



Application of the produced microbial citric acid as a leachate for uranium from El-Sebaiya phosphate rock

R. A. Ghazala^a, W. M. Fathy^b and F. H. Salem^a

^aProduction Division, Nuclear Materials Authority, Cairo, Egypt; ^bFaculty of Engineering, Mining and Petroleum Department, Al-Azhar University, Cairo, Egypt

ABSTRACT

Increasing uranium exhaustion of the classical uranium ores in the last century as well as the urgent need for discovering clean nuclear energy would recently reorient research attention toward the nonconventional resources. The latter include mainly phosphate ore. This work is design to produce of citric acid by *Aspergillus niger* via optimization of the nutritional parameters. The medium used was supplemented with different concentrations of sucrose. It was found that sucrose (60 g/l) at pH of 3 to yield about 44.16(%) citric acid. The work is then successive to recover uranium from El-Sebaiya phosphate rock through its bioleaching which adopts on the citric acid produced from the *A. niger* metabolism extractant. The impure precipitate of bioleach liquor was purified via Amberlite I.R.A-400 anion exchanger resin and a marketable product of ammonium diuranate was prepared

KEYWORDS

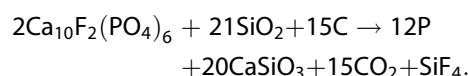
Citric acid production; *Aspergillus niger*; El-Sebaiya phosphate rock; uranium leaching

1. Introduction

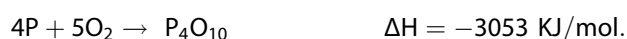
Phosphorites are actually considered as important alternates or secondary sources for uranium with an average uranium content of 50–200 ppm. The discovery of phosphatic rocks in Egypt dates back to the end of the 19th century (Hermina, 1972). Since then, these deposits have been subjected to intensive studies due to their economic and geological significance. Mining and production of the phosphorite ores on a commercial scale represent a main target for the phosphatic fertilizer industries in Egypt. During 2008, about 2.2 million tons of phosphorite ores were mined: 2 million tons from Nile Valley and Red Sea coast areas, in addition to 0.2 million tons only from Abu Tartur area. Half of the mined phosphorite was manufactured as phosphatic fertilizers and 30,000 tons as phosphoric acid, while the rest is exported (AFA Report, 2008).

Generally, to use the phosphates rock either in fertilizers or in recovery of valuable metals, for example (uranium or lanthanides), it must be converted to phosphoric acid. Phosphoric acid is produced either by the wet process or by the electric furnace process. The production of phosphoric acid by the thermal process is carried out in two stages. First, elemental phosphorus is produced from phosphate rock; an electric furnace is used to smelt the phosphorous ore using coke and silica. The silica added to the furnace charge behaves as a strong acid at a high temperature, about 1500°C, and combines with the

calcium constituent of the phosphate rock to form calcium silicate (Schipper et al., 2001; Joseph, 2008; Mark, 2011; International Atomic Energy Agency [IAEA], 1987). The overall reaction may be well expressed in the following equation:



In the second stage, the elemental phosphorus is oxidized with air to become phosphorous pentoxide (P_2O_5). The P_2O_5 is then hydrated to produce a mist of high-quality phosphoric acid which is collected. The reaction was expressed as follows:

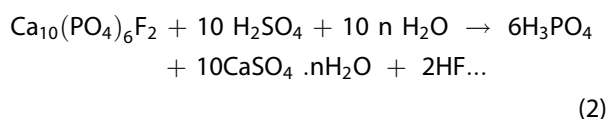


The wet process presents 90% of the world current phosphoric acid production. In this process, there are three possible subgroups depending on the mineral acid that is used for the acidulation. This may be sulfuric, nitric, or hydrochloric.

Phosphoric acid is produced by complete acidilution of the phosphate ore by using sulfuric acid. The acidilution process also produces an insoluble precipitate (calcium sulfate). Calcium sulfate can exist in one of the following forms: dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), hemihydrate ($\text{CaSO}_4 \cdot 1/2\text{H}_2\text{O}$), or the anhydrous CaSO_4 depending on the phosphate ore, the reaction temperature, P_2O_5 concentration, free sulfate content, and the quantity of water

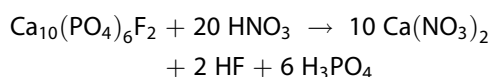
available in the reaction (Abdel-Aal, 2004; Abdel-Aal, Mahmoud, El-Shall, & Ismail, 2007; Chojnacki, Chojnack, & Gorecki, 2005; Fukuma, Denadai, & Quinelayo, 2003; Saueia, Mazzilla, & Favaro, 2005).

