ISSN : 0974 - 7443 Volume 9 Issue 6

CHEMICAL TECHNOLOGY

An Indian Journal

Full Paper

CTAIJ 9(6) 2014 [212-217]

Potentiality of uranium solubilization from phosphate rock sample using mixture of organic acids of fermented media

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ABSTRACT

Testing of El-Sibaiya phosphate using the fermented media of the *Penicillium Simplicissimum (P. simplicissimum)*, in which both citric and oxalic acids are considered the main leaching agents. About 82% of the original uranium content was leached out of the ore. The applied leaching conditions included S/L ratio, stirring time and the working temperature. On the other hand, only 64% of the phosphate uranium content was leached using a synthetic mixture of both citric and oxalic acids in simulated concentrations as the natural ones. The difference between the leachability ratios in both cases was attributed to the presence of some proteins in the fermented media which was assured by the chemical analysis. Such proteins were responsible about the dissolution of 18% of the uranium content from the target ore.

KEYWORDS

Phosphate ore;
Uranium;
Organic acid;
Penicillium simplicissimum;
Chemical leaching;
Protein.

INTRODUCTION

Uranium is one of the strategic metals which is used as fuel in nuclear power generators and extracted from its ores by traditional chemical methods either by acid or alkali leaching[1,2]. The microbial recovery of uranium from both high and low-grade ore can be considered "green technology" with low cost, low energy requirements and environmentally safe.

The phosphate is commonly called "rock" in sedimentary deposits and apatite in igneous deposits. Generally, the phosphatic rocks contain an appreciable amount of uranium in the range of 0.005 - 0.03% U_3O_8 mainly as isomorphous substitution for calcium[3]. The uranium content in the Egyptian phosphates ranges, more or less, between 40 and 100 ppm, and in some types reaches 200 ppm[4]. Although the variation in their uranium contents, the phosphate rocks from the different Egyptian localities exhibit more or less similarity in the mineralogical composition where the hydroxyl apatite, carbonate apatite, calcite, dolomite, hematite and quartz are the main mineralogical constituents[5]. Uranium occurs in phosphate rocks mainly in two valences i.e as U^{4+} in the apatite crystal and as U^{6+} which adsorbed to phosphate minerals, or fixed on phosphate ions to form secondary uranium minerals[6].

Recently, efforts are being made to utilize acidophilic heterotrophs (bacteria and fungi) for uranium extraction from bearing ores due to production of organic acids (e.g. citric and oxalic) that can serve as leaching agents for the solubilization of metals. The use of fungi was thought to be interesting alternative[7]. *A. niger* and *P. simplicissimum* are two widely used species of fungi in the bioleaching of metals. Citric and oxalic acids are the major organic acids produced by these organisms[8,9]. For this purpose citric and oxalic were used in chemical leaching experiment to mimic the chemical action of *P. simplicissimum* culture in order to understand and compare between chemical leaching with organic acids and bioleaching on uranium solubilization from phosphate ore.

Some metabolic products other than organic acids might involve in the bioleaching of metals, such as extracellular protein or proton extrusion[10]. This view represents one of the main aims of this work. Proteins are fundamental components of all living cells and include many substances, such as enzymes, hormones and antibodies. They consist of long chains of amino acids connected by peptide bonds.

MATERIALS AND METHODS

Rock phosphate

The studied phosphate sample collected from El-Sibaiya Nile Vally. This sample was ground to less than 200 mesh sizes then a representative sample was subjected to complete chemical analysis of both major and trace elements contents were determined by wet chemical analysis[11] where; the spectrophotometer was used to measure SiO_2, Al_2O_3, TiO_2 and P_2O_5 while both K_2O and Na_2O were determined using the flame photometer technique. On the other hand, the traditional titration method was used for Fe_2O_3, MgO and CaO measurements. Trace elements were estimated using a Unicam atomic absorption spectrophotometer and finally the loss on ignition (LOI) was assigned gravimetrically. Uranium was analyzed by the oxidimetric titration method using ammonium metavanadate[12]. The measurements showed analytical error better than ±1 %. All these analyses are carried out in the Nuclear Materials Authority laboratories.

