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Fungal Leaching of Uranium from Low Grade Ore and its Waste Using Asp.Lentulus at Allouga Locality, Southwestern Sinai, Egypt

Harpy N.M [1], El Dabour, S.E [1], Nada, A.A [2], Sallam, A.M [3], El Feky M.G [1] and El Aassy A.E [1].*

[1]Nuclear Materials Authority.
[2]Faculty of Girls for Art, Science and Education, Ain Shams University.
[3]Physics Department faculty of Science Ain Shams University.

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Abstract: The capacity of the micro-organisms in ore transformations makes bioleaching technique a very interesting alternative to conventional processes. The ore sample is a prepared sample, typically like the sample in the original processing site for determining the behavior of uranium during processing by biotechnology (*Asp.Lentulus*) instead of conventional techniques in the ore sample and its resulting waste after processing. The bioleaching efficiency of *Asp.Lentulus* in the ore sample is lesser than its resulting residual due to its enrichment in organic matter and deficiency in sulphur content relative to waste. The investigated samples proved that the optimum conditions of uranium leaching are: 7 days incubation time for ore and 8 for waste, 3% pulp density, 30^{oC} incubation temperature and pH 4. By applying the studied optimum conditions for uranium bioleaching from the investigated waste sample using *Aspergillus Lentulus* species on other waste samples rather than the studied one (samples W1, W2, W3 and W.4). The high bioleaching efficiency (reach up to 80.2%) in all waste samples, could be attributed to the presence of high sulphate contents in these samples that converted by the assistance of microorganisms into sulphuric acids, which increase the bioleaching efficiency. The fungal leaching technique demonstrated an adequate recovery of uranium with an efficient and cost-effective means from waste samples. This may help in reuse (reprocessing) of waste for environmental and economic purposes.

Keywords: Uranium, Southwestern, Asp.Lentulus.

1 Introduction

Uranium is considered the heaviest strategic element, naturally occur in their ore bodies (rocks, soil, sand and waste water…etc) and has a great importance in nuclear industries [1, 2].

It is the most abundant element of all the naturally occurring actinides. The average concentration of uranium in the Earth crust is 2.8 mg kg^{-1} [3]. Natural uranium is mainly composed of two long-lived radioactive isotopes: ^{235}U = 0.72% and ^{238}U = 99.27% and exists in two oxidation states having different solubility. Under oxidising conditions, U is typically present as soluble hexavalent uranyl ion $UO_2^{\ 2+}$ which under reducing conditions U occurs in the insoluble complexes tetravalent state UO_2 [4]. ^{238}U is a radionuclide present in the earth's crust that provides 65.9% of annual average radiation dose [3].

Radioactive waste is generated from activities that utilize nuclear materials such as nuclear medicine or power plants. Depending on their half-life, they emit radiation continuously, ranging from seconds to millions of years. Exposure to ionizing radiation can cause serious harm to humans and the environment. Therefore, special attention is paid to the management of radioactive waste in order to deal with its large quantity and dangerous levels. Current [5]. Uranium produced from different activities such as nuclear power/weapon production, mining of ore and various industries applying radioactive isotopes [6].

Uranium tailings are waste by-product of uranium milling. In mining, raw uranium ore is brought to the surface and crushed into fine sand. The valuable uranium-bearing minerals are then removed via heap leaching with the use of acids or bases, and the remaining radioactive sludge, called "uranium tailings", is stored in huge impoundments. Uranium tailings can retain up to 85% of the ore's original radioactivity [7].

The abandonment of mines with waste rocks disposed at open-pit poses a serious environmental risk to adjacent

*Corresponding author e-mail: mglal_99@hotmail.com

ecosystems even after decades of abandonment. The disposed waste rock at open pit of Pedra Verde mine still contains high levels of copper even after 30 years of exposure, with Cu mainly associated with carbonates, oxides and sulphides [8]. Mining of gold in the Welkom and Virginia areas of the Free State Province in South Africa has produced contamination of groundwater by potential harmful elements in to human health from gold mine tailings [9].

Harpy et al. have been determined natural radioactivity in samples from Um Bogma Formation by Hyper-pure germanium (HPGe) in waste samples collected after acidic heap leaching. Fairly, they found that all investigated tailing waste samples do not satisfy the universal standards [10].

