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Bioleaching of uranium from el-sella ore material using epicoccum nigrum

Azhar M Elsayad[1], *Shimaa S Hussien[2], Mohamed G Mahfouz[2], Abdou A El Mougith[1] and Wesam A Hassanien[1]

[1]Department of Botany, Faculty of Science, Zagazig University, Egypt
[2]Ore Processing Department, Nuclear Materials Authority, Cairo, Egypt
Corresponding author e-mail: mash_1997@yahoo.com (Shimaa S. Hussien)

Abstract. Uranium (U) is one of the strategic elements and essential for many applications as a fuel in nuclear power plants and nuclear weapons. Bioleaching of uranium was carried out with Epicoccum nigrum isolated from El-Sella ore material. El-Sella area is located in the southern eastern desert of Egypt. The maximum bioleaching of U(VI) was found to be 76.6 % at these optimum conditions: 9 days incubation period, 0.5% pulp density, 30 °C incubation temperature at shaking speed 175 rpm. Glucose and NH4Cl were considered the best carbon and nitrogen sources for fungal growth. E. nigrum exhibits good potential in generating varieties of organic acids which effective for bioleaching of uranium. It produced 112.3 and 23.5 µg/ml from gallic and ellagic acids in the culture filtrate under the optimum conditions. This work addresses the area of beneficiation of the used mineral to solubilize U(VI) through the biotechnological route in Egypt, where the bioleaching method is more effective than the chemical one using organic acids.
Keywords: Bioleaching; uranium; organic acids; Epicoccum nigrum**.**

1. Introduction

Bioleaching has been successfully used in commercial metal mining for decades. It uses microbes to biosolubilize metal-containing inorganic compounds such as metal oxides and sulfides [1]. Benefiting from lower operational costs and energy requirements than do hydrometallurgical and pyro-metallurgical processes in metal recovery [2]. The use of microorganism to dissolve metals from ores can be environmentally friendly and a low cost alternative to traditional methods that lead to high cost because of either energy consumption or bioremediation of polluted environment [3]. Several mechanisms may be involved in bioleaching these include, acidolysis, complexolysis, redoxolysis and bioaccumulation. Acidolysis is the main principal mechanism in bioleaching of metals where, the fungus and bacterium produce varieties of organic acids as citric acid, oxalic and gluconic acids during the bioleaching [4]. Fungus has four advantages in the leaching of uranium ore [5]. Firstly, chemo-organo-heterotrophic fungi can use utilize organic carbon source as substrate and can dissolve uranium at high pH. And the secreted organic acids can react with calcium, aluminum, iron, and other elements in gangue to form complexes with higher solubility. Secondly, in addition to acidic ore, fungi can transform uranium oxides, carbonates, and phosphates to form uranium complexes with carboxylic acids. Thirdly, fungi are the heterotrophic microorganisms characterized by rapid growth rate, large biomass and short extraction cycle **[6]**. Fourthly, fungal leaching has low anti-corrosion requirement for equipment, since the organic acids produced by fungi are mainly weak acids, which show less environmental hazards than H_2SO_4 and other strong acids and can be degraded by environmental microorganisms. As an environment-friendly method, fungal leaching is of huge development potential and wide application prospect and has been extensively studied [7]. Hussien *et al*.[4] observed that the highest percentages of bioleached uranium from the tested samples directly by *Penicillium purpurogenium* were found to be 72.49, 55.60 % at a pulp density 300 g/L after 9 days of incubation at 30 ^{o}C. The main objective of this study is the use of *Epicoccum nigrum* for uranium solubilization from El-Sella ore material.

2. Materials and Methods

2.1. *Characterization of the tested sample*

El-Sella area is located in the southern eastern desert of Egypt, between Latitudes 22°14′ 30$^{//}$ and 22° 18′ 36$^{//}$N and Longitudes 36° 11′ 45$^{//}$ and 36° 16′ 30$^{//}$ E, at a distance of about 60 km south west of Abu Ramad according to [8].