Fungal strain and growth media

Strain of *P. simplicissimum* was isolated from the tested sample. The media composition for growth of isolated organism is as follows: $NaNO_3$ (2 g/l), KH_2PO_4 (1 g/l), $MgSO_4.7H_2O$ (0.5 g/l), KCl (0.5 g/l), $FeSO_4.5H_2O$ (traces), sucrose (30 g/l) and yeast extract (5 g/l)[13]. The growth media was sterilized at 121 °C and 1.5 atm. for 20 min. The pH of medium was adjusted to 6.5. Inoculation of *P. simplicissimum* strain was made in the shaking flask containing growth media. This flask was incubated at 30 °C and 150 rpm for 7 days.

Bioleaching studies of rock phosphate

Bioleaching experiment was carried out in three conical flasks (250 ml). One gram of phosphate sample was added to each flask which contained (50, 100 and 200 ml) of tested fungal metabolite. The initial pH of *P. simplicissimum* metabolite was in range 2.5 - 3. All flasks were mixed well on shaker at 100 rpm for 24 hours. In time course, samples were removed and centrifuged to get rid solid suspension. Supernatants were analyzed for pH, dissolved uranium, secreted proteins and organic acids consumed.

Characterization of organic acids in fermented media

The concentrations of organic acids produced by *P. simplicissimum* strain and organic acids consumed during bioleaching process were determined by high performance liquid chromatography (HPLC) at the Regional Center for Mycology and Biotechnology, Al Azhar Univ., Cairo, Egypt.

Factors affecting uranium boleaching

To conduct the relevant condition of uranium leaching process using *P. simplicissimum* metabolite, several experiments have been performed. The factors affecting leaching included S/L ratio, stirring time and applied temperature.

Chemical leaching process

Chemical speciation studies were done by taking the concentration of oxalic and citric acids (synthetic acids) which produced by *P. simplicissimum* together. By applying the optimum conditions for uranium bioleaching (1/3 S/L ratio, 5 hours and 50 °C) the supernatant was removed and analyzed for uranium.

Effect of proteins secretion on uranium solubilization

The protein content in the clear culture filtrate was determined by the method described by Lowry et al.[14] using bovine serum albumin as the standard protein.

RESULTS AND DISCUSSION

Chemical analyses of rock sample

Chemical analyses of El-Sibaiya phosphate rock sample showed high content of CaO and P_2O_5, which represented 39.4% and 30.5% respectively and moderate content of SiO_2 and Al_2O_3 which represented 13.74% and 4.35% respectively. While, the other oxides such as TiO_2, MgO, Na_2O, K_2O and Fe_2O_3 were detected in low quantities ranging between 0.07 and1.65 2% TABLE 1. The loss on ignition (LOI) was recorded as 6.26%. The uranium concentration of the sample was 120 ppm while, the content of other metals were detected in high concentrations representing 1272, 664, 184, 174, 136 and 86 ppm for Sr, Ba, Zn, V, Cr and Y respectively. On the other hand Ni, Cu, Zr, Pb and Rb were detected in very low quantities, less than 17 ppm TABLE 2. The distribution of most of these elements within phosphate sample may be related to their affinity to be adsorbed on the apatite grains.

TABLE 1 : Chemical analyses of the studied phosphate sample

Components	Conc. (%)	Components	Conc. (%)
SiO_2	13.74	Na_2O	0.65
TiO_2	0.07	K_2O	0.07
Al_2O_3	4.35	P_2O_5	30.5
Fe_2O_3	1.65	L.O.I.*	6.26
MgO	1.51		
CaO	39.4		
Total		98.6	

***L.O.I. = loss on ignition**

TABLE 2 : Trace elements analysis

Trace element	Conc. (ppm)	Trace element	Conc. (ppm)
Ba	664	Pb	4
V	174	Cr	136
Ni	16	Sr	1272
Cu	11	Zr	9
Zn	184	U	120
Rb	11		
Y	86		

Change in pH during fungal growth

The production of organic acids by *P. simplicissimum* is markedly influenced by pH values. Periodic decrease in pH was observed up to seven days of incubation from 6.5 to 2.52 TABLE 3, where it considered as an indicator of microbial growth[15]. The organic acid production via incomplete oxidation of glucose by *P. simplcissimum* strain was the main responsible for the drop in pH.