If uranium tailings are stored aboveground and allowed to dry out, the radioactive sand can be carried for a great distances by the wind, entering the food chain and bodies of water [11]. The accumulation of mine tailings on Earth is a serious environmental challenge. The importance for the recovery of heavy metals, together with the economic benefits of precious and base metals, is a strong incentive to develop sustainable methods to recover metals from tailings. Currently, researchers are attempting to improve the efficiency of metal recovery from tailings using bioleaching, as a more sustainable method compared to traditional methods [12].

Use of microbiological methods for environmental remediation has come up as an appreciable concept, which often acts as an eco-friendly approach [13]. Biological processes have to be integrated into future industrial processes [14]. Bio-hydrometallurgy deals with the application of biotechnology in mining industry. In fact, microorganisms can be successfully used for the extraction of metals (e.g., copper, zinc, cobalt, lead, uranium) from low grade ores. Mining with microbes is both economic and environmental friendly. Regarding sustainability, biological processes can contribute to a large extent to future technologies, including also waste treatment. In general, bioleaching is a microbiological process described as being "the dissolution of metals from their mineral source by certain naturally occurring microorganisms" or "the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered through it [15].

There are three main groups of microorganisms which have been used for bioleaching process; autotrophic bacteria (e.g. *Thiobacilli* spp.) [16] heterotrophic bacteria (e.g. *Pseudomonas* spp., *Bacillus* spp.) [17] and heterotrophic fungi (e.g. *Aspergillus* spp., *Penicillium* spp.) [18].

The current study focus on the investigation of the ability of isolated fungi in bioleaching of uranium from the ore and its resulting residual (W6) and optimization of some uranium bioleaching parameters.

2 Experimental

2.1 Collection and Characterization of Ore And Waste Samples

Sinai's important geographical and strategic position is attracting researchers to explore opportunities to maximize exploitation of its treasures, especially in the area of sustainable development [19].

The uraniferous ore sample was collected from Um Bogma Formation at Allouga locality (Fig. 1). The lower and middle member of Um Bogma Formation consist of carbonaceous black shale, sandy- dolostone, limonitic marl, and siltstone and are exposed in the face of Allouga Quarry, with several mineralized layers. [20] reported the presence of both primary coffinite and brannerite and secondary autunite and uranophane uranium mineral.

The studied ore sample (Q8) occurs at the base of the quarry and composed mainly of black shale with yellow and pale yellow uranium mineralization. This sample was also in situ investigated radiometrically using in situ γ-ray spectrometer. The dose rate of the sample reach up to 7.26 μSv/h suggesting high radioactivity.

The studied sample represents the youngest waste sample. All waste samples (W1, W2, W3, W4, W,5 and W6) are placed above the ground. The resulting solid waste after processing (W6) was collected from the sites of waste accumulation. Waste materials were stored after acidic heap leaching and agitated tank leaching of sedimentary rock ores. The resultant wastes were grouped in piles and generally open-air stacked. The studied waste piles were stacked in a rectangular shape with defected sides (Fig. 2). The studied waste sample was collected from the surface of their waste pile.

The effect of microorganisms on the leaching of uranium from waste materials, which 50-60% of uranium was previously leached by classical chemical hydrometallurgical procedure has been investigated. The ore and waste samples weighting 50 Kg were collected in sterile polyethylene bags from the prospective area in southwestern Sinai, Egypt.

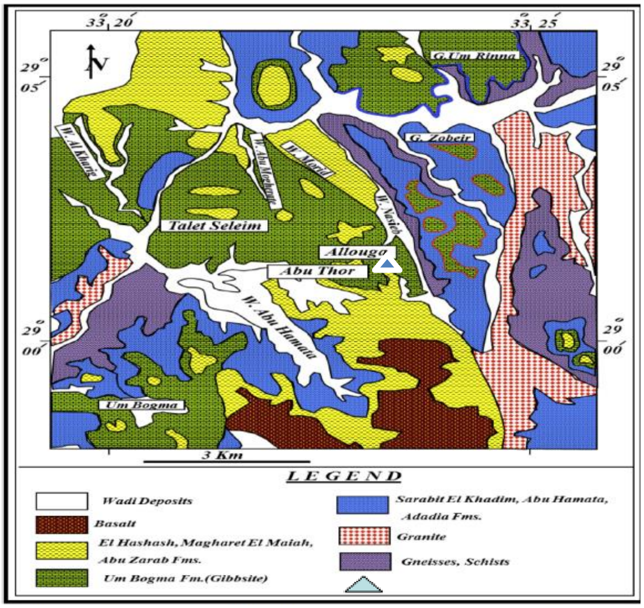

Fig.1: Geological map Showing the studied locality and location of tailing wastes After [21]