The general reaction of phosphoric acid production from the Fluorapatite is as in the following equation:

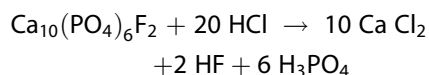


Most of the wet-process phosphoric acid produced in the world is obtained by the classical (Dihydrate (DH) Process) Drawbacks of this process are as follows:

Gypsum contains lattice P_2O_5 , causing a low phosphate efficiency. A dilute acid is obtained (Ca. 30% P_2O_5). Concentrated acid (Ca. 52% P_2O_5) is obtained by evaporation. Gypsum is not suitable for the building industry and cannot be used as a cement retarder. Gypsum as well as the phosphoric acid contains Ra, As, Cd, and other heavy metals. When phosphate rock is treated with nitric acid, phosphoric acid and soluble calcium nitrate are formed according to the following equation:



The reaction between phosphate rock and hydrochloric acid yield phosphoric acid and calcium chloride according to the following equation:



Uranium dissolution during the acidilution process of phosphate rocks depends primarily upon the nature of the produced phosphate material. If the latter is in the form of mono calcium phosphate $\text{Ca}(\text{H}_2\text{PO}_4)_2$, about 1/3 of uranium content of Egyptian phosphate will be transferred to the phosphate product after dissolution. However, if the product is the phosphoric acid (30% P_2O_5), almost all the uranium content will be transferred to the phosphoric acid. On the other hand, if the phosphoric acid is produced by the hemihydrate process (50% P_2O_5), uranium could be dissolved, however, it would be almost probably associated with the calcium sulfate by-product. It is practically possible to keep uranium in the concentrated phosphoric acid during the acidilution process by using an oxidant (Hurst & Arnold, 1982).

The main object of this study is to recover uranium from the El-Sebaiya phosphate rock via nonconventional process by using citric acid. In this regard, several studies have previously been done using sulfuric acid leaching and have proven that uranium is amenable to acid leaching.

However, this has been realized on the expense of relatively gypsum formation (Orabi, El-Sheikh, Mowafy, Abdel-Khalek, & El Kady, 2015; Orabi et al., 2016; etc.). On the other hand, separation of U from its leach liquors in these studies has mainly been done by several techniques, for example, solvent extraction, anion impregnated resins, etc.

To realize the objectives of this work, a technological sample assaying 120 ppm uranium has been collected from El-Sebaiya phosphate rock. To avoid consumption of sulfuric acid and gypsum formation and to separately leach U, citric acid as an organic acid is produced via microbial way.

Citric acid (2-hydroxypropane-1, 2, 3-tricarboxylic acid) is an intermediate of tricarboxylic acid cycle, which is obtained when carbohydrates are oxidized to carbon IV oxide. It has three carboxylic acid functional groups with three pKa values at pH 3.1, 4.7, and 6.4. It is a ubiquitous intermediate product of metabolism and its traces are found in virtually all plants and animals (Papagianni, 2007).

Recently citric acid is commercially produced by submerged (liquid state) fermentation (SmF) using *Aspergillus niger* (Barrington & Kim, 2008; Lofty, Ghanem, & El-Helow, 2007). Many microorganisms such as fungi, bacteria, and yeast can produce citric acid. A large number of these microorganisms have been employed for citric acid production, but only a few of them can produce citric acid in industrial scale (Socol, Luciana, Berghe, Cristine, & Pandey, 2006). Lofty et al. (2007) is reported that *Aspergillus niger* is almost exclusively used for industrial-scale production of citric acid. This is due to its high citric acid productivity at low pH, without secretion of toxic metabolites, ease of handling, and ability to ferment a variety of cheap raw materials. Citric acid is commercially produced by large-scale fermentation mostly using selected fungal or yeast strains in aerobic bioreactors. The major physico-chemical parameters, which influence the growth of *A. niger* and its production of citric acid on submerged liquid media are type of carbon source, carbon source concentration, ethyle alcohol concentration, incubation period, and pH (Bari, Alam, Muyibi, Jamal, & Mamun, 2010; Ellaiah, Srinivasulu, & Adinarayana, 2004; Lofty et al., 2007).

The aim of this work is to recover uranium from El-Sebaiya phosphate rock through bioleaching, which adopts on the extracted citric acid. The extracted citric acid is extract from the *A. niger* metabolism.

2. Materials and methods

2.1. Fermentation medium

Strain of *A. niger* was isolated from soil. The media for growth of isolated organism was composed from the following compounds: NaNO_3 (2 g/l), KH_2PO_4 (1 g/l),

MgSO₄·7H₂O (0.5 g/l), KCl (0.5 g/l), FeSO₄·5H₂O (traces), sucrose (30 g/l), and yeast extract (5 g/l). The growth media was sterilized for 20 min at 121°C within 1.5 atm. The pH of the medium was adjusted at 6.5. The latter was incubated for 7 days and 150 rpm at 30°C.