2.2. *Microorganisms and growth conditions*

Seven fungal species were isolated from El-Sella ore material using Czapek´s-Dox agar medium by serial dilution method. Aspergillus terrus, A. niger sp1, A. niger sp2, A. sulfurous, Penicillium chrysogenum, P. notatum and Epicocuum nigrum. The fungal strain Epicoccum nigrum was found to be one of the most acid producer fungal isolate through lowering the pH of the growth medium. E. nigrum was sub-culturing using Modified Czapek's- Dox agar (MCDA) for 7-10 days at 30 ^{o}C. It was identified as described by **[9]**. E. nigrum was grown in the absence and the presence of different weights of El-Sella ore material. E. nigrum cultures were incubated at 28 °C for 7 days then the dry weights were determined as mg/mL.

2.3 Microbial leaching techniques

A. Direct bioleaching process: In direct bioleaching, 100 ml of sterilized Modified Czapek's–Dox broth (MCDB) containing 1g of El-Sella ore material inoculated with 1mL (approximately 1×10^{7} spores/mL) fungal spore suspension. Culture was incubated in a rotary shaking incubator at 175 rpm at 28°Cfor 7days [10].

B. Indirect bioleaching process: The production of organic acids can be performed by an indirect bioleaching process. *E. nigrum* was cultivated in the corresponding medium under the previous growth conditions. All cultures were incubated in a rotary shaking incubator at 175 rpm at 28°C for 7 days .After separation of the fungal biomass, 1g of sterilized El-Sella ore material was added to the filtrates (1 g/100 ml) at shaking 150 rpm for 24 h. For each bioleaching the final pH of filtrates and percentages of bioleached uranium in supernatant was determined [11].

2.4 Estimation of uranium (VI) by arsenazo III

The bioleached liquors were filtered through a filter paper (Whatman No. 41) and the concentration of U(VI) in the solution was measured before and after equilibrium by Metertech Ino model Sp-5001, UV- Visible spectrophotometer) using arsenazo III **[**12**]**

2.5 Production and estimation of organic acids by high performance liquid chromatography (HPLC)

The efficiency of the tested strain to produce organic acids was examined by adding 0.5 g/L of $CaCO_3$ to the growth agar media [13].Diameters of the clear zone were determined. The organic acids produced in the presence of El- Sella ore material with the organism were estimated using high-performance liquid chromatography (HPLC).Samples of 10 µL were injected onto C18 column (4.6 mm*250mm in length) at a flow rate of 3.0 mL /min (at room temperature).The mobile phase consisted of acetonitrile and water (80:20) was detected with UV detector at 254 nm for gallic and ellagic acids estimation [14].

2.6 Factors affecting the uranium bioleaching from El-Sella ore material

The optimum conditions for maximum of uranium bioleaching from El-Sella ore material were evaluated. Sample concentration from (0.1-1) g/50 mL, pulp density from (0.1-1.5) %, incubation periods from (5-12) days, incubation temperatures frpm (20-40) ^{o}C, shaking speed from (120-175) rpm and different carbon and nitrogen sources were examined for direct bioleaching of uranium. Erlenmeyer flasks (250 ml), each containing 50 ml of the used MCDB medium in presence of 0.3 g of ore sample .These conical flasks were autoclaved then inoculated with 1 mL of fungal spore suspension.

3 Results

3.1. Ore sample characterization

The chemical composition of El-Sella ore material was shown in table 1. The results indicated that U(VI) concentrate was reached 1173.4 ppm**.**

Table 1. Chemical analysis of El-Sella ore material and the residue of leaching.

Wt. Percent, %	Oxides											
	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	MnO	K_2O	Na_2O	TiO_2	P_2O_5	LOI	Total
Sample	68.76	15.07	5.21	1.03	0.53	0.04	1.25	0.37	1.07	0.57	5.78	99.86
Residue	62.64	12.41	3.07	3.28	2.48	0.03	1.79	0.96	1.89	0.47	1.43	99.45

3.2. Effect of El-Sella ore material on the growth of E. nigrum

This experiment was carried out to study the effect of sample weights on the fungal biomass. At the end of the incubation periods 9 days results were obtained and are shown in figure 1. The dry weights of the tested organism were clearly decreased as the weight of El-Sella ore material was increased as compared to control.