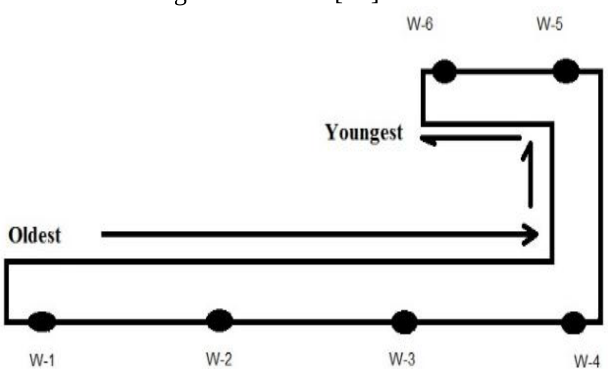

Fig.2: Sketch showing the waste piles distribution in the studied area after [22].

2.2 Uranium Estimation

The uranium contents of the solid samples and lech liquors were determined according to the method described by [23] and developed and modified by Farag et al. 2015 [24] and established by Nuclear Materials Authority laboratories, Egypt. The uranium concentration was determined through titration against 0.1% ammonium metavanadate and will be calculated according to the following equation:

$$U\ (mg/L) = (T \times V_1 \times 10^3 / V). \qquad (1)$$

Where, T is the titration intensity of NH_4VO_4 [Ammonium meta-Vandate] solution, V and V_1 is the volume of the measured sample and NH_4VO_4 solution consumed, respectively.

2.3 Microbiological Studies

Fungi artificial medium

(Dox liquid) used for fungi isolation from ore sample Q8 and Uranium tailings (waste byproduct) and in the bioleaching of the studied samples. It has the following composition (g/l):1$NaNO_3$, 2 KH_2PO_4, 1Mg SO_4.7 H_2O, 0.5 KCl, 0.5 $FeSO_4$.5 H_2O and trace, sucrose, 30; agar, 15. 5g, yeast extract was added to initiate fungal growth. The pH value of the medium was adjusted at 6.5 before autoclaving at 1.5 atmospheres for 20 min [25].

Fungal isolation, Purification and identification

The direct-plating technique was used in fungal isolation from the ore, in which fine ore-powder was spread directly on the surface of Dox agar plates under aseptic conditions. The agar plates were incubated at 28 $^oC \pm 2$ until the fungal colonies appear well [26, 27]. Hyphal tips of each colony were removed and plated on the surface of agar plates. The developed colonies were examined with a microscope to detect contamination. The pure isolated fungi were identified according to [26 - 29]. The tested fungi were identified based on macroscopic and microscopic characteristics according to [30 -32] up to species level.

3 Results and Discussion

3.1 Geochemical Characteristics of the Studied ore and Waste Samples

The chemical analyses were carried out for major oxides and trace elements of the ore and waste samples in ACME analytical Laboratories of Vancouver, Canada by ICP-emission spectrometry (ICP-ES) and ICP-mass spectrometry (ICP-MS). The obtained results showed in (Fig. 3) indicate that the ore and waste samples are mainly composed of SiO_2, Al_2O_3, Fe_2O_3, CaO, MgO, SO_3^{--}, in addition to loss on ignition (L.O.I). Some oxides of major elements such as SiO_2, Fe_2O_3, MgO, SO_3^{--} and Al_2O_3 display enrichment in the ore and waste samples. The waste sample has lower CaO and L.O.I contents suggesting lower carbonate and so organic carbon contents. Later, the tow samples were also analyzed chemically by [22].

The waste sample has nearly double value of the SiO_2 in the ore sample due to the presence of gangue minerals. On the other hand, MnO, K_2O, Na_2O TiO_2 and P_2O_5 show low contents in the ore and waste samples. Uranium is analyzed chemically. Uranium contents reach up to 1650 and 302 ppm in the ore and waste samples, respectively.

