



Microbial Leaching of Uranium from Low Grade Ore and Waste Sample of Northern Part of Gabal Gatter, Egypt Using *Penicillium Purpurogenium* and *Pseudomonas Fluorescens* SHA 281

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Abstract

Uranium (U) is one of the strategic elements and essential for many applications as a fuel in nuclear power plants and nuclear weapons. Microbiological leaching has been used as an alternative approach to conventional hydrometallurgical methods of uranium's recovery from low grade ores and waste samples. *Penicillium purpurogenium* and *Pseudomonas fluorescens* SHA 281 were exhibited a good potential in generating varieties of organic acids effective for bioleaching uranium. Efficiency of bioleaching was studied by varying parameters like pulp density and incubation period. Indeed, it was 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 °C and 57.47, 60.06 % by *P. fluorescens* SHA 281 after 8 days of incubation at 35 °C using shaking incubator at 175 rpm from (T-2)80 and waste sample (W1), respectively.

Keywords: Bioleaching; Uranium; Organic Acids; *Penicillium Purpurogenium* and *Pseudomonas Fluorescens* SHA 281.

1. Introduction

Bioleaching has emerged as an important branch of biotechnology in recent years. Microbial technology helps in case of recovery of ores which cannot be economically processed with chemical methods, because they contain low grade elements. Therefore, large quantity of low grade ores are produced during the separation of high grade ores. The metal-solubilization process is due to a combination of chemistry and microbiology: chemistry, because the solubilization of the metal is considered to be mainly a result of the action of ferric iron and/or acid on the mineral, and microbiology, because microorganisms are responsible for producing the ferric iron and acid. Microbially metal-extraction processes are usually more economical and ecofriendly than physicochemical processes [1]. It does not use large amounts of energy and don't produce hazardous chemicals as sulphur dioxide and another harmful gas [2]. One possible solution is to develop other leaching processes, such as bioleaching. 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 [3]. The ability of a variety of microorganisms to mobilize and leach metals from solid materials is based on three principles, namely (i) the transformation of organic or inorganic acids (protons); (ii) oxidation and reduction reactions and (iii) the excretion of complexing agents. Metals can be leached either directly (i.e. physical contact between microorganisms and solid material) or indirectly (e.g. bacterial oxidation of Fe^{2+} to Fe^{3+} which catalyses metal solubilization as an electron carrier) [4].

[5] Mentioned that in situ fungal leaching of black shale with different fungal strains in the presence of molasses as growth substrate resulted in highest leaching yield of uranium by *Phoma tropica* (57.73%) compared to *Penicillium chrysogenum* (32.30%), *Penicillium citrinum* (25.59%) and *Aspergillus niger* (24.23%). [6] Stated that the extraction of uranium from low-grade ore was collected from Radoniow's 'small' dump, Poland using the bioleaching of uranium achieved a maximum extraction of 75 ± 15 % w/w after 55 days by *Acidithiobacillus* bacteria.

Compared with *P. fluorescens* SHA 281 and *P. purpurogenium* this fungus has four advantages in the leaching of uranium ore [7]. Firstly, chemoorganoheterotrophic 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 [8]. 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 [9].

The main objective of this study is the use of *P. purpurogenium* and *P. fluorescens* SHA 281 for uranium solubilization from low grade ores and waste samples.

2. Materials and Methods

2.1 Characterization of Tested Samples

Seven samples are chosen, two from high radioactive channel samples and the other from moderate and lower radioactive samples. These samples are due to the mineralization of the Hammamat sedimentary rocks along the fault contact of G-V, uranium occurrence in the northern part of G-V. These rocks are blackish green on the fresh surface, which the altered and mineralized rocks are whitish green due to bleaching by hydrothermal solution. Sometimes are hematitized showing red color.

The rocks with these alterations are strongly radioactive with secondary uranium mineralization. The unaltered rocks are lowering radioactive. The alteration of the Hm. Sed. Roc. Along the fault contact between them and the younger granite of Gabal Gattar are due to intrusion of mineralized hydrothermal solutions, that carry uranium, and leach uranium from the younger granite and precipitated there leads of uranium within Hm. Sed. Roc. Thus, it was necessary to use the microbial bioleaching methods, for extracting uranium side by side with the chemical leaching method that working now in G. Gattar.

2.2. Isolation of Microorganisms from the Waste Samples

Modified Czapek's- Dox agar (MCDA) was used for isolation of the tested fungi. The medium composition is as follows (g/L): sucrose 30; $NaNO_3$ 3; KH_2PO_4 1; $MgSO_4 \cdot 7H_2O$ 0.5; KCl 0.5; $FeSO_4$ 0.01; yeast extract 10 and agar-agar [10]. While *P. fluorescens* SHA 281 was obtained from previous work [11]. *P. fluorescens* SHA 281 was inoculated into sterile nutrient agar medium (NA) [12]. Nutrient agar (NA) medium composed of (g/L): beef extract 3; bacteriological peptone 5; yeast extract 1; sodium chloride 5 and agar-agar 20 The Fungal cultures were incubated at 28 °C for 7 days and *P. fluorescens* SHA 281 cultures were incubated at 35°C for 3 days then the dry weights were determined as mg/50mL [4].

2.3. Microbial Bioleaching of Uranium from Tested Samples

Microbial leaching test was carried out in two Erlenmeyer flasks with 500 mL modified Czapeck-Dox and nutrient acid broth media, separately. The flasks were sterilized by autoclave at 121 °C for 20 min. five mL (approximately 1×10^7 spore's mL^{-1}) of each fungal-spores suspension (3-5 days age) or 1mL (approximately 1×10^7 spores/mL) while bacterial suspension (1-3 days age) or 1mL (approximately 5×10^8 cells/mL) was added to each flask separately. Fungal flasks were incubated at 30 °C for 5 and 10 days [13] while, bacterial flasks were incubated at 35 °C for 4 and 8 days [14]. All flasks were shaken using rotary shaker at 175 rpm in order to keep everything in a homogenous slurry form. After 5 and 4 days of incubation, the solution was containing organic acids produced by fungi and bacteria respectively. 100 g/L from the tested samples was added to the previous solution which contains organic acids and incubated for 8 days. At regular time intervals, the culture from each flask were filtered and the filtrate was analyzed for uranium concentrations. Control experiment was also carried out.

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 [15].

2.5. Estimation of Organic Acids by High Performance Liquid Chromatography (HPLC)

The organic acids were estimated using high-performance liquid chromatography (HPLC) with a Pack column (6.0mm×150mm in length) at a flow rate of 0.8mL/min (room temperature). The mobile phase of 0.01N H₂SO₄ was detected with UV detector at 210 nm for citric and oxalic acid estimation [16]. While, 0.1% ortho-phosphoric acid used as a mobile phase for gluconic acid [17] under the same conditions. The organic acids were identified by comparing the retention times and quantified on the basis of beaks areas of standards.