2.2. Citric acid production and determination

2.2.1. Citric acid production

Fermentation was carried out using 250 ml Erlenmeyer flasks containing 100 ml of fermentation medium. Spore suspension of *A. niger* was inoculated to fermentation medium at an inoculum volume of 1.0 ml/100 ml media. Experiments were carried out at a constant temperature (30°C). To conduct the relevant conditions of citric acid production using *A. niger*, several experiments have been performed. This metabolism (mainly citric acid) used to accomplished the process of uranium bio-leaching.

These factors controlled citric acid production included: type and concentration of carbon source, ethyle alcohol concentration, and incubation period and pH value. All experiments were performed in triplicate and repeated three times for accuracy and precision. Mean values of results were graphically plotted.

2.2.2. Determination of citric acid

Citric acid was determined titrimetrically using 0.1 NaOH and phenol phthalein as an indicator and was calculated in percentage according to equation 1 (AOAC, 1995).

2.2.3. Extraction of citric acid

Recovery of citric acid from liquid fermentation has been described by Colin (1960). Citric acid extracted by this method has been recommended for use in food and drugs. Precipitation, the classical method, was performed by the addition of calcium oxide hydrate (milk of lime) to form the slightly soluble tri-calcium citrate tetrahydrate. This precipitate was removed by filtration and washed several times with water, then treated with sulfuric acid forming calcium sulfate, which was filtered off. Mother liquor containing citric acid was applied on the phosphate rock sample for uranium bioleaching.

2.3. El-Sebaiya characterization

A phosphate rock sample collected from El- Sebaiya Nile Vally. The sample was grounded to less than 200

mesh size and was then subjected to complete chemical and uranium analysis. Uranium was analyzed by the oxidimetric titration method using ammonium metavanadate (Mathew, Bürger, Ogt, Mason, & Narayanan, 2009). All measurements were carried out in the Nuclear Materials Authority laboratories.

2.3.1. Uranium bioleaching

Uranium bioleaching process were accomplished by mixed extracted citric acid with phosphate sample under the optimum condition of (Amin & Ghazala, 2014). The latter namely, S/L(1/3), pH2.5, at room temperature and shaking for 1 day in an orbital shaker at 100 rpm.

3. Results and discussion

3.1. Factors of citric acid production

In order to improve the quality of the citric acid production, several series of experiments have first been performed to optimize the different factors.

3.1.1. Effect of carbon sources (sugar type)

For studying the effect of different types of carbon sources (40 g) for fermentation of *A. niger* to produce citric acid, three series of experiments have been performed in which citric acid production by *A. niger* grown on fermentation medium with different types of carbon sources (sucrose, glucose and starch) within incubation period of 5 days at 30°C.

The obtained results are plotted in Figure 1. From the latter results, it is noticed that the medium supplemented with sucrose gave the highest citric acid values (36.26%), while the other two types of carbon sources glucose and starch achieved only 31.8 and 25.11, respectively.

The obtained data are actually in agreement with Kubicek (1998) who explained that the nature of carbon source has a marked effect on citric acid production by fungi. This might be due to the fact that simple carbon compounds are assimilated directly while complex ones (disaccharide (sucrose) and polysaccharides (starch)) must be converted into simpler forms before use as the carbon source. Monosaccharide and disaccharides are the preferred carbon source as they are more rapidly metabolized by the fungus than polysaccharides,

$$\text{Citric acid \%} = \frac{\text{Normality X Volume of 0.1 M NaOH X equivalent weight of citric acid X dilution factor}}{\text{Weight of sample (g) X 10}} \quad (1)$$

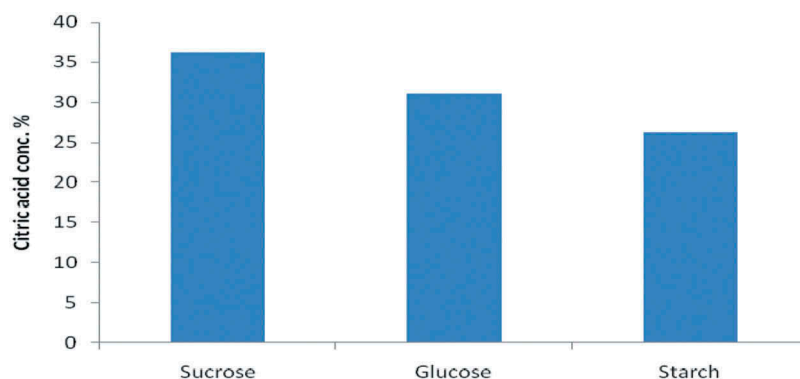


Figure 1. Citric acid production by *A. niger* in by using of different types of 40 g sugar.

thus producing higher yield of citric acid (Sati & Bisht, 2006).

Polysaccharides are not suitable as the raw material since the decomposition process takes long time to meet the rate of sugar catabolism necessary for the production of citric acid. The slow rate of polysaccharide hydrolysis is due to reduced enzymatic activity, which affects the pH in the fermentation medium (Papagianni, Wayman, & Matthey, 2005).