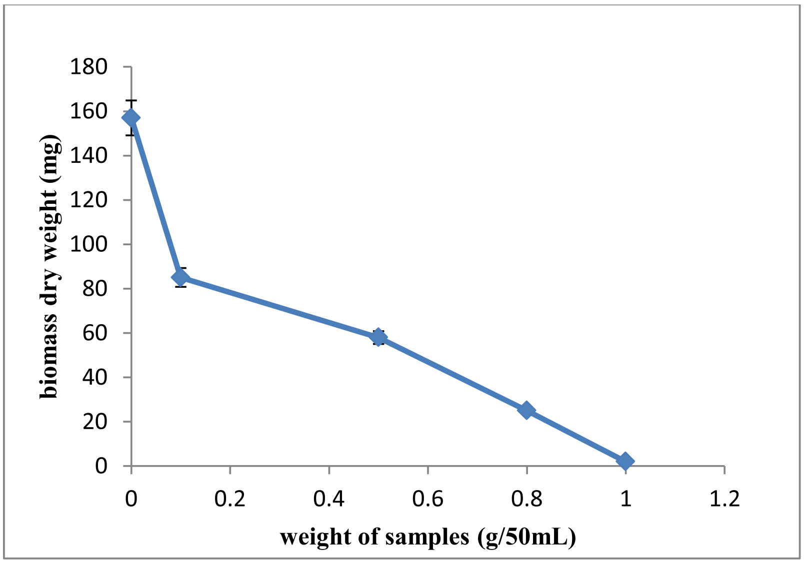

Figure 1. Growth of *E.nigrum* in the presences of different weight of El-Sella ore material.

3.3 Microbial leaching techniques

This experiment was carried out to study the bioleaching technique by direct and indirect method. The result obtained in Table 2, indicated that the highest bioleaching percentage was obtained by direct method. The bioleaching efficiency of uranium was about 41.3 % by direct method.

Table2. Uranium bioleaching percentages by *E. nigrum* using direct and indirect methods.

Fungus isolate	% of bioleached uranium			
	Final pH	Direct method	Final pH	Indirect method
E. nigrum	5.1	41.3±0.14	5.31	30.9±0.91

3.4 Production and estimation of organic acids by high performance liquid chromatography (HPLC)

Table 3, 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 containing calcium carbonate as an indicator for acid production after 9 days incubation period. In the second method, organic acids produced in the fungal and bacterial filtrates were analyzed using HPLC in presence of El-Sella ore material after 9 days. It was found that the main acids produced by *E. nigrum* were gallic acid 112.9 (µg/ml) followed by ellagic acid 23.5 (µg/ml).

Table 3. Acid productions of the microorganisms after 7 days for clear zone, while after 9 days for HPLC.

fungus	Diameter of clear zone (cm)	Organic acid produced (µg/ml)	Final pH filtrate
E. nigrum	7.6	Gallic acid 112.9 Ellagic acid 23.5	4.3

3.5. Factors affecting the uranium bioleaching from El-Sella ore material by E. nigrum.

The effect of different factors as weight of samples (g/50ml), pulp density, incubation periods (days), incubation temperatures (°C), shaking speed (rpm), on bioleaching of uranium from the tested sample were evaluated.

The maximum bioleaching percentages of U(VI) from El-Sella ore material was found to be about 76.6 % at weight of sample (0.5g/50 mL), pulp density (0.3 %), incubation period (9 days), incubation temperature (30°C), shaking speed (175 rpm) as shown in figures (2-6).

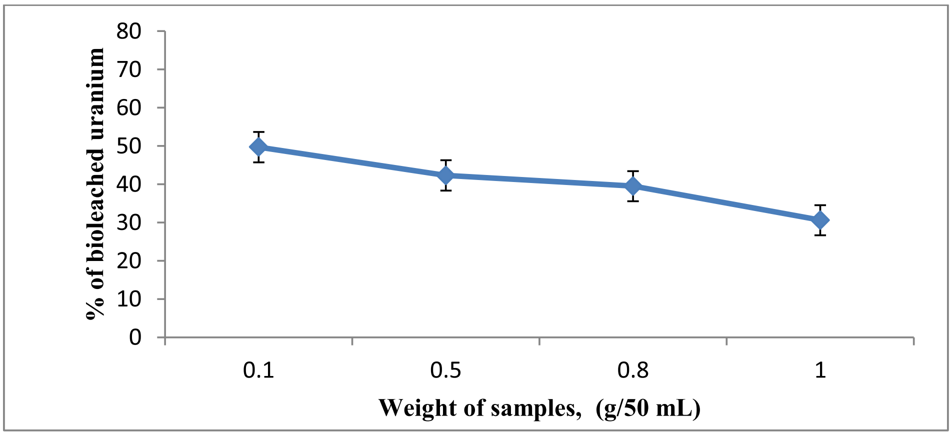

Figure 2. Effect of sample weights (g/50mL) on bioleaching of uranium from El-Sella ore material by *E. nigrum.*