2.6. Effect of Important Factors on Microbial Leaching of Uranium

To determine the optimum incubation period, shaking speed and incubation temperature and sample concentrations for direct bioleaching of uranium. Erlenmeyer conical flasks (250 mL), each containing 50 mL of MCD medium (for fungal growth) and 50 mL NB medium (for bacterial growth) in presence of 5 g of tested samples were used. These conical flasks were autoclaved then, inoculated with 1mL of fungal spore suspension or 1mL of bacterial suspension.

2.7. Recovery of Uranium from the Bio-Leached Liquor of Gattar Waste Sample

These experiments were carried out to precipitate uranium that complexed with organic acid as sodium diuranate. The bioleached liquor after optimum conditions was centrifuged at 4100 rpm. Then the filtrate contained uranium precipitated as sodium diuranate as described by [18]. Finally, the obtained sodium diuranate characterized by scanning electron microscope (EDEX) and X-ray diffraction (XRD).

3. Results

3.1 Characterization of the Tested Waste Samples

Seven samples were taken from the northern part of Gabal Gattar (G-V) for determination the uranium content as appeared in (Table 1).

Table1. Uranium content of the tested samples

Samples	(T-2)16	(T-2)80	(T-2)82	(T-7)97	(T-7)80	(T-7)81	(T-7)83
Uranium (ppm)	698	350	680	404.2	577.8	696.3	676

The chemical composition of low grade ore (T-2)80 and waste sample mainly presented in oxides as shown in (Table 2). The results indicated that, we chose low grade ore sample (T-2)80 which contains of 350 ppm U(VI) while waste sample contains of 202 ppm U(VI).

Table2. Chemical analysis of the tested sample

Elements (%)	Samples	
	(T-2)80	Waste sample
SiO ₂	51.90	49.40
Al ₂ O ₃	16.30	15.50
CaO	8.50	7.90
MgO	6.86	6.10
Fe ₂ O ₃	8.80	8.20
TiO ₂	1.50	1.30
Na ₂ O	1.20	2.10
K ₂ O	0.97	1.50
MnO ₂	0.70	0.95
P ₂ O ₅	0.96	1.72
U ₃ O ₈	350 ppm	202 ppm
ThO ₂	21 ppm	20 ppm
LOI (Loss of ignition)	2.20	5.10
Total	99.71	99.80

3.2. Identification and Characterization of Highest Acid Producer Microorganisms

Five isolates of fungi were obtained from the (T-2)80 and waste sample besides one identified bacterial strain. The fungal isolates were identified as *Helminthosporium solani*, *Penicillium purpurogenium*, *Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus flavus*. While, the used bacteria identified as *Pseudomonas fluorescens* SHA 2. The morphological characters and microscopically examination were appeared in (Figure1).

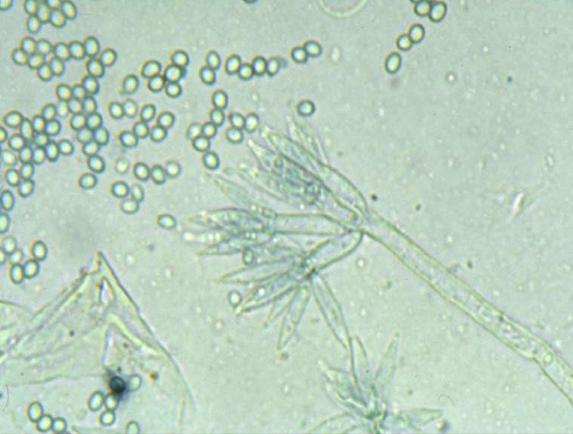
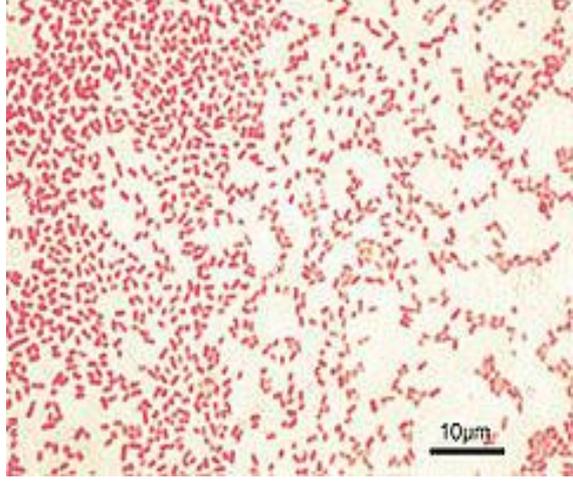
	
<p>Colony morphology</p> <p>White colonies mycelium turns to dark green when old. Powdery dark green. Compact colony. Secret a red pigment.</p>	<p>Microscopic Examination</p> <p>Branched conidiophores, Branched metulae, giving a brush-like appearance. Conidia were green with thicked wall.</p>
	
<p>Colony morphology</p> <p>The growth of the bacteria characterized by secreting of green fluorescent pigment on nutrient agar medium.</p>	<p>Microscopic Examination</p> <p>It is gram negative, aerobic, a rod shaped bacterium with unipolar motility.</p>

Fig. (1): Morphological characters and microscopically examination of *P. purpurogenium* and *P. fluorescens* SHA 281.

For studying the efficiency of fungal isolates and tested bacterial strain to produce organic acids 0.5 g/L of CaCO_3 was added to MCD and NA agar media as acid production indicator. It was found that the most organic acid fungal producer was *Penicillium purpurogenium*.