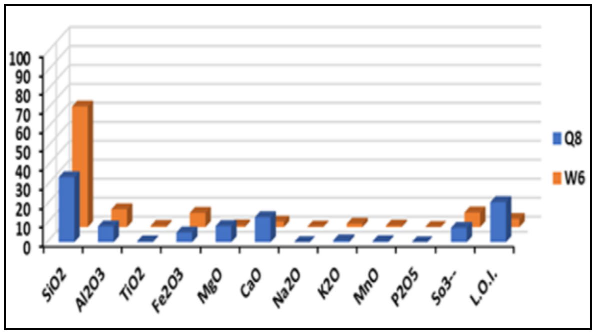

Fig.3: Concentrations of major oxides (wt%) and Uranium contents (ppm) in the studied ore (Q8) and waste samples (W6).

3.2 Microbiological Investigations

Five species were successfully isolated, identified and their leaching efficacies were showed in (Fig. 4). The isolates were also identified phenotypically up to species level and their leaching efficacies by El Dabour et al. 2019 [22].
All isolated revealed that they belong to the genus *Aspergillus*(*Asp. Lentulus*, *Asp. flavus*, *Asp. Niger*, *Asp. Felis*, and *Asp. fumigatus*). The tested isolates belong to 5 species were examined for the possibility of microbial leaching of uranium (U) from waste sample [W6]. Five previously isolated and identified native microorganisms, *Aspergillus*, were used to make U-bioleaching using cells (direct). The tested isolates showed variable U-bioleaching efficiencies and the highest results was attained via the direct method by *Asp. Lentulus* (68%).

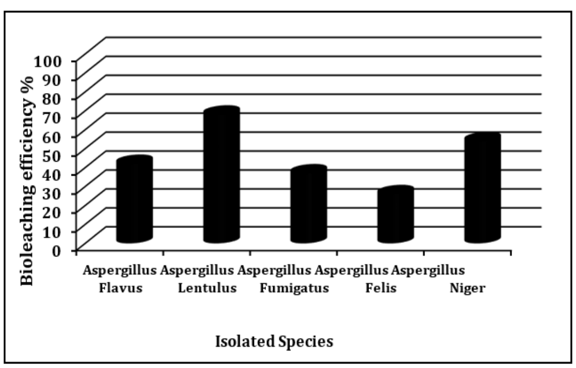

Fig.4: Leaching efficiencies for the isolated species from the studied samples.

3.3 Optimization of Uranium Bioleaching Parameters

Different microorganisms have been studied for bioleaching of uranium ores. Among these microorganisms, fungi, especially genus *Aspergillus*, were found to be effective in extracting uranium and other valuable metals from their ores using an effective, cheap and eco-friendly method [33, 34, 4, 35, 36].

Several factors were investigated with the aim of obtaining the optimum conditions for uranium solubilization by *Asp. Lentulus* i.e. Effect of different ore concentrations (1 to 9%), incubation period (3 to 10 days), incubation temperature (20 to 45 ^{o}C) and pH values (1 to 10).

Effect of different ore concentrations on isolated *Asp.Lentulus* fungus biomass

The effect of different ore concentrations (1 to 9%), on *Asp.Lentulus* indicate that *Asp.Lentulus* could grow in the presence of different ore concentrations up to 7 % but at 9% no growth occurs. The growth of isolated *Asp.Lentulus* being decreased with elevated concentrations of the used ores. This may be attributed to toxic effect of some metal ions which may be released to the media such as U, Al, Mg and Ca ions that increased in the culture filtrate with elevated ore concentrations.

Effect of pulp density (PD) on the bioleaching of uranium:

One hundred ml of Dox liquid medium was placed in 250 ml Erlenmeyer flasks. The flasks were supplemented with different ore concentrations (1, 3, 5, 7 and 9 % [w/v]). The flasks were autoclaved at 1.5 atm and 120 ^{o}C for 20 min, after cooling the flasks were inoculated with 0.5 ml of spore suspension of *Asp.Lentulus* and finally incubated at 30 ^{o}C for 8 days. At the end of the incubation period the mycelial mats were harvested, the liquid media were filtered using Whatman No.1 filter paper, finally U was analyzed and their leaching efficiencies were calculated and given in (Fig. 5). It seems that ore concentration is effective in the bioleaching process.