The HPLC results of 7 days of incubation indicate that among organic acids produced, citric and oxalic acid are the more predominant (378.5 g/l) and (517 g/l) respectively TABLE 4. After seven days of incubation, pH value of fermented media was increased due to consumed of all glucose during the growth of fungal strain, then started to utilize organic acids produced in order to satisfy their nutritional requirement[16].

TABLE 3 : Effect of incubation time upon the pH of fermented media

Time/days	pH
3	5.43
4	5.13
5	4.64
6	3.45
7	2.52
8	3.76
10	5.54

TABLE 4 : The effect of organic acid produced by *P. simplicissimum* on uranium leaching efficiency

Original organic acids Conc. (%)		Treatment		Consumed Acids (%)		U Leaching Efficiency (%)
Citric	Oxalic	Ore Wt. (g)	Volume (ml)	Citric	Oxalic	
		1	50	1.03	2.87	41
37.85	51.7	1	100	1.44	3.73	58
		1	200	2.48	6.27	83

Bioleaching process of rock phosphate

Role of citric and oxalic acids in uranium bioleaching

Citric acid contains three carboxylic groups (pK_{a2}= 10.82 and pK_{a2}= 4.76 and pK_{a2}= 6.39) and one hydroxyl group (pK_{a2}= 10.82). The possible complex is:

$$C_6H_8O_7 \leftrightarrow C_6H_5O_7^{3-} + 3H^+$$

$$3UO_2^{2+} + 2C_6H_5O_7^{3-} \rightarrow (UO_2)_3(C_6H_5O_7)_2$$

Similarly oxalic acid contains two carboxylic groups (pK_{a2}= 1.20 and pK_{a2}= 4.20) at 25°C, which explains the complex of uranyl ion (UO_2^{2+}) with oxalate ion[16].

$$C_2H_2O_4 \leftrightarrow C_2O_4^{2-} + 2H^+$$

$$UO_2^{2+} + C_2O_4^{2-} \rightarrow UO_2C_2O_4$$

TABLE 4 explains the effect of different concentrations of organic acids (citric and oxalic) in fermented media of *P. simplicissimum* and the leaching efficiency of uranium from the ore sample. From the results, it is found that the percentage of citric and oxalic acids concentration was directly proportional with the percentage of consumed organic acids and uranium leached.

Optimization of bioleaching parameters

Different effective bioleaching parameters such as: sample/liquid (S/L) ratio, stirring time and temperature were investigated.

Effect of solid/liquid ratio (amount of organic acid) on uranium leaching efficiency (S/L ratio)

To study the effect of this factor on the uranium leaching, 1000 ml of Dox liquid medium in 2 liter measuring flask was autoclaved at 1.5 atm. for 20 min. After half an hour of the proper cooling, the flasks were inoculated with 10 ml of *P. simplicissimum* spore suspension then they were incubated at 30 ºC for 7 days. The bioleaching process was tested under variable solid/*P. simplicissimum* metabolite ratios (1/1 to 1/5) at adjusted pH 2.5 in an orbital shaker at 100 rpm for one day.

From the obtained data TABLE 5, the leaching efficiency proportionally increases with the solid/liquid ratio to reach its maximum values (95 %) at S/L ratio 1/3. Over than this ratio, the leaching efficiency starts to decrease. Such behavior could be ascribed to the solubility of other metals with increasing of the organic acids amount which negatively affects the opportunity of the uranium leaching from its bearing sample[9].

TABLE 5 : Effect of S/L ratio upon U leaching efficiency

S/L Ratio	U leaching efficiency (%)
1/1	66
1/2	83
1/3	95
1/4	91
1/5	73

Effect of stirring time upon uranium leaching efficiency

Under the conditions of S/L (1/3), pH 2.5, at room temperature and shaking for one day in an orbital shaker at 100 rpm, the effect of the stirring time was examined regularly from 1 to 7 hours.

Increasing of the stirring time was accompanied by gradual increase in the uranium leaching efficiency which reached its maximum (62.5%) value with the fifth hour TABLE 6. At the fifth hour, the leaching efficiency started to go down. Decreasing of the uranium leachability over 5 hours may be attributed to releasing of some metal ions to the medium.