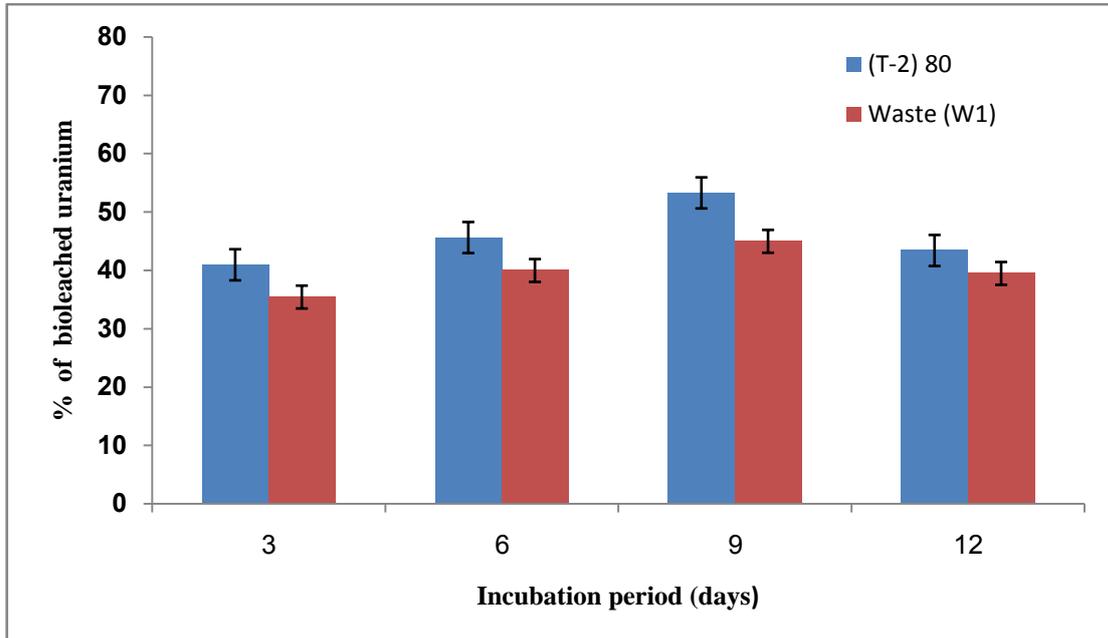
3.3. Production of Organic Acids by *P. Purpurogenium* and *P. Fluorescens* SHA 281

The produced organic acids in the fungal and bacterial filtrates were analyzed using HPLC after 6 and 5 days for *P. purpurogenium* and *P. fluorescens* SHA 281, respectively. The main acids produced by *P. purpurogenium* were citric acid followed by oxalic acid. The highest concentrations of citric and oxalic acids were 16.5 g/L and 3.4 g/L, respectively while, the main acid produced by *P. fluorescens* SHA 281 was gluconic acid (8.4 g/L).

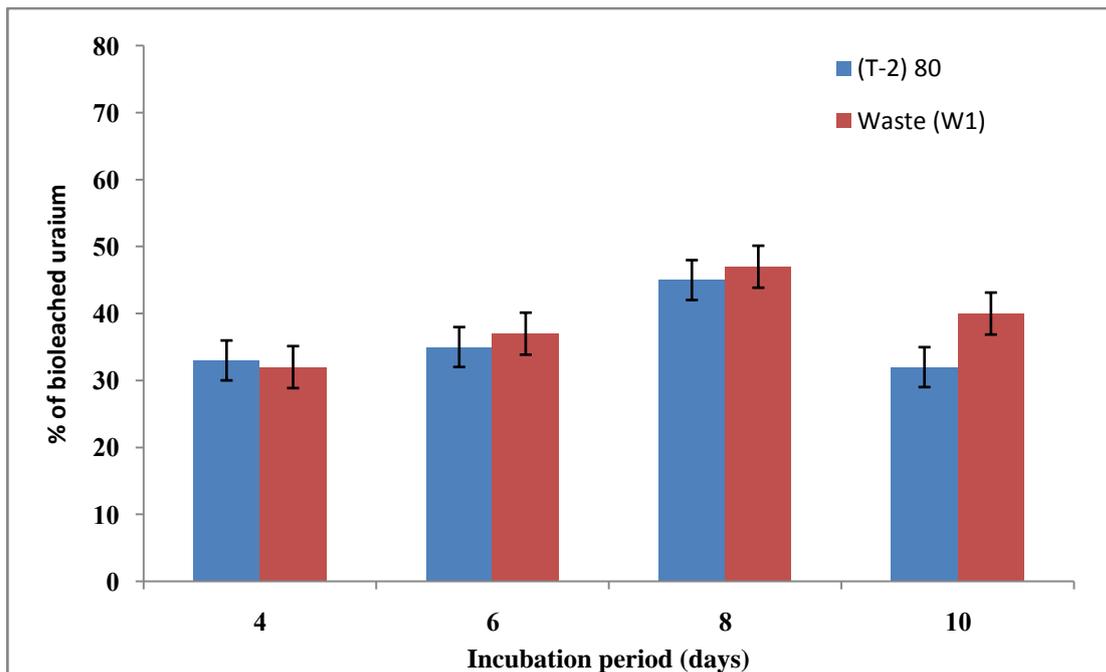
3.4. Effect of Important Factors on Microbial Leaching of Uranium

The effect of different incubation period (days), shaking speed (rpm), weight of samples (gram) and incubation temperature ($^{\circ}\text{C}$) on bioleaching of uranium from tested samples was studied. As illustrated in (Figure 2). The highest

efficiency of bioleached uranium was obtained by *P. purpurogenium* and *P. fluorescens* SHA 281 (53.30, 45.00 %) and (45.00, 47.00 %) after 9 and 8 days from (T-2)80 and waste sample (W1), respectively.



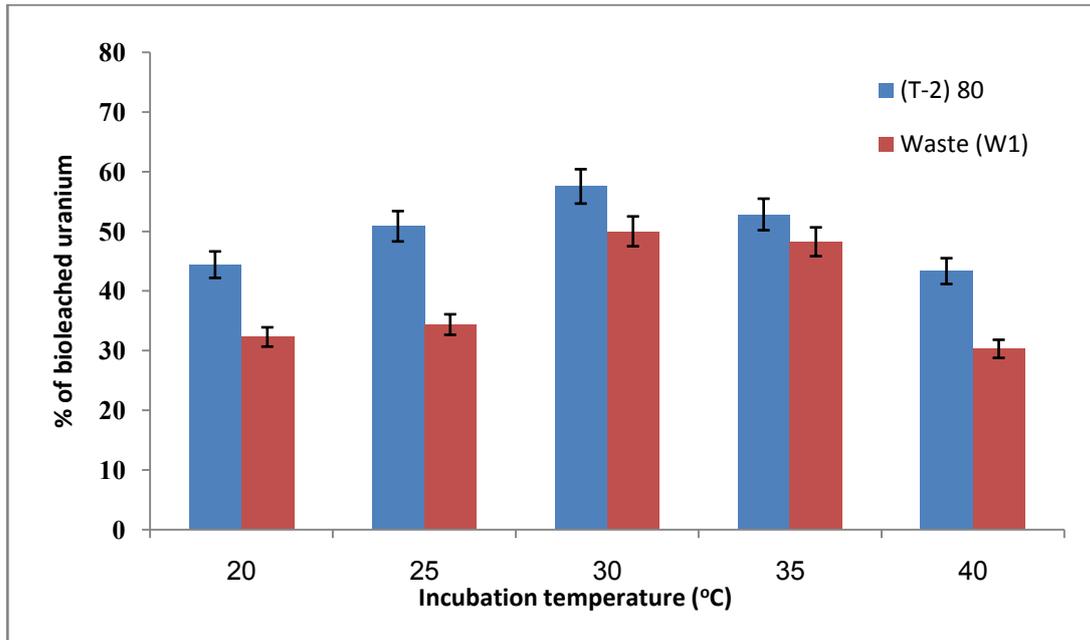
(A)