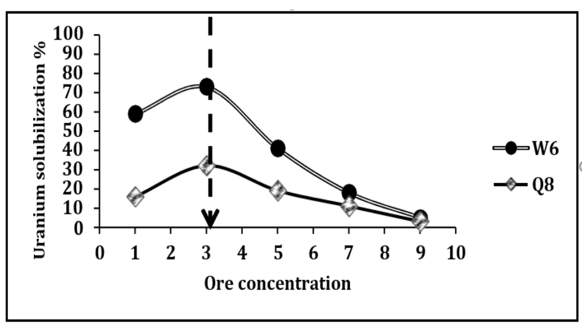

Fig.5: Relationship between different ore concentrations on uranium solubilization by *[Asp.Lentulus] grown* on Dox liquid medium at 30 ^{o}C for 8 days.

The amount of released uranium by *Asp.Lentulus* was increased with increasing ore concentrations in the growth media up to 3% at which, the highest percentage of solubilized uranium (Q8 or W6) in the growth media. The solubility of uranium from Q8 ranged between 11 and 32 % in the growth media. *Asp.Lentulus* at concentration of 3%

of Q8 has the highest solubility conditions (32%). On the other hand, the solubility of uranium for the waste sample (W6) ranged from 18 to 73 % in the growth media. *Asp.Lentulus* at 3% concentration of W6 has the highest solubility of uranium (73%), too.

The best leaching of uranium occurred only when the final pH of the control media was shifted toward acidity (pH=4). The increasing values of final pH may lead to the increasing in U concentration in the growth medium, the highest leaching of uranium occurred at 3% ore concentration for two samples (Q8 and W6).

After that the leaching of uranium is decreased due to the fact that the fungi can't produce amino acids due to the increasing in ore concentration of the media or may be attributed to the solubilization of other metals that consumed organic acids produced by the tested fungi. The amount of solubilized uranium increased with increasing ore concentrations in the growth media up to 3% and decreased above this concentration, this could be attributed to two reasons: (I) raising the final pH which perhaps decrease the solubilization efficiency (II) the high binding capacity of the fungal mycelium at higher concentrations of the ore.

The obtained results also support the probability that cell contact with the radionuclides and/or heavy metals in rock sample may cause inhibition of the production of secondary metabolites by some of the tested microorganisms and eventually result in reduced U concentration in leach liquor [19]. Also, the present study agrees with that of Rashidi et al. (2014) who attributed the limitation in bioleaching with increasing PD to several factors including oxygen requirement, nutrient availability, effect of agitation, inhibitory effect of ions, decrease in suspension homogeneity due to aggregation of fine particles, reduction in oxidant to reductant ratio and turbulence on the cells [37]. Also, Wang et al. 2018 indicated that increasing pulp density caused a decrease in U-bioleaching using *Acidithiobacillus ferrooxidans* as low pulp densities make uranium minerals easily extracted to the aqueous solution [38].

The high bioleaching efficiency of the studied fungi in the waste sample in comparison with the ore sample could be related to the presence high contents of organic matter in the ore sample (L.O.I. =21.14 wt.%), which may inhibit uranium mobility or could be related quantity of amino acids in the sample that consumed by organic matter, additionally, the presence of high sulphur contents in the waste samples.

Effect of incubation temperature

Uranium leaching from its ores was found to be highly affected by the incubation temperature, at the previous fixed conditions beyond varying temperatures of culture filtrate (*Asp.Lentulus*) from (20ºC to 45 ºC), the bioleaching efficiency was investigated. Results of leaching efficiencies of U are given in (Fig. 6). It was found that, in both Q8 and W6 samples, uranium leaching efficiencies increased by increasing the temperature up to 30ºC after that the bioleaching efficiency decrease, indicating that the maximum uranium leaching efficiency of both Q8 and W6 reach up to 42% & 79%, respectively at 30ºC. The growth of isolated *Asp.Lentulus* being decreased with increasing incubation temperatures, nearly, no growth for fungus at 45ºC.