3.1.2. Effect of sucrose concentration

Effect of different sugar concentrations on citric acid production by *A. niger* was studied in the range from 15 to 200 g/l sucrose while other condition is fixed at incubation period of 5 days at 30°C and pH of 3.3 and 1% alcohol.

From the obtained result shown in Figure 2, it was found that: the maximum amount of citric acid production (41.86%) was obtained in the medium containing 60 g/l sucrose. On other hand, the consumption of sugar and dry mycelial weight were 48.13 and 11.87 g/l, respectively.

It is important to mention herein that decreasing in citric acid formation was observed when the sugar concentration of sucrose from 70 to 200 g/l was increased. It may be due to the over growth of the mycelium, which resulted in high viscosity of

the medium. Matthey and Allan (1990) described that with the increase of mycelial formation in the medium, there is a reduction in the yield of citric acid. Pazouki, Felse, Sinha, and Panda (2000) pointed out that sugar concentration higher than 16–18% leads to greater amount of residual sugars, making the process uneconomic, while lower sugar concentration leads to lower yields of citric acid due to the accumulation of oxalic acid in the culture broth.

A parallel relationship between citric acid production and sugar consumption was also observed. This result agreed with the report of El-Holi and Al-Delamy (2003), the production of citric acid is approximately paralleled to the consumption of sugar. At the end of the fermentation process, a significant decrease in residual sugar from 68.1 g/kg to 4.5 g/kg was obtained; this result agreed with the report of Hang and Woodams (1987).

3.1.3. Effect of ethyl alcohol concentration

Ethanol is the major controlling factor in the final yield of citric acid supplementation ethanol because it regulates the citric acid production positively and stimulating using of carbon sources. Effect of ethanol on citric acid production under previously

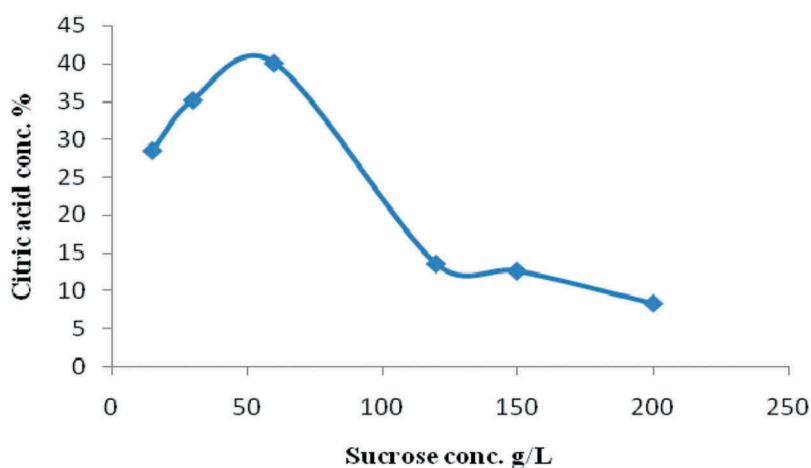


Figure 2. Citric acid production by *A. niger* of different concentration of sucrose.

mentioned condition of incubation period of 5 days at 30°C and pH of 3.3.

The results are shown in Figure 3 where the maximum citric acid production (44.01%) was obtained at 2% ethanol concentration. An increase in citric acid production at 2% ethanol concentration is agreement with Hossain, Brooks, and Moddax (1984) who stated that the presence of ethanol in fermentation media may increase citric acid production using *A. niger*. The inductive effect of ethanol for citric acid production may be due to reduction of the inhibitory effects of metal ions Kiel, Gurin, and Henis (1981). Moyer (1953) discovered the use of low molecular weight alcohols, that is, methanol, isopropanol as adjuncts to the culture medium which greatly increases citric acid production in both surface and submerged cultures. Guebal and Torres (2000) suggested that ethanol increases the permeability of cell wall which allows

to enter citrate out the cell. Maximum biomass was also obtained at 2% ethanol concentration and decreased at more than this concentration.

3.1.4. Effect of initial pH value

As a matter of fact, the most important parameter that affects upon fermentation of citric acid is actually the pH value. Therefore, the effect of pH has been studied in the range of 1–6 while other condition is fixed at incubation period of 5 days at 30°C.

The obtained results in Figure 4 revealed that maximum amount of citric acid (44.16%) was achieved when the initial pH of fermentation medium was adjusted at 3 value. Increasing pH than 3 citric acid production will be decreased. This result agrees with the observations of Pessoa, Diasde, and Angela (1982) and Shadafza, Ogawa, and Fazell (1976); they reported that a higher initial pH leads to the accumulation of oxalic acid.