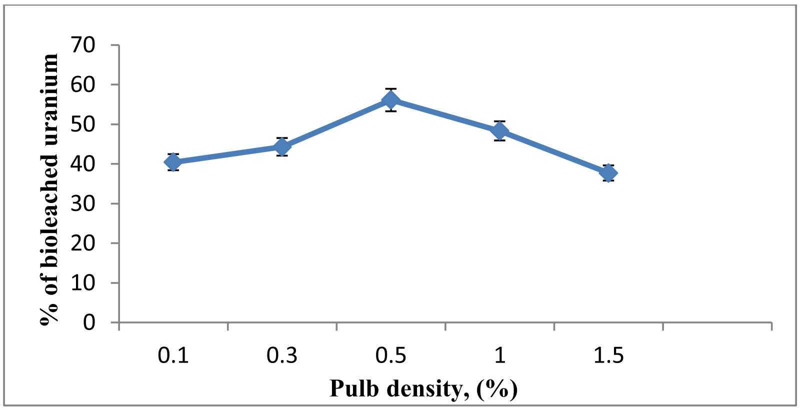

Figure 3. Effect of pulp density on bioleaching of uranium from El-Sella ore material by *E. nigrum.*

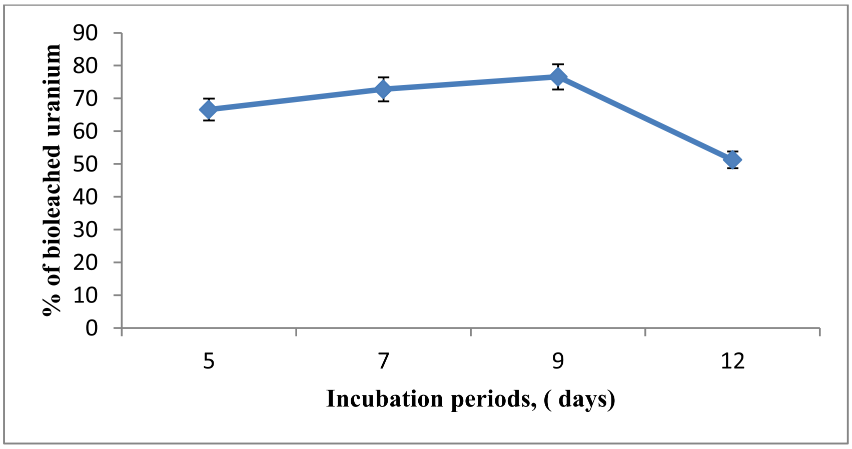

Figure 4. Effect of incubation periods (day) on bioleaching of uranium from El-Sella ore material by *E. nigrum.*

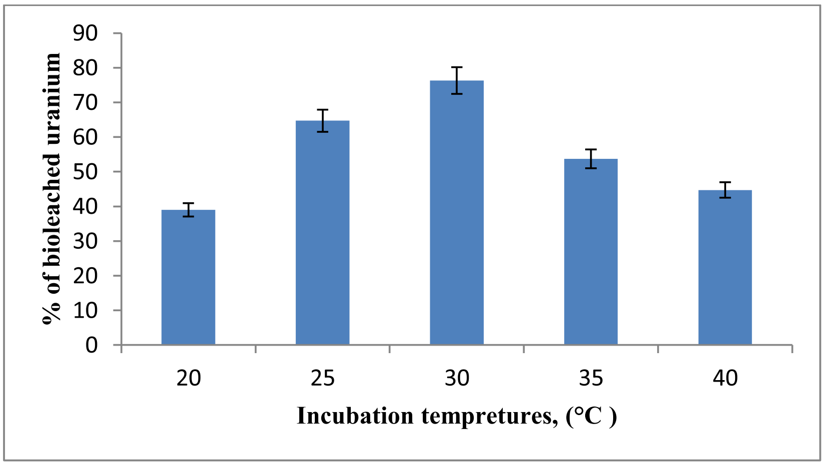

Figure 5. Effect of incubation temperatures (°C) on bioleaching of uranium from El-Sella ore material by *E. nigrum.*