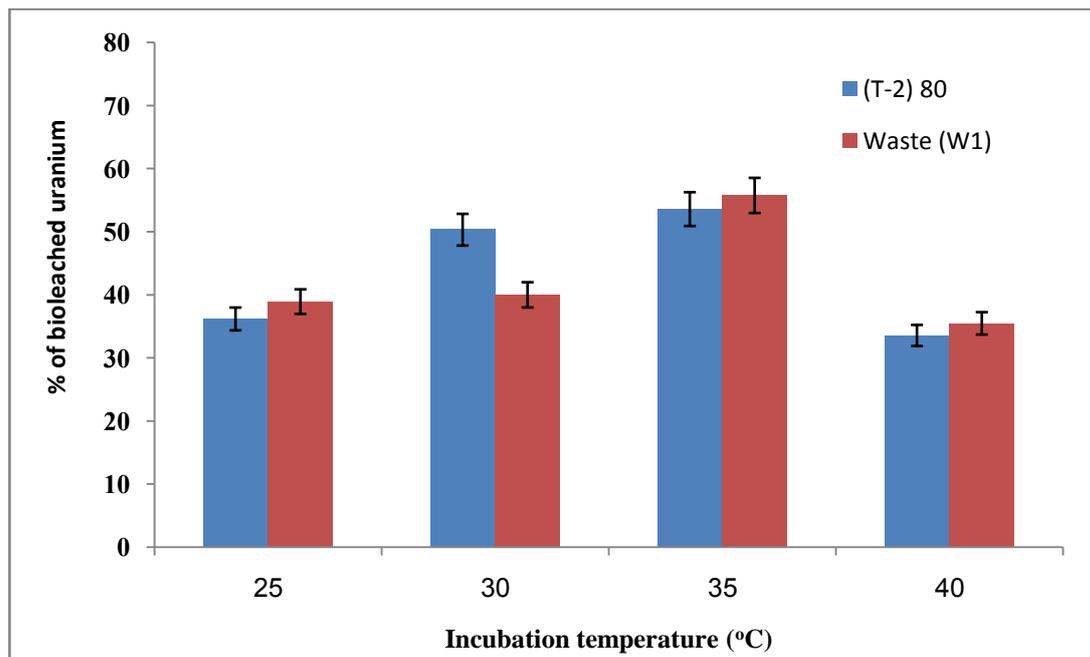
(B)

Fig (2): Effect of incubation periods (day) on bioleaching of uranium from the tested samples by *P. purpurogenium* (A) and *P. fluorescens* SHA 281 (B).

Besides, 30 and 35 °C were considered the most optimum temperature for maximum uranium bioleaching from the tested samples were *P. purpurogenium* (57.53, 50 %) and *P. fluorescens* SHA 281 (53.60, 55.76 %) from (T-2)80 and waste sample (W1), respectively. as appeared in (Figure 3).



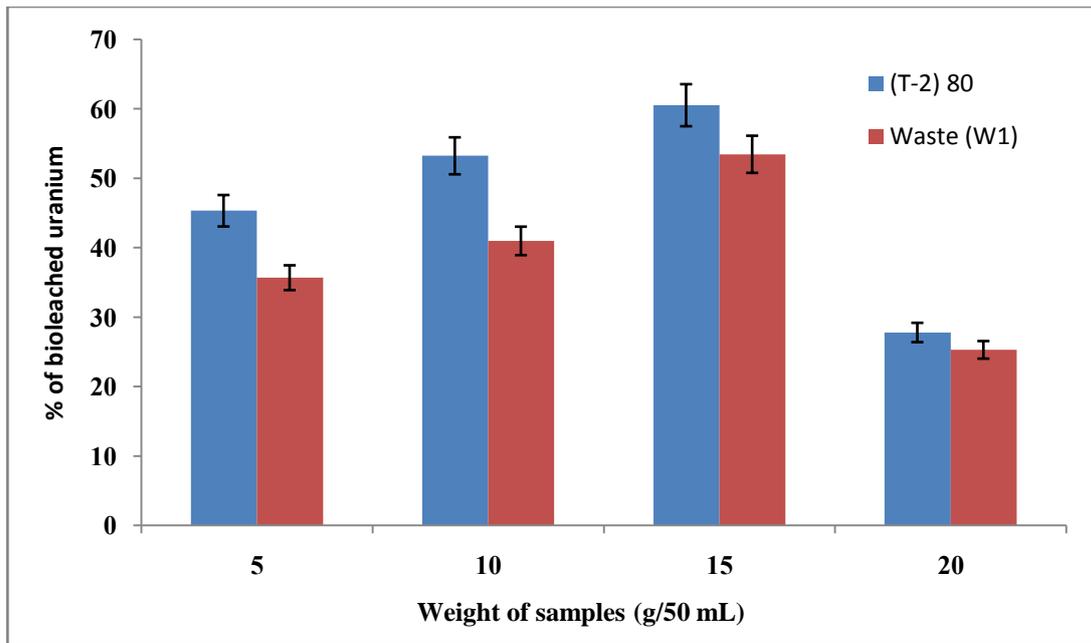
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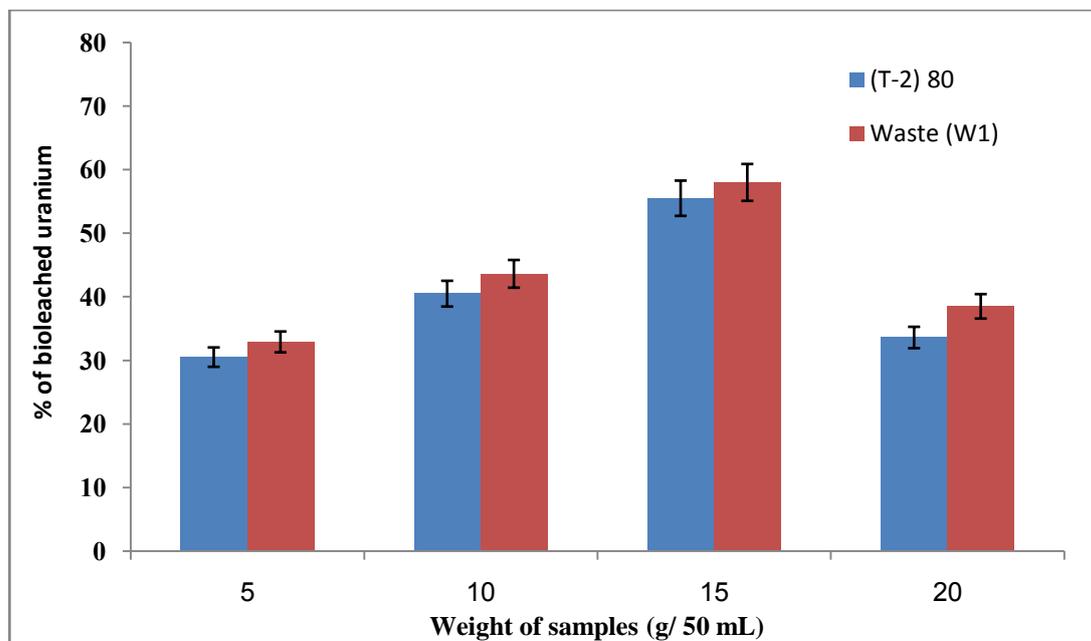
(B)