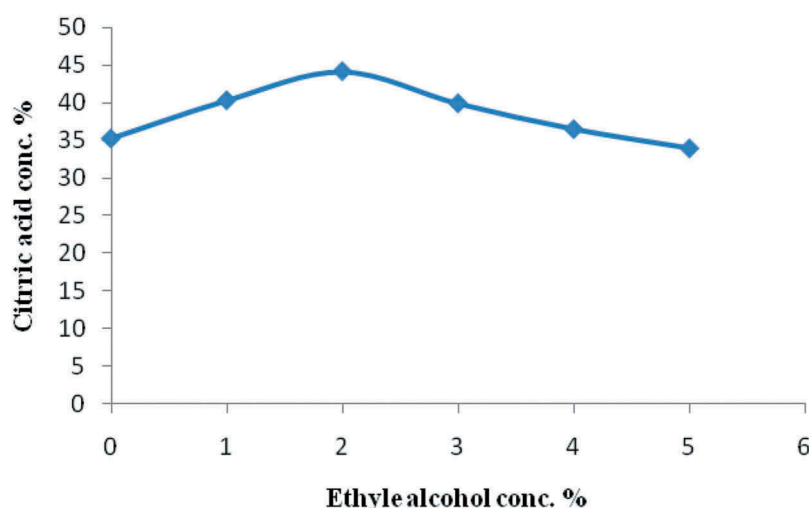


Figure 3. Citric acid production by *A. niger* of sucrose media with different concentration of ethyl alcohol.

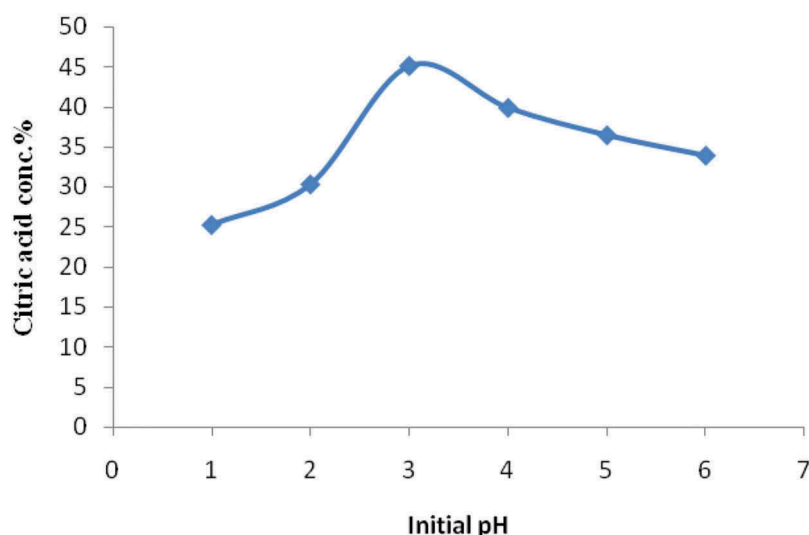


Figure 4. Citric acid production by *A. niger* of sucrose media with different pH value.

From the above-studied optimization factors of the citric acid production, it can be concluded that the optimum conditions for production about 44.16% would be summarized as follows:

carbon sources: sucrose
concentration of sucrose 60 g/l
ethanol concentration: 2%
pH : 3
incubation period: 5 days
Temp.: 30°C.

3.2. Recovery of uranium from El-Sebaiya phosphate rock sample

3.2.1. Characterization of El-Sebaiya phosphate rock

As mentioned above, the optimum conditions of U leaching using extracted citric acid produced by *A. niger* were applied for uranium leaching from El-Sebaiya phosphate material. It is important to mention herein that citric acid is extracted as mentioned above at 2.2.3. in experimental section. The chemical composition of the latter was determined as shown in Table 1.

3.2.2. Leaching of uranium from El-Sebaiya phosphate rock sample

After prepared citric acid by *A. niger* with an studied optimum condition. The work then shifted to study the potentiality of citric acid after extraction process from metabolism upon uranium leaching from El-Sebaiya phosphate rock. The latter process were applied using different solid/liquid ratio from citric

acid solution and phosphate ore sample (1/5, 1/10, 1/20, and 1/30) under the other fixed optimum leaching conditions of 5 h incubation period and 50°C according to (Amin & Ghazala, 2014). From the obtained leaching efficiencies given in Table 2, it was found that beyond 1/20 S/L ratio, only a slight steady increase in the leaching efficiencies of uranium has been achieved. Accordingly, a solid ratio of 1/20 would be considered as optimum at which the leaching efficiency of uranium attained 91.1%.

3.2.3. Preparation of uranium product

Under the optimum leaching condition of uranium from half kilo gram El-Sebaiya phosphate rock study using the prepared citric acid has been performed. As a matter of fact, uranium is the first to precipitate from the obtained 10 l of bioleach liquor after arising its pH to 7.5 using 10% NaOH and the obtained product has then been subjected to proper analysis using the ESEM-EDX. From the latter shown in Figure 5, it was found that U assay in the product amounted to only 65.24% U and which is associated with several impurity elements, namely, Si, Ca, and P and some Fe.