TABLE 6 : Effect of stirring time upon U leaching efficiency

Time (hours)	U leaching efficiency (%)
1	25
2	37
3	48
4	55
5	62.5
7	51

Effect of temperature upon uranium leaching efficiency

Leaching efficiency was investigated against variable temperatures starting with the room temperature (25 °C) up to 50 °C. Under fixed conditions of the other factors, the temperatures play an important role in uranium leachability TABLE 7.

TABLE 7 : Effect of incubation temperature upon U leaching efficiency

Temp. (°C)	U leaching efficiency (%)
25	62.5
30	64
50	68.3
80	44
100	32.5

The obtained data revealed that the leaching efficiency was improved with the rise of temperature, where it reached its maximum value (86.3%) at 50 °C, above this value the efficiency decreased. The organic acid structure suffers partial thermal decomposition at high temperature. Working at room temperature is economically and more favorable, because the variation between the maximum leaching efficiency of uranium at 50 °C and at room temperature is small.

From the present study, it could be inferred that, uranium in phosphate representative ore sample was highly dissoluted by bioleaching applications with fermented

TABLE 8 : Bioleaching and chemical leaching of uranium from phosphate ore sample

Leach liquor	U leaching efficiency (%)
Bioleach liquor	81%
Synthetic leach liquor	64%

media of *P. simplicissimum* prepared in 5 hours at S/L ratio of 1/3 and at room temperature however the pH value of the produced leach liquor was 2.5.

By applying these optimum conditions upon 100 g of the ore sample added to 300 ml of fermented media of *P. simplicissimum* and 300 ml of synthetic solution (mixture of citric and oxalic acids with concentration 37.8% and 51.7 % respectively) yielded bioleach liquor contains 9.15 mg U and synthetic leach liquor contains 7.68 mg U with leaching efficiencies 81% and 64%, respectively TABLE 8. The leaching efficiency of uranium when applying *A. niger* fungus on phosphate rock sample is 48.1%[17].

Extracellular protein secretion in fermented media of *P. simplicissimum*

Referring to the obtained data in TABLE 9, the protein amount decreased from 1.88 µg/ml in the control to 1.04 µg/ml with increasing uranium leaching. This could reflect the role of proteins in the leaching process where some of them was exhausted in the uranium leaching[18]. Such explanation seem to reasonable with the capability of excreted amino acids to solubilize some metals from the bearing ores e.g. Cu, Zn, Au, U, Ni and antimony[19-22]. The above achieved conclusion explains the higher microbial U leaching efficiency comparing to the chemical one TABLE 9. The difference in the leaching efficiency in both methods is easily attributed to the positive impact by excreted proteins by fungal activity. The excretion of amino acids as additional complexing agents besides citric acid exceeded the solubility of zinc from industrial filter dust by fungus *P. simplicissimum*[10]. The well noticed thing is the quantities of secreted organic acids can be saved as the exploited is only 2.5 and 6.2% from the secreted citric and oxalic acids respectively.

TABLE 9 : The effect of organic acids and extracellular proteins production in uranium leaching efficiency

Original acid Conc. (%)		Protein secretion (µg/ml)	Treatment		Consumed acids (%)		Protein (µg/ml)	U leaching efficiency (%)
			Ore Wt. (g)	Volume (ml)	Citric	Oxalic		
Citric	Oxalic	1.88	1	50	1.03	2.87	1.55	41
37.85	51.7			100	1.44	3.73	1.26	58
				200	2.48	6.27	1.04	83

CONCLUSION

Traditionally, the metals bioleaching using the fungal activity is usually ascribed to the effect of the organic acids, citric and oxalic, which are naturally produced in the fungus metabolism and provide the appropriate low pH medium for the leaching process. However, using of simulated concentrations of synthesized citric and oxalic acids under similar optimum leaching conditions (S/L ratio, stirring time and applied temperature) resulted in lesser uranium leachability comparing to those of natural production where the uranium leaching ratios were 64% and 82% respectively from the original uranium content in El-Sibaiya phosphate rocks.

This phenomena point to presence of another additive agent in the fungus metabolism supports the capability of the metals leaching. The chemical analysis of the produced metabolism using the spectrophotometer technique at the relevant wavelength (750 nm) showed the presence of some proteins which are known by their ability to absorb the metals from their ores. Accordingly, it could be said that the proteins which excreted by *P. simplicissimum* recovered about 18% of the original uranium in the target ore.

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