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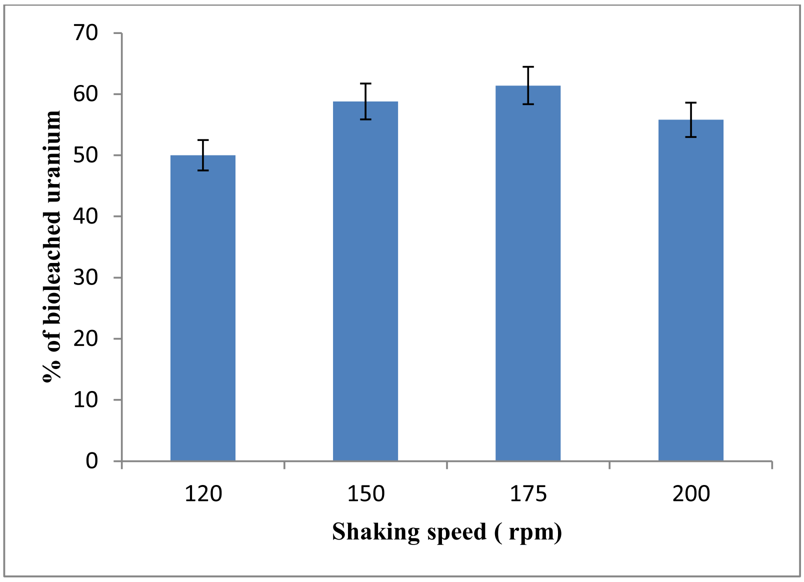

Figure 6. Effect of shaking speed (rpm) on bioleaching of uranium from El-Sella ore material by *E. nigrum.*

Also, glucose and ammonium chloride were found to be the best carbon and nitrogen source, respectively, as appeared in figures (7-8).

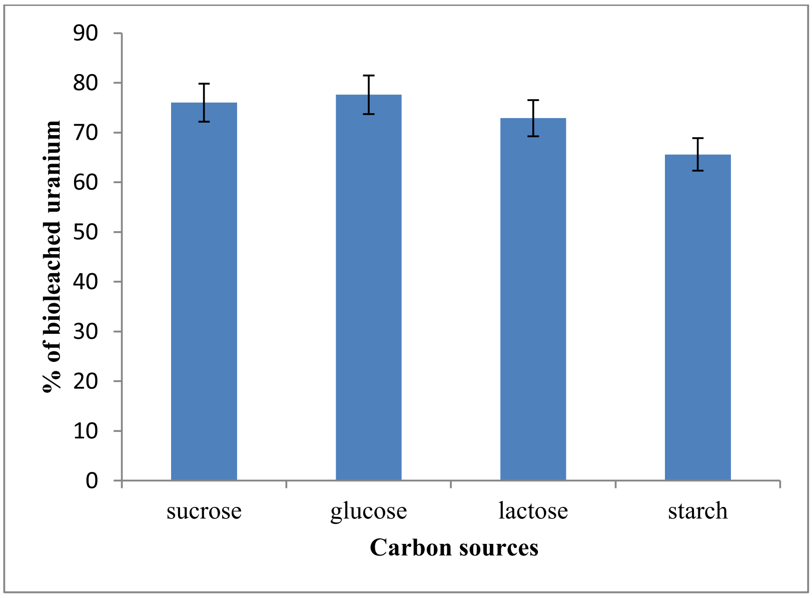

Figure 7. Effect of different carbon sources on bioleaching of uranium from El-Sella ore material by *E. nigrum.*

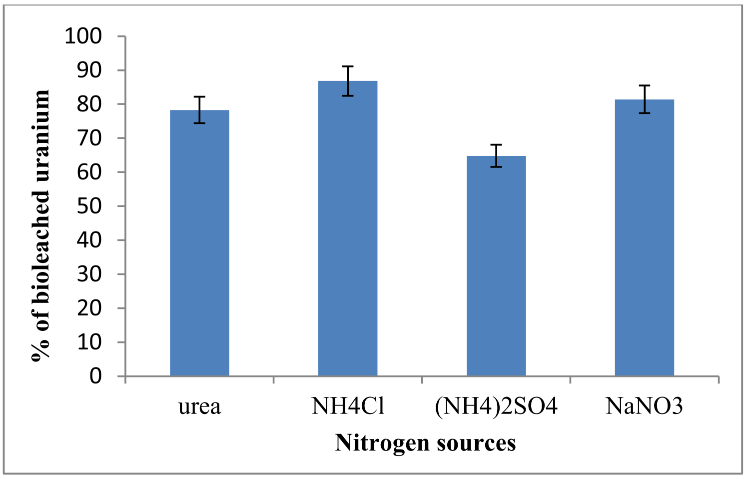

Figure 8. Effect of different nitrogen sources on bioleaching of uranium from El-Sella ore material by *E. nigrum.*