In order to improve the quality of the obtained impure uranium concentrate, the latter was dissolved in 1 molar sulfuric acid and the obtained solution was subjected to proper concentration and purification using anion exchange resin which is quite selective for uranium recovery from the obtained sulfate leach liquor. In the latter solution, the competing anions include mainly SO_4^{2-} and HSO_4^- and therefore uranium would be better adsorbed at pH value exceeding 1.8 while below this value, HSO_4^- would be strongly adsorbed (Merriitt, 1971; Preuss & Kurrin, 1965).

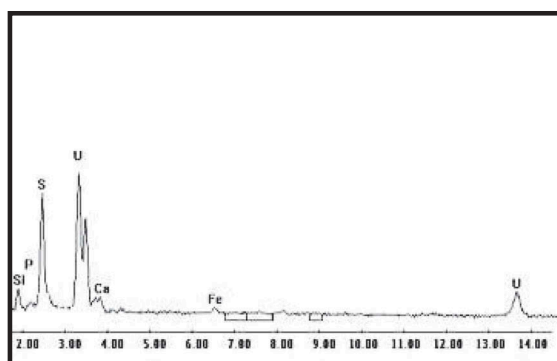
Table 1. The chemical analysis of the working El-Sebaiya phosphate ore sample.

Component	wt. %	Component	wt. %
SiO ₂	7	CaO	40
TiO ₂	0.02	K ₂ O	0.14
Al ₂ O ₃	1.6	P ₂ O ₅	35
Fe ₂ O ₃	3	*L.O.I. (1000 °C)	9
MgO	0.7	U	0.0120
Na ₂ O	1.2		

*L.O.I. = loss on ignition

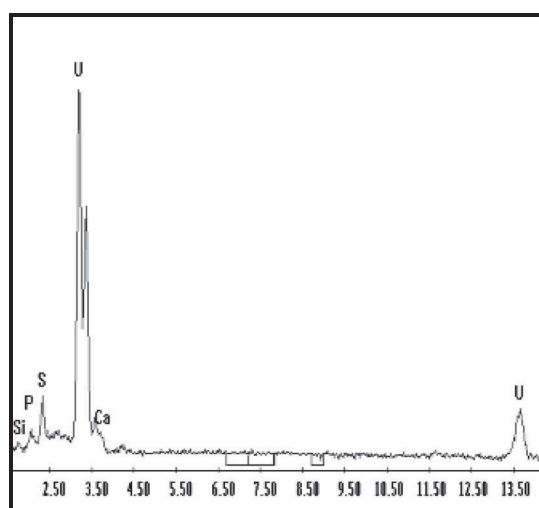
Table 2. Effect of ore/citric acid (solid/liquid ratio) upon uranium leaching.

S/L ratio	U (%)
1/5	33.12
1/10	83.27
1/20	91.10
1/30	91.21



Element	Wt%
U	65.24
s	17.23
p	6.06
Si	7.11
Ca	4.12
Fe	0.24

Figure 5. ESEM-EDX analysis of uranium concentrate sodium diuranate product directly precipitated from the bioleach liquor at pH 7.5.



Wt%	Element
U	88.40
s	4.56
p	2.19
Si	1.98
Ca	2.87

Figure 6. ESEM-EDX analysis of ammonium diuranate precipitated from Amb. I.R.A. 400 eluted at pH 5.5.

In this work, 2.5 ml of wet settled resin (wsr) Amberlite I.R.A 400 anion exchanger was packed in a suitable Pyrex glass column (0.5 cm diameter) over a glass wool plug. The prepared uranium sulfate solution was firstly treated with 5% of NaOH to adjust its pH to 1.8 followed by its passing through the prepared resin column using a contact time of 3 min (1.3 ml/min). The loaded uranium was then eluted from the resin using 1N NaCl acidified with 0.1M sulfuric acid. An eluate volume of 100 ml was collected at the end of elution process and was found to assay 550 ppm resulting to an elution efficiency 99%. From the obtained eluate, uranium was precipitated by NH_4OH as ammonium diuranate at pH 5.5 and the product was subjected to analysis using ESEM-EDX (Figure 6) from which U assay increased up to 88.4%.

3.2.4. Comparison between different types of citric acid in uranium leaching from El-Sebaiya phosphate rock sample

Citric acid contains three carboxylic groups ($\text{pK}_{a1} = 10.82$ and $\text{pK}_{a2} = 4.76$ and $\text{pK}_{a3} = 6.39$) and one hydroxyl group ($\text{pK}_{a2} = 10.82$). The possible complex is:

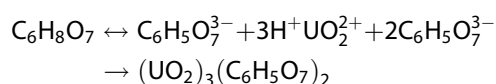


Table 3. Uranium leaching efficiency % using different types of citric acid in uranium leaching from El-Sebaiya phosphate rock sample.