Fig (3): Effect of incubation temperature (°C) on bioleaching of uranium from the tested samples by *P. purpurogenium* (A) and *P. fluorescens* SHA 281 (B).

As shown in (Figure 4) the most suitable weight of samples was (15g/50mL) for highest efficiency of bioleached uranium where *P. purpurogenium* (60, 53, 53.64 %) and *P. fluorescens* SHA 281 (55.49, 57.98 %) from (T-2)80 and waste sample (W1), respectively.



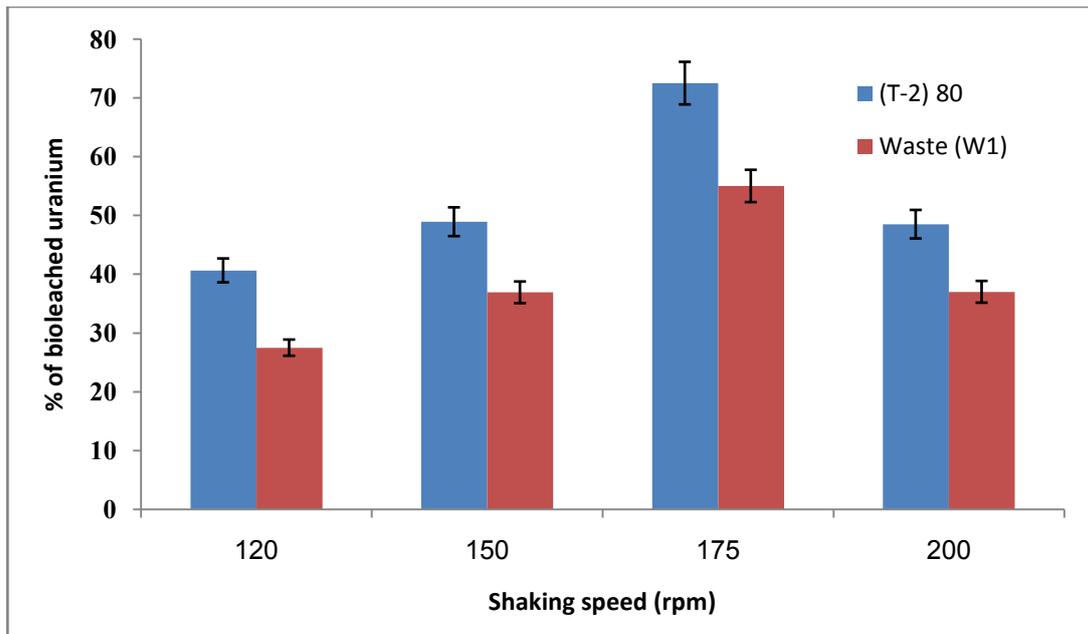
(A)



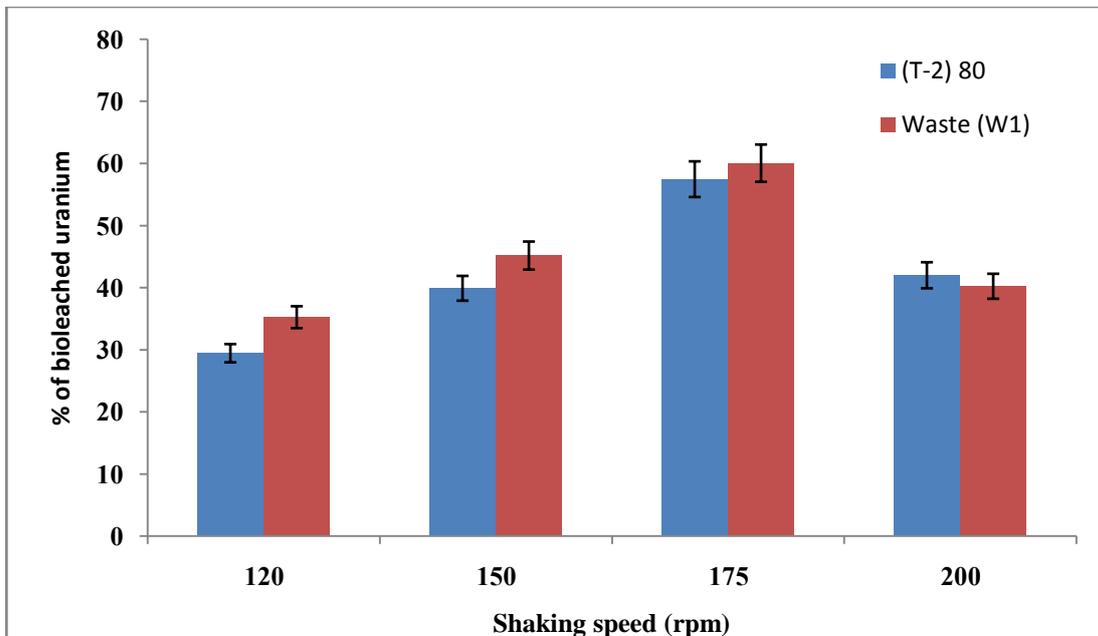
(B)

Fig (4): Effect of weight of sample (g) on bioleaching of uranium from the tested samples by *P. purpurogenium* (A) and *P. fluorescens* SHA 281 (B).

Also, the optimum shaking speed 175 rpm for *P. purpurogenium* (72.49, 55.60 %) and *P. fluorescens* SHA 281 (57.47, 60.06%) from (T-2)80 and waste sample (W1), respectively as appeared in (Figure 5).