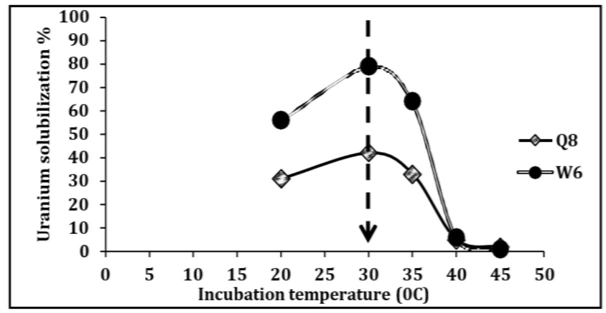

Fig.6: Relationship between different incubation temperatures on uranium solubilization from 3% ore concentrations of samples W6 and Q8 by *[Asp.Lentulus]* grown on Dox liquid medium for 8days.

James et al. stated that temperature was a regulatory factor in the secondary metabolism of *Streptomyces thermaviolaceus* [39]. Mohapatra et al. reported that at extreme temp., the metabolic rate of organism decreases and also the organism might not survive [40].

It is worthy to mention that *Asp.Lentulus* was affected by the high content of organic matter in sample Q8, as noticed after roasting the sample at 1000 ^{0}C, the leachability reached up to 73%, suggesting the role of organic matter in uranium immobility.

It is also noticed that the final pH in counteractive relation with the leaching efficiency of U; where, the highest leaching efficiency of U in both samples Q8 and W6 was occurred at the highest final pH value (7.19 and 8.12, respectively). The U-bioleaching efficiency started to decrease with decreasing the final pH, which nearly the same pH of preparing media.

Effect of the incubation period.

This factor was studied by putting 100 ml of the Dox liquid medium in six 250 ml measuring flasks with the ore sample at optimal ore concentration (3%) and pH of 6.5. They were autoclaved at 1.5 atm. and 120 ^{o}C for 20 min. After cooling, the flasks were inoculated with 0.5 ml of spore suspension of *Asp.Lentulus* and finally incubated at 30ºC for several different days (3, 5, 7, 8, 9 and 10). U was determined and their leaching efficiencies were calculated and recorded in (Fig. 7).

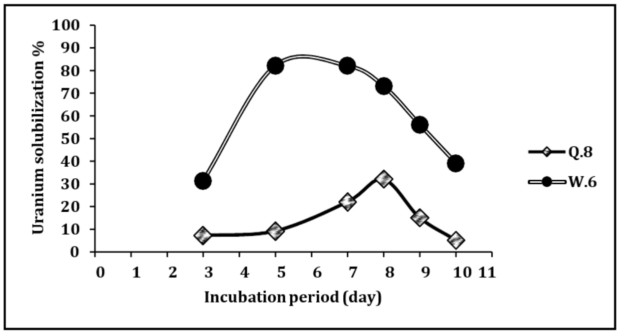

Fig.7: Relationship between different incubation periods on uranium solubilization (%) from 3% ore concentrations of samples Q.8 and W.6 using *Asp.Lentulus* grown on Dox liquid medium at 30 ^{o}C

Results in (Fig. 7) indicated that U leaching efficiency from Q8 and W6 were highly affected by the incubation time where the maximum leaching efficiencies attained 32% for Q8 at 8 days and 82% for W6, from 5th to 7th day of incubation for *Asp.Lentulus* culture**.** It was noticed that the leaching percentage of U from Q8 rapidly decreased after 8 days but after 7 days from W6. This phenomenon may be attributed to the effect of some interfering metal ions that might dissolved and the amount of organic acids production may be decreased with time.
This may be due to that these periods were required for the full growth and good adaptation of tested strains in the growth medium as previously described by [41] or may be attributed to the effect of some metal ions released to the medium which effect on the activity of organism. The maximum antibiotic production was achieved on 7th to the 8th days of incubation of *Actinomyces lavendulae* culture [42].

The reduction in the bioleaching of total solubilized metals after the optimum period may be due to: 1- The accumulation of leached material on the surface of fungal hyphae [43]. 2-The production of volatile metabolites instead of organic acids and in-activation of enzymes of the fungus following its decline phase [44]. 3- A deficiency of available substrate for culture [45].

Effect of pH values

The acidity of culture filtrate of *Asp.Lentulus* is one of the most important factors affecting the bioleaching of the working samples. However, the culture was adjusted at different pH values; 1, 3, 4.5, 7, 9 and 10 and mixed with the ore sample at pulp (3 % (w/v), 30 ºC temperature and incubated for 8 days for Q8 and 7 dayes for W6. U was determined and their leaching efficiencies were calculated.

