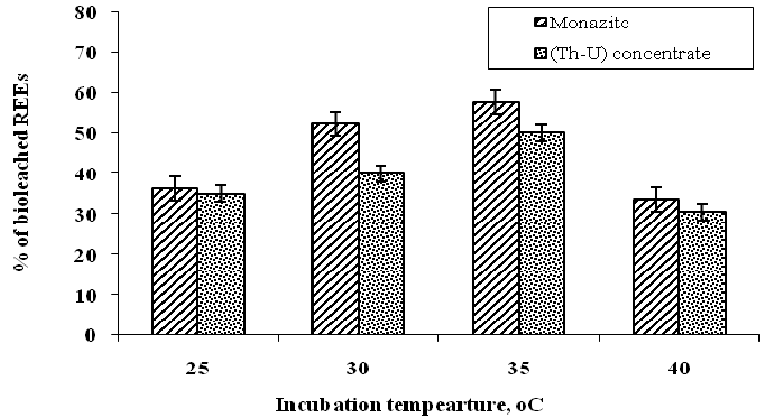
(a)


Monazite
(Th-U) concentrate
% of bioleached REEs
Shaking speed, rpm
0
10
20
30
40
50
60
70
80
120
150
175
200

(b)


Monazite
(Th-U) concentrate
% of bioleached REEs
Weight of sample, g/50 mL
0
10
20
30
40
50
60
70
80
0.1
0.3
0.6
0.9

(c)



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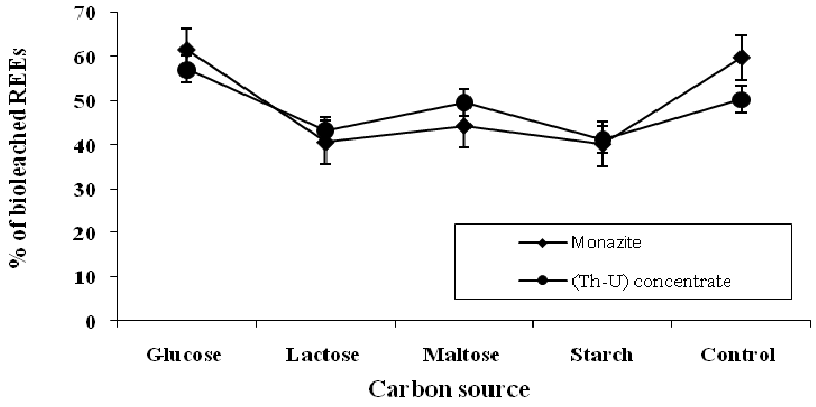
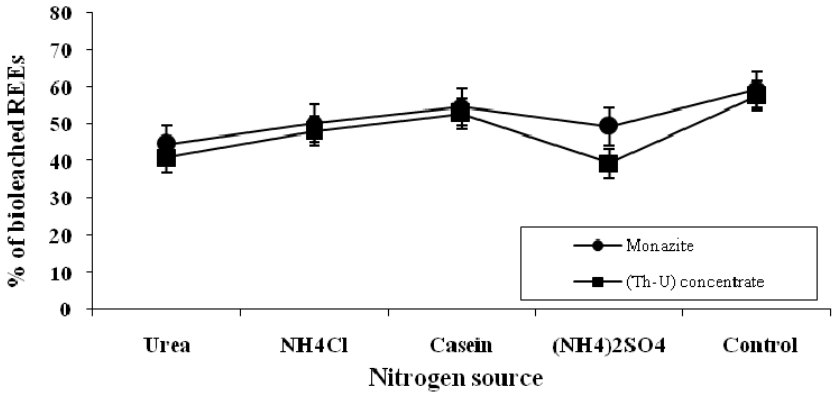


(e)



(f)

Figure 3 Factors affecting the bioleaching efficiency of some REEs from samples by *P. aeruginosa* where incubation periods range from 4 - 10 days (a), shaking speed from 120 - 200 rpm (b), incubation temperature from 25 - 40 °C (c), weight of sample from 0.1 - 0.9 g/ 50 ml (d) carbon sources (e) and nitrogen sources (f).

Table 4 Effect of different concentration of citric, oxalic and 2-ketogluconic acids on leaching of some REEs from monazite and (Th-U) concentrate.