Types of citric acid	U efficiency %
Citric acid (in metabolism)	83
Synthetic – Commercial citric acid	64
Extracted citric acid	91

Comparison between different types of citric acid in uranium leaching from El-Sebaiya phosphate rock sample. The citric acid types are, namely, (i) citric acid present in metabolism, (ii) commercial citric acid, and (iii) extracted citric acid using *A. niger* using the optimum condition of mentioned bioleaching of 5 h incubation period and 50°C and S/I ratio of 1/20. The obtained data tabulated in (Table 3) revealed that the extracted citric acid produced by *A. niger* metabolism gives the highest efficiency percent attaining about 91%. Finally, the phosphate content is changed to 34% in phosphate rock residue after the recovery of uranium.

4. Conclusion

Cultural conditions for citric acid production by *A. niger* has been optimized by incubation period of 5 days, 60 g/l sucrose, 2% ethanol, pH of 3, and 30°C to produce about 44% citric acid. It is important to mention herein that the citric acid then extracted using calcium oxide hydrate. The optimum conditions of U leaching using extracted citric acid produced by *A. niger* were applied for uranium leaching from El-Sebaiya phosphate material. The impure precipitate of bioleach liquor was purified via Amberlite I.R.A-400 anion exchanger resin and a marketable product of ammonium diuranate was prepared.