(A)



(B)

Fig (5): Effect of shaking speed (rpm) on bioleaching of uranium by *P. purpurogenium* (A) and *P. fluorescens* SHA 281 (B) from the tested samples.

3.5. Application for Recovery of Uranium from Tested Samples, the Northern Part of Gabal Gattar, Egypt

The bio-leach liquor ($\frac{1}{2}$ k/L) of the working Gattar samples (T-2) 80 and waste (W1) with highest uranium leaching after filtration has (pH=2.1 and 2.3) were studied for precipitating uranium as sodium diuranate using 20 % sodium hydroxide. Firstly, at pH=3-3.3 we noticed appearance the granules of iron hydroxide which removed by filtration. Then pH was raised to 5.5-6.5 at which uranium precipitated as (175 & 90 mg) sodium diuranate ($\text{Na}_4 \text{U}_2 \text{O}_7$) from the tested sample (T-2)80 and waste (W1), respectively which subjected to XRD and EDEX analyses. The recovery efficiency for (T-2) 80 sample was 66.01% while waste sample (W1) was 57.11 %. Finally, at pH=8-8.3 using 10 oxalic acid, rare earth elements were precipitated as REEs oxalate. These previous results were proposed to be applicable in semi-pilot plant that show the promising aspects of microbial leaching as alternative process for recovering of uranium low grade ores.

The precipitated crystals of sodium diuranate were calcinated at 550 °C to obtain its oxides which subjected to X-ray diffraction analysis to ascertain the chemical nature of it as illustrated in (Figure 6). It appears distinct peaks which indicating the deposition of crystallized U^{6+} . Diffractogram of the calcinated sodium diuranate displayed maximum number of peaks to U^{6+} between 2θ of 8.48° , 2θ of 11.64° , 2θ of 21.04° , 2θ of 31.84° and 2θ of 45.38° . Significantly the D values of most the peaks corresponding to sodium diuranate. This obtained result indicates that U^{+6} in this precipitate is assay up to about 85 %.

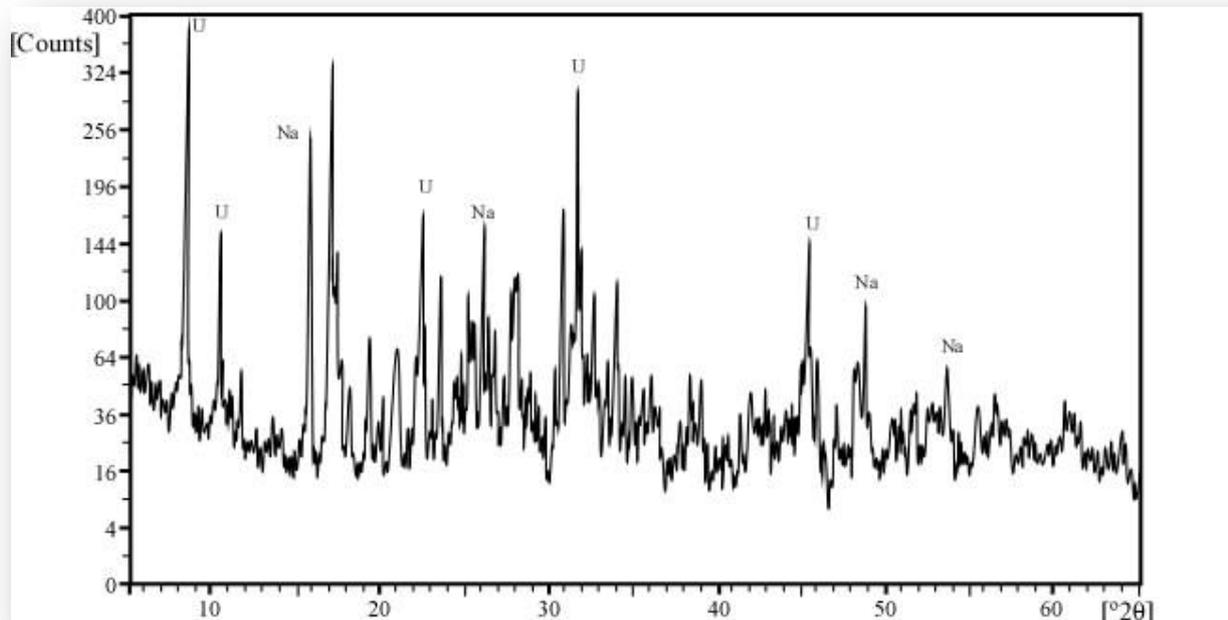
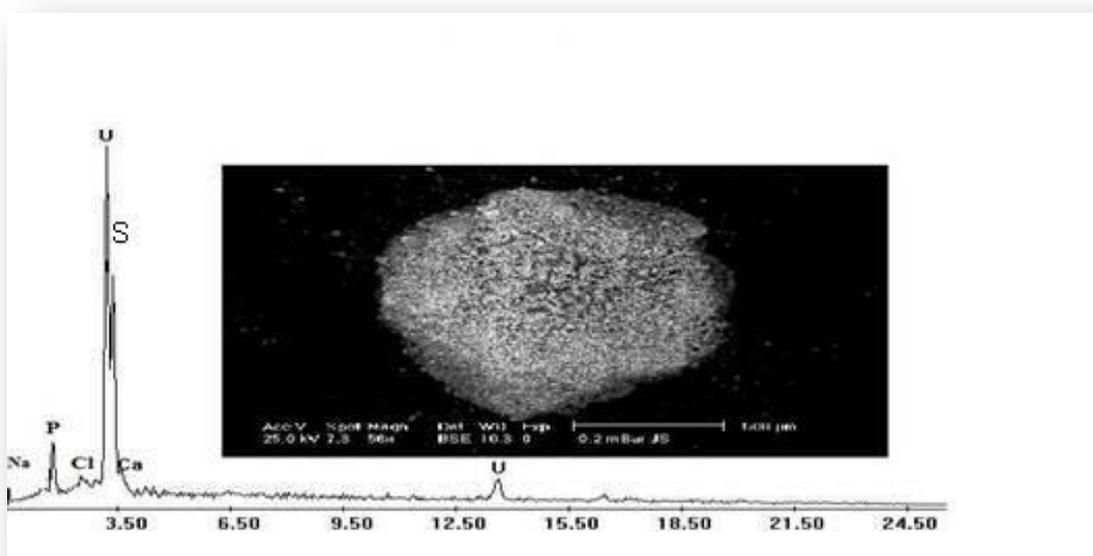


Fig. (6): XRD diffraction data of the product ($Na_4U_2O_7$) ASTM card No. 18-1234.