Organic acid concentrations (g/l)		% of REEs leaching efficiency	
		Monazite	**(Th-U) concentrate**
Citric	**12.3**	45.6±1.5	32.5±1.3
	14.3	55.7±1.3	39.4±0.8
	16.3	50.9±1.2	25.5±0.6
Oxalic	**1.0**	24.8±0.5	12.5±0.8
	1.4	26.0±0.4	15.1±0.5
	1.8	15.2±0.5	10.7±0.7
Citric+ Oxalic	**12.3+1.0**	42.3±1.0	55.6±0.6
	14.3+1.4	45.4±1.0	58.8±0.8
	16.3+1.8	35.5±1.0	56.5±1.0
2-ketogluconic	**4.2**	39.9±1.0	29.5±1.0
	6.2	45.6±1.0	35.3±0.9
	8.2	43.5±1.0	34.5±1.0

Discussion

Concerning the chemical analysis of tested monazite and (Th-U) concentrate samples, the results were similar to those obtained by [18]. *Aspergillus* and *Pseudomonas* genera are capable of dissolving heavy metals from the tested mineral and surface of the rocks [3]. It was observed that there was a gradual decrease in the biomass of *A. facuum* and *P.aeruginosa* as the concentration of monazite and (Th-U) concentrate increased in the growth medium. That may have been due to the toxic effect of heavy metals present in the mineral [19]. Similar effects have also been reported in the bacterial leaching of pyrite using *Sulfolobus metallicus* [20].

During the present study in the direct bioleaching process, the filtrate pH decreased from 3.9 to 3.0 during the growth of *A. ficuum* and from 7.8 to 6.0 during the growth of *P. aeruginosa* due to the excreted metabolites, which included H^+ from organic acids, amino acids and other metabolites. These metabolites dissolve metals by displacement of metal ions from the solid minerals by hydrogen ions or by the formation of soluble metal complexes and chelates [21]. The final pH for the microbial cultures was increased more in the presence of monazite and (Th-U) concentrate than the controls due to its toxicity towards *A. ficuum* and/or *P. aeruginosa* then organic acids production was reduced due to the consumption of organic acids for complex formation with the leached metals [22]. In the indirect bioleaching process, the final pH was increased within 24 h because of the proton consumption while converting the oxides in samples into soluble metal salts [23]. It appears that, organic acids were the most important leaching agents in bioleaching process using fungi and bacteria [22]. During the growth studies of *A. ficuum* and *P. aeruginosa*, the substrates undergo microbial oxidation which resulted in the production of organic acids, citric, oxalic, tartaric and gluconic acids, that play a fundamental role in the environmental mobility of metal ions [24]. Some of these acids act as complexing agents for metal ions. Citric acid is a tricarboxylic acid which contains three carboxylic groups and one hydroxyl group (*pka* = 6.39) as a possible donor of protons (H^+) at 25 °C. When rare earth element cations (Re_2O_3) are present in a system and citric acid is fully dissociated in aqueous solution, a complexation reaction may take place;

$C_6H_8O_7 \longrightarrow (C_6H_3O_7)^{3-} + 3H^+$ (pka = 6.93)

$(C_6H_5O_7)^{3-} + RE_2O_3 \longrightarrow RE\ (C_6H_5O_7)$ **Rare earths citrate complex**

Also, oxalic acid contains 2 carboxylic groups (pka = 1.20 and pKa = 4.20) at 25 °C. So, the possible complexes of rare earths reaction with oxalate anion are;

$C_2H_2O_4 \longrightarrow (C_2HO_4)^{1-} + H^+$ (pka = 1.20)

$3(C_2HO_4)^{1-} + RE_2O_3 \longrightarrow RE\ (C_2HO_4)_3$ **Rare earths oxalate complex**

$C_2H_2O_4 \longrightarrow (C_2O_4)^{2-} + 2H^+$ (pka = 4.20)

$3(C_2O_4)^2 + 2RE_2O_3 \longrightarrow RE_2\ (C_2O_4)_3$ **Rare earths oxalate complex**

Similarly, gluconic acid contains one carboxylic group (pka = 3.66) at 25 °C. So, the possible complexes of rare earths reaction with gluconate anion are;

$C_6H_{12}O_7 \longrightarrow (C_6\,H_{11}O_7)^{1-} + H^+$ (pka = 3.66)

$3(C_6H_{11}O_7)^{1-} + RE_2O_3 \longrightarrow RE\ (C_6H_{11}O_7)_3$ **Rare earths gluconate complex**