Acknowledgments

Authors are thankful to Dr. M. Amine for suggesting this point and for her guidance and continued support.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Abdel-Aal, E. (2004). Crystallization of phosphogypsum in continuous phosphoric acid industrial plant. *Crystal Research and Technology*, 39, 123–130.
- Abdel-Aal, E., Mahmoud, M., El-Shall, H., & Ismail, A. (2007). Increasing the filtration rate of phospho-gypsum using surfactant. *Hydrometallurgy*, 85, 53–58.
- AFA Report. (2008). *Arab fertilizers association report* (Issue no. 51). Cairo. pp. 20–35.
- Amin, M. M., & Ghazala, R. A. A. (2014). Potentiality of uranium solubilization from phosphate rock sample using mixture of organic acids of fermented media. *An Indian Journal*, 9, 212–217.
- AOAC. (1995). *Official methods of analysis* (16th ed.). Washington, DC: Association of Official Analytical Chemist.
- Bari, M. N., Alam, M. Z., Muyibi, S. A., Jamal, P., & Mamun, A. (2010). Statistical optimization of process parameters for the production of citric acid from oil palm empty fruit bunches. *African Journal of Biotechnology*, 9, 554–563.
- Barrington, S., & Kim, J. W. (2008). Response surface optimization of medium components for citric acid production by *Aspergillus niger* NRRL 567 grown in peat moss. *Bioresource Technology*, 100, 3113–3120.
- Chojnacki, A., Chojnack, K., & Gorecki, H. (2005). Utilization of spent petrochemical sulfuric acid in the production of wet-process phosphoric acid. *Journal of Chemical Technology & Biotechnology*, 80, 1331–1338.
- Colin, P. (1960). Extraction of citric acid from aqueous solution. *Fr. Pat.*, 1, 66.
- El-Holi, M. A., & Al-Delamy, K. S. (2003). Citric acid production from whey with sugars and additives by *Aspergillus niger*. *African Journal of Biotechnology*, 2, 56–59.
- Eliaiah, P., Srinivasulu, B., & Adinarayana, K. (2004). Optimization studies on neomycin production by a mutant strain of *Streptomyces marinensis* in solid state fermentation. *Process Biochemistry*, 39, 529–534.
- Fukuma, H., Denadai, F., & Quinelayo, A. (2003). Natural radionuclide-free phosphoric acid production. *Journal of Radioanalytical and Nuclear Chemistry*, 257, 117–121.
- Guebal, D. V., & Torres, N. V. (2000). Optimization of citric acid production by *Aspergillus niger* through amietabolic flux balance model. *Process Biotechnol*, 4, 123–127.
- Hang, Y. D., & Woodams, E. E. (1987). Microbial production of citric acid by Solid-state fermentation of Kiwi fruit peel. *Journal of Food Science*, 52, 226–227.
- Hermina, M. (1972). Review of the phosphate deposits of Egypt. In *2nd Arab Conference of Mineral Resource Conference papers* (pp. 109–149). Egypt.
- Hossain, M., Brooks, J. D., & Moddax, I. S. (1984). The effect of the sugar source on citric acid production by *Aspergillus niger*. *Applied Microbiology*, 1, 393–397.
- Hurst, F., & Arnold, W. (1982). A discussion of uranium control in phosphogypsum. *Hydrometallurgy*, 9, 69–82.
- International Atomic Energy Agency (IAEA). (1987). *IAEA-TECDOC-533*. Vienna: The recovery of uranium from phosphoric acid.
- Joseph, A. (2008). Best available technology for producing high grade phosphoric acid from lower zone phosphate ore in the Southern extension. *The 23rd annual regional phosphate conference*. Florida: Lakeland.
- Kiel, H., Gurin, R., & Henis, Y. (1981). Citric acid fermentation by *Aspergillus niger* on low sugar concentration and cotton waste. *Applied Environmental Microbiology*, 42, 1–4.
- Kubicek, C. P. (1998). The role of sugar uptake and channeling for citric acid accumulation by *Aspergillus niger*. *Food Technology and Biotechnology*, 36, 173–175.
- Lofty, W. A., Ghanem, K. M., & El-Helow, E. R. (2007). Citric acid production by a novel *Aspergillus niger* isolate: II. Optimization of process parameters through statistical experimental designs. *Bioresource Technology*, 98, 3470–3477.
- Mark, V. (2011). Phosphate environmental footprint of the improved hard process for the manufacture of phosphoric acid. *The 26th Annual Regional Phosphate Conference*. Lakeland, FL: The Lakeland Center.
- Mathew, K. J., Bürger, S., Ogt, S. V., Mason, P. M. E. M., & Narayanan, U. I. (2009). Uranium assay determination using Davies and Gray titration. *Proceedings of The Eighth International Conference on Methods and Applications of Radio analytical Chemistry (Marc VIII)* (pp. 5). Kailua- Kona, Hawaii.
- Mattey, M., & Allan, A. (1990). Glycogen Accumulation in *Aspergillus niger*. *Biochemical Society Transactions*, 18, 1020–1021.
- Merritt, R. C. (1971). *Extractive metallurgy of uranium*. Golden, CO: Colorado school of Mines Research Institute.
- Moyer, A. J. (1953). Effect of alcohols on the mycological production of citric acid in surface and submerged culture. *Applied Microbiology*, 1, 1–6.
- Orabi, A. H., El-Sheikh, E. M., Mowafy, A. R., Abdel-Khalek, M., & El Kady, M. Y. (2015). Studies on the selectivity of cetrimide for uranium extraction from wet process phosphoric acid. *International Journal of Mineral Processing*, 137, 26–33.
- Orabi, A. H., El-Sheikh, E. M., Saleh, W. H., Youssef, A. O., El-Kady, M. Y., & Shalaby, Z. M. (2016). Potentiality of uranium adsorption from wet phosphoric acid using amine-impregnated cellulose. *Journal of Radiation Research and Applied Sciences*, 9, 193–206.
- Papagianni, M. (2007). Advances in citric acid fermentation by *Aspergillus niger* biochemical aspects, membrane transport and modeling. *Biotechnology Advances*, 25, 244–263.
- Papagianni, M., Wayman, F., & Mattey, M. (2005). Fate and role of ammonium ions during fermentation of citric acid by *Aspergillus niger*. *Applied and Environmental Microbiology*, 71, 7178–7186.
- Pazouki, M., Felse, P. A., Sinha, J., & Panda, T. (2000). Comparative studies on citric acid production by *Aspergillus niger* and *Candida lipolytica* using molasses and glucose. *Bioprocess Engineering*, 22, 353–361.
- Pessoa, D. F., Diasde, C., & Angela, C. (1982). Production of citric acid by *Aspergillus niger*. *Microbiology Reviews*, 13, 225–229.
- Preuss, A., & Kurrin, R. (1965). *A general survey of types and characteristics of ion exchange resin used in uranium recovery* (Technical Reports Series No. 359). Vienna: International Atomic Energy.
- Sati, S. C., & Bisht, S. (2006). Utilization of various carbon sources for the growth of water borne conidial fungi. *IndiaMycologia*, 98, 678–681.
- Saueia, C., Mazzilla, B., & Favaro, D. (2005). Natural radioactivity in phosphate rock, phosphogypsum and

- phosphate fertilizers in Brazil. *Journal of Radioanalytical and Nuclear Chemistry*, 264, 445–448.
- Schipper, W., Klapwijk, A., Potjer, B., Rulkens, W., Temmink, B., Kiestra, F., & Lijmbach, A. (2001). Phosphate recycling in the phosphorus industry. *Environmental Technology*, 22, 1337–1345.
- Shadafza, D., Ogawa, T., & Fazell, A. (1976). Comparison of citric acid production from beet molasses and date syrup with *Aspergillus niger*. *Hakko Kogaku Zasshi*, 54, 65–75.
- Soccol, C. R., Luciana, P. S., Berghe, V., Cristine, R., & Pandey, A. (2006). Citric acid production. *Food Technology and Biotechnology*, 44, 141–149.