4 Discussion

The sample was taken from El-Sella area in the eastern desert in Egypt for determination of uranium. The fungal isolate was identified according to their features and their frequency of occurrence according to [9,15]. *E. nigrum* is capable of dissolving heavy metals from the tested ore material and surface of the rocks as previously mentioned by [16]. It was observed that there was a gradual decrease in the biomass of *E. nigrum* as the concentration of U(VI) concentrate increased in the growth medium. These obtained results may be due to the toxic effect of heavy metals present in the tested ore material. During the growth studies of bioleaching process by *E. nigrum*, glucose was considered the substrate undergo microbial oxidations which resulted in the production of organic acids as gallic acid and ellagic acid. Organic acids play a fundamental role in the environmental mobility of metal ions. Concerning the growth medium of *E. nigrum,* it was found that glucose as carbon and energy while ammonium chloride as nitrogen were considered the best sources for maximum growth conditions. Hussien [17] evaluates that the maximum bioleaching of uranium from El-Sella mineralisation by *Aspergillus clavatus* was achieved under two-step bioleaching process when a total concentration of 3% (w/v) El-Sella mineralisation was applied. The decrease in pH was observed due to the organic acids production via in complete oxidation by tannase enzyme produces gallic and ellagic acids as in Equations 1 and 4.

$$\underset{\text{(Tannic acid)}}{C_{27}H_{24}O_{18}} \xrightarrow[C_6H_{12}O_6]{\text{Tinnase enzyme}} \underset{\text{Gallic acid}}{3(C_7H_6O_5)} \tag{1}$$

$$\underset{\text{Gallic acid}}{C_7H_6O_5} \xrightarrow[-H]{\text{Dissociation}} \underset{\text{llate}}{C_7H_5O_5^{-1}} \quad {}_{P}Ka = 4.4 \tag{2}$$

$$2C_7H_5O_5^{-1} + UO_2^{+2} \longrightarrow (C_7\,H_5O_5)_2UO_2 \tag{3}$$

$$\underset{\text{(Tannic acid)}}{\overset{\text{Gallate}}{C_{27}H_{24}O_{18}}} \xrightarrow{\text{Tinnase enzyme}} \underset{\text{ellagic acid}}{\overset{\text{Uranyl gallate}}{C_{14}H_6O_8}} \quad (4)$$

$$\underset{\text{Ellagic acid}}{C_{14}H_6O_8} \xrightarrow{\text{lactonization}} \underset{\text{Ellagate}}{C_{14}H_4O_8^{-2}} \quad {}_pKa = 5.54 \quad (5)$$

$$\underset{\text{Ellagate}}{C_{14}H_4O_8^{-2}} + UO_2^{+2} \longrightarrow \underset{\text{Uranyle ellagate}}{C_{14}H_4O_8UO_2} \quad (6)$$

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 [18]. Generally, the bioleaching (direct or indirect) process of U(VI) from the tested sample using *E. nigrum* will be more effective on presence of some organic acids as gallic and illagic acids. These obtained results are in agreement with that reported by [4]. They concluded that bioleaching using organic acids produced by organisms would become more effective using organic acid as a leaching agent, due to higher leaching efficiency and lower capital cost.

5 Conclusions

The present investigation has demonstrated the effect of different weight sample on growth, acids production and leaching behaviour of *E. nigrum*. Tested organism was produced organic acids result in high bioleaching efficiency of U(VI) from tested samples. In direct process, the highest U(VI) dissolution was76.6 % from El-Sella ore material by *E. nigrum*. The bioleaching process showed better U(VI)bioleaching and reduced cost. These results indicate the challenges but also the promising aspects of biological leaching as alternative process for recovering U(VI) from El-Sella ore material.

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