Low-molecular weight organic acids (LMWOAs) are comprised of aliphatic compounds with 1 - 3 carboxylic acid functional groups and methoxy, hydroxy, carboxylic acid substituted benzoic and cinnamic (aromatic) acids [25]. Because they form stable complexes with metals, LMWOAs play a significant role in mineral transformation. Organic acids promote mineral dissolution by (1) donating H^+ to proton-promoted dissolution processes, (2) forming inner-sphere surface complexes that dislodge structural metals from the mineral surface , and (3) the formation of aqueous metal-ligand complexes that reduce the relative solution saturation with respect to minerals undergoing dissolution [26]. Citric, oxalic and gluconic acids have chelating properties and have a higher affinity for Cr, Ni, Zn and REEs [27]. The amounts of obtained organic acids produced were in similarity to that obtained by [28] when *A. niger* and *P. notatum* were tested.

In comparison between the one and 2-step bioleaching methods, the single step bioleaching experiments showed several advantages. Higher leaching efficiencies occurred in the one-step process than in the 2-step process. These results were probably due to 2 reasons; firstly, microbial cells, live or dead, and their products can be highly efficient bioaccumulators of both soluble and particulate forms of heavy metals, which alter the equilibrium metal concentration in the culture [29]. Secondly, different metabolites were involved in medium leaching in addition to citric, oxalic, gluconic acid and others [30]. The direct bioleaching process showed high efficiency, which reduces the operating costs. In the indirect bioleaching process, producing organic acids in a separate step can facilitate the production of metal leaching acids, and avoid the difficulties related to: (a) maintaining optimum fungal culture conditions in the field, and (b) the toxicity of soil involved in the treatment process. However, capital costs would increase due to the requirement for extra bioreactor tanks to produce the organic acids [31].

Bioleaching, like any other process involving living beings, is influenced by the environmental conditions of the organisms. The recommended optimum environmental and nutritional conditions for maximum bioleaching of some REEs by tested *A. ficuum* were in agreement with that previously recommended by [17] when they used *A. niger* for bioleaching cadmium, lead and zinc from a contaminated soil. The recommended conditions for tested *P.aeruginosa* for bioleaching of some REEs were in agreement with [32] when they used *Leptospirillum ferroxidans* as a bioleaching organism for copper from chalcopyrite.

Concerning chemical leaching, the results indicate that chemical leaching using citric acid is more effective than oxalic acid. This greater recovery of REEs is due to the formation of REEs citrate complex in the solution and thus enhances the solubilization of the metal ions [26], while in the presence of oxalic acid, a complex of REEs oxalates may be formed which have low solubility, making the leached precipitation of REEs precipitate. Also, it appears that chemical leaching using citric/oxalic acid mixture is more effective than using an individual acid , and these results are in agreement with [33]. They showed that chemical leaching of copper and nickel by citric acid is more effective than oxalic acid. Therefore, the leaching process is mainly the reaction of an oxide with protons, even though citrate, which is an important metal coordinating agent, is certainly instrumental in keeping the metal ions soluble.

Generally, the bioleaching (direct or indirect) process of REEs from tested samples using *A. ficuum*, *P. diversum* and *P. aeruginosa* appeared to be more effective than chemical leaching using citric/oxalic or 2-ketogluconic acids. This may be due to the ability of the tested microorganisms to produce other organic compounds, which may behave as synergistic effects on the bioleaching activity. These obtained results are in agreement with that reported by [17]. They concluded that bioleaching using organic acids produced by organisms would become more effective than chemical leaching using organic acid as a leaching agent, due to higher leaching efficiency and lower capital cost.

Conclusions

The present investigation has demonstrated the effect of different concentration of tested samples on growth, acids production and leaching behaviour of *A. ficuum* and *P. aeruginosa*. Tested organisms were produced high kinds of organic acids result in high bioleaching efficiency of REEs from tested samples. In one step process, the highest REEs dissolution were 60.6 and 52.6 % from monazite by *A. ficuum* and *P. aeruginosa*, respectively. During 2 step bioleaching process, the highest bioleaching efficiency of REEs from monazite were 55 and 47.7 % by *A. ficuum* and *P. aeruginosa*, respectively. Compared to chemical leaching the bioleachin process showed better REEs bioleaching and reduced cost. These results indicate the challenges but also the promising aspects of biological leaching as alternative process for recovering REEs from monazite.

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