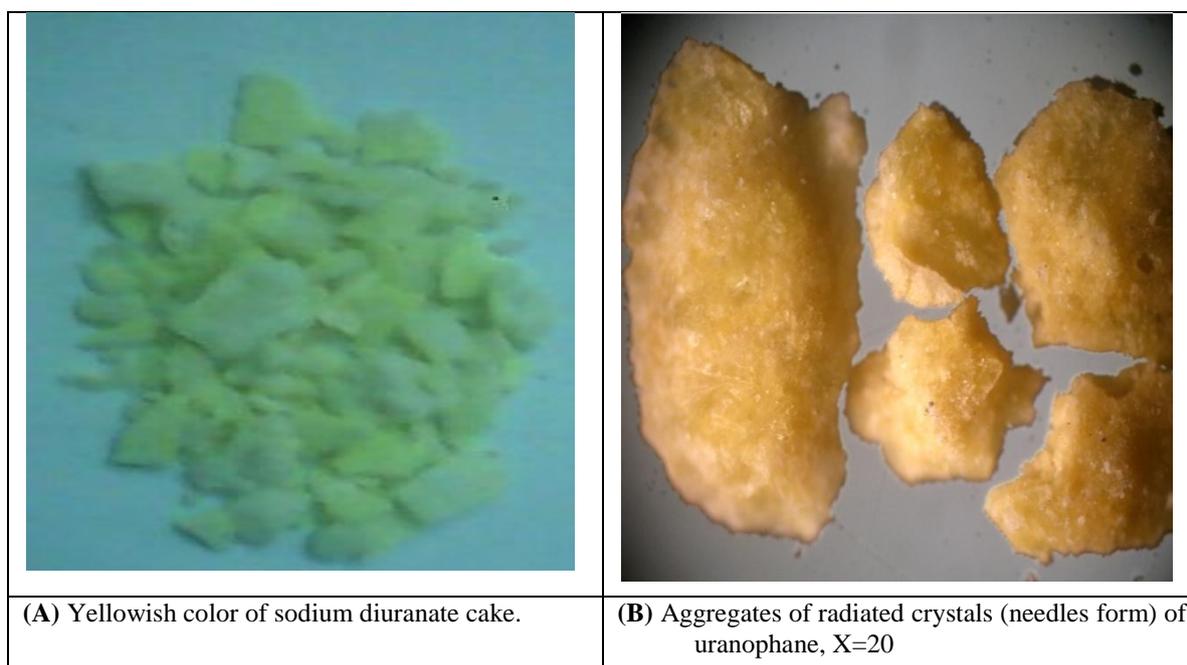
(Figure 7) illustrates SEM micrograph and the corresponding EDX spectrum of calcinated sodium diuranate where, the percentage of U_3O_8 was 64.50%.



Elements	Wt %	Elements	Wt %	Elements	Wt %	Elements	Wt%
Na ₂ O	8.85	P ₂ O ₅	5.35	Cl ₂ O	4.97	U ₃ O ₈	64.50
CaO	6.10	SO ₂	10.23	Total	100.00		

Fig. (7): SEM micrograph and the corresponding EDX spectrum of calcinated sodium diuranate.

Also, (Figure 8B) illustrates stereo-photograph for sodium diuranate which may be; Aggregates of radiated crystals (needles form) of uranophane.



+Fig.(8): Stereo-photographs sodium diuranate cake (A) Yellowish color of sodium diuranate cake and (B) Aggregates of radiated crystals (needles form) of uranophane.

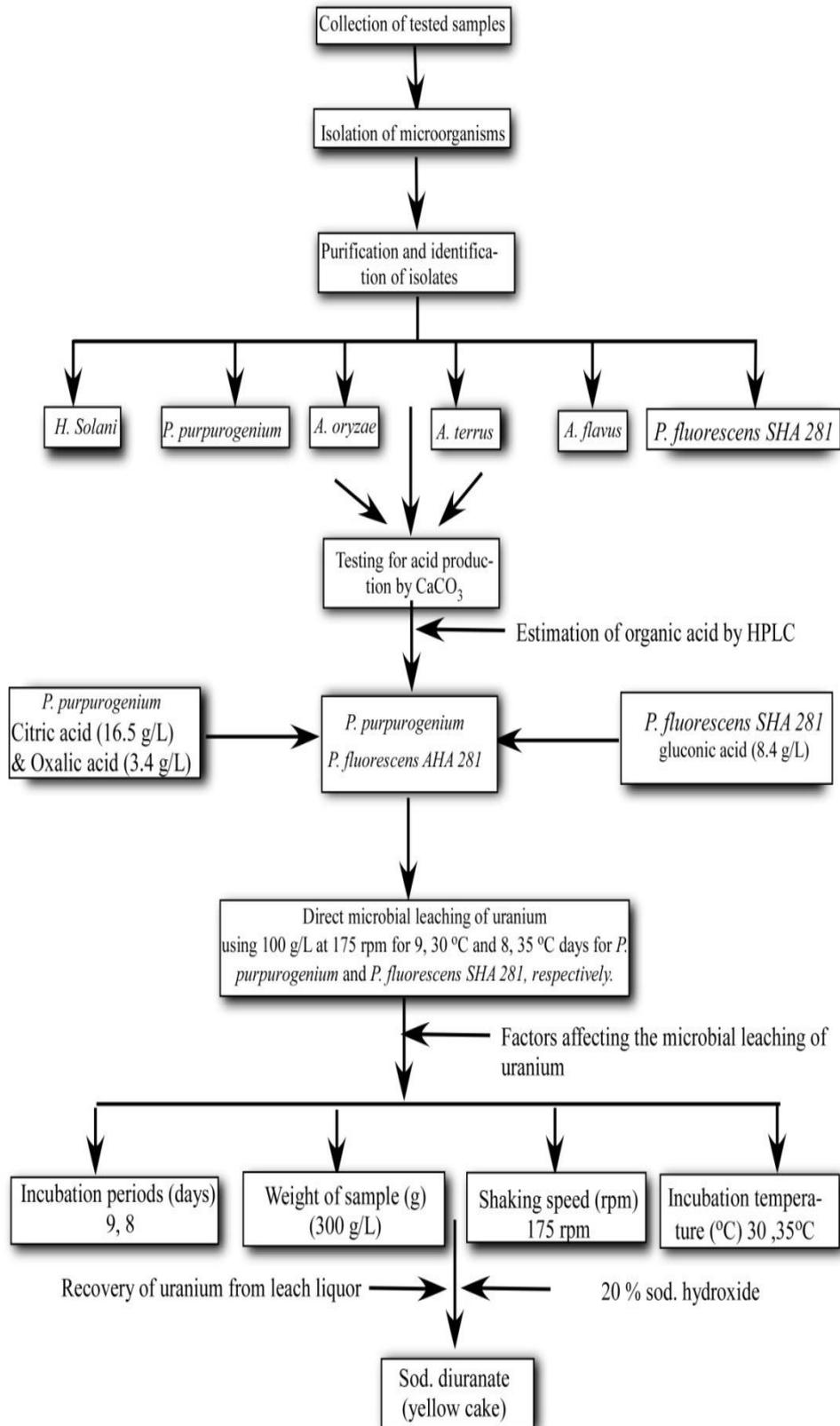
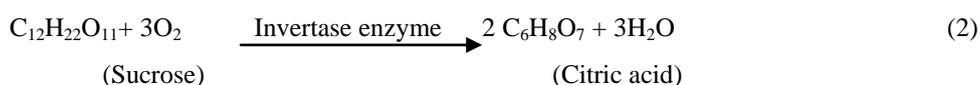
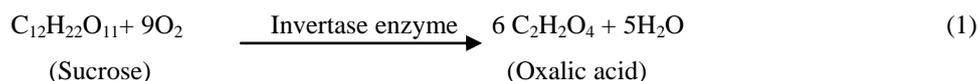


Fig. (9): Flow chart showing all the experiments to obtain the yellow cake product.

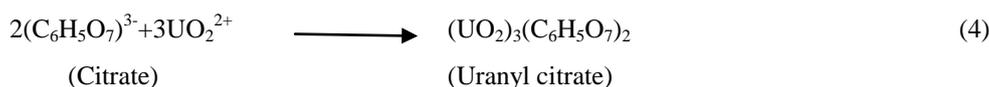
4. Discussion

All samples were taken from the northern part of Gabal Gattar (G-V) for determination of uranium. The chemical analysis of tested samples, the results were similar to those obtained by [19]. The fungal isolates were identified according to their features and their frequency of occurrence according to [20]. While, Bacteria identified as *Pseudomonas fluorescens* SHA 281 according to [11]. It was observed that *Penicillium purpurogenium* and *Pseudomonas fluorescens* SHA 281 have the ability to produce organic acids according to [21].

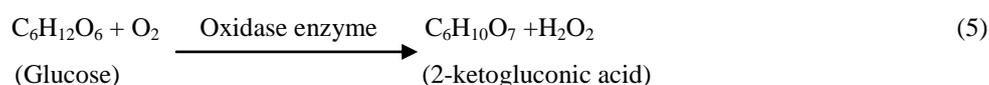
During the growth studies of *Penicillium purpurogenium* and *Pseudomonas fluorescens* SHA 281, the substrates undergo microbial oxidation which resulted in the production of organic acids, citric, oxalic, tartaric and gluconic acids, that play a fundamental role in the environmental mobility of metal ions. Concerning *P. purpurogenium* MCDB contained sucrose as a carbon source and energy source. The decrease in pH was observed due to the organic acid production via incomplete oxidation by invertase enzyme to citric and oxalic acids [22] as Eq. 1 and 2:



Citric acid is a tricarboxylic acid which contains three carboxylic moieties and one hydroxyl group (pKa = 6.39) as possible donor of proton (H⁺) at 30 °C.



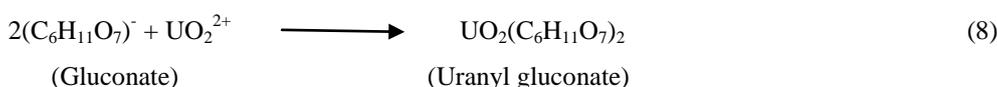
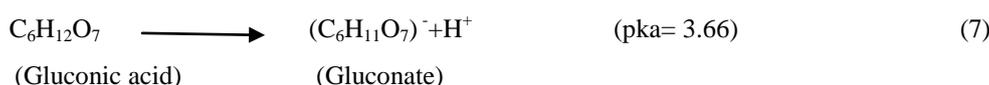
In doing so, *P. fluorescens* SHA 281 NB medium contained glucose as a carbon source and energy source. Glucose oxidase enzyme was used for oxidation of glucose this produced gluconic acid and H₂O₂ (Eq. 5) [23] as follows:



The H₂O₂ released during this process reacted with Fe(II) and oxidized Fe(II) to Fe(III) according to Eq. (6) [24]:



Similarly, gluconic acid contains one carboxylic moiety (pKa = 3.66) at 30 °C. So, the possible complex of uranium with gluconate anion is:



The recommended optimum environmental and nutritional conditions for maximum bioleaching of some REEs by tested *Penicillium purpurogenium* were in agreement with that obtained by [25] they stated that the bioleaching of tungsten from spent hydro cracking using *P. simplicissimum* when it incubated in sucrose medium for 14 days with (5% w/v) of spent hydrocracking using an orbital shaking incubator (120 rpm) at 30 °C for 14 days. While for *Pseudomonas fluorescens* SHA 281 the recommended conditions were in agreement with that described by [26] where they used *Leptospirillum ferrooxidans* as a bioleaching organism for copper from chalcopyrite when 100 mL of basal medium containing 2g of chalcopyrite concentrate incubated with the tested ore at 30 °C for 30 days using shaking incubator (100 rpm).

Moreover, X-ray diffraction analysis illustrated that diffractogram of the calcinated sodium diuranate displayed maximum number of peaks to U₆₊ between 2θ of 8.48°, 2θ of 11.64°, 2θ of 21.04°, 2θ of 31.84° and 2θ of 45.38°. This obtained result indicates that U⁺⁶ in this precipitate is assay up to about 85 % and similar to obtained by [19].

The EDX analysis for crystals of sodium diuranate indicated that the percentage of U₃O₈ was 64.50%. Also, the physical properties of the produced sodium diuranate were studied using BSM. It was found that the stereo-photographs of

Aggregates of radiated crystals (needles form) of uranophane at X=20. These results were similar to that obtained by [27].

5. Conclusions

Worldwide reserves of high-grade ores are decreasing at alarming rate due to a rapid increase in the demand for metals. However, there exist large stockpiles of the low grade ores. Bioleaching a 'green technology' is an emerging technology refers to the conversion of metals into their water soluble forms by microorganisms. Our investigation has demonstrated the bio-acid production and leaching behaviour of *P. purpurogenium* and *P. fluorescens* SHA 281. Organisms were produced different kinds of organic acids result in high bioleaching efficiency of uranium from the tested samples. The highest uranium dissolution percentages were 72.49% and 60.06 % by *P. purpurogenium* and *P. fluorescens* SHA 281 from (T-2)80 and waste sample (W1), respectively.

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