The data shown in (Fig. 8) emphasized that, the bioleaching efficiency of U was increased with the increasing of the initial pH of the medium up to pH 4 and decreased above this pH. Leaching of U occurred in the acidic range, whereas, it was highly decreased in the alkaline medium.

The maximum leaching efficiencies of U from Q8 and W6 were 46% and 82%, respectively, by culture filtrate of *Asp.Lentulus* conducted by increasing the acidity of the culture filtrate up to pH4. It was found that, increasing the pH value of the culture filtrate has an opposite effect on leaching efficiencies of U in both samples. This might be attributed to the role of medium acidity in increasing the leaching of different elements.
The well noticed thing is that, there is no growth of *Asp.Lentulus* at pH1 and decrease in the growth of *Asp.Lentulus* up to pH7 and nearly no growth at pH10. The change in pH of the culture medium induced production of new substance that affects secondary metabolite production [46].
The obtained results are in agreement with the most researchers, whether working with bacteria or fungi, pointed out that low pH and acid production increases U-bioleaching [47, 37, 48] and Amin 2008 who mentioned that the maximum leaching efficiency of uranium occurred at pH 4 [49]. On the other hand, Abdelsalam et al. [19] who mentioned the optimum pH for growth and also for U-bioleaching by *Aspergillus niveus* EGY2 was 9 which was not agreement with the optimum pH for growth and also for U-bioleaching in this study and was not in agreement with the results obtained by other authors who indicated that bioleaching can be done by using molds, yeasts or bacteria that can often tolerate high pH values **[**50].
In all previous factors, it was observed that there is notable variation in final pH, which sometimes decreased, could be due to the growth of the organism, which excreted metabolites included H and organic acids [51**]**. On the other hand, final pH sometimes, increases at the end of the bioleaching process and this could be related to the following reasons:

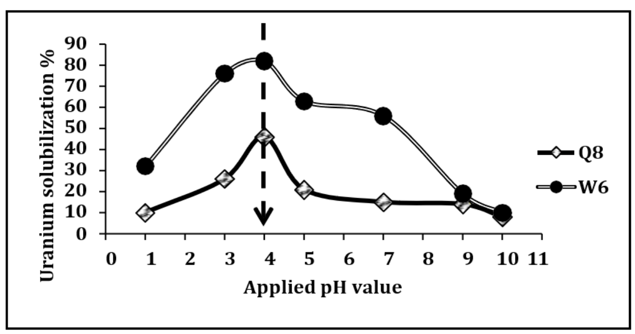

Fig. 8: Relationship between different pHs on Uranium solubilization (%) from 3% of ore and waste (Q8 and W6) by *Asp.Lentulus* grown on Dox liquid medium at 30 ^{o}C for 7days.

(1) The adsorption of hydrogen ions from aqueous solutions by fungal biomass or neutralization of H^+ with OH^- released from the biomass [52, 53].
(2) Increasing concentration of Na^+ in the growth medium

as a result of bioleaching process of Na_2O found in the ore [54].
(3) Complete utilization of glucose, then fungi started to use its own metabolite [55].
However, the acidity of the waste sample (W6) must be greater than ore sample (Q8), due to its previous processing by sulphuric acid. In contrast, the final pH of the waste sample during bioleaching was more alkaline than the ore sample (Q8) pH. This may be due to the presence of arsenate and phosphate radicals in waste sample that leading to the hydrolysis of the solution in the leach liquor and consequently increase the alkalinity of the solution [56].

3.4 Applying the Optimum Conditions for Uranium Bioleaching on the Other Waste Samples

The wastes are providing the biggest concern because they are hazardous for both humankind and surrounding environments. High levels of radiation exposed can cause the death and radioactive contamination to groundwater, ocean, etc. may indirectly effect the food chains. Wastes must be classified and treated with physical, chemical or biological methods before disposal to serve the human and environment safety regulations [5].

By applying the studied optimum conditions for uranium bioleaching from waste sample using *Aspergillus Lentulus species* on other waste samples rather than the studied waste (samples W1, W2, W3 and W.4). The most important results were: (i) 8 days incubation time for waste; (ii) 3% pulp density; (iii) 30 ^{o}C incubation temperature and ph ***4***. The bioleaching efficiencies resulted from applying these conditions on the other waste sample show its minimum in W3 sample (75.4 %) and reach up to its maximum in W2 sample (80.2%), (Table 1) and (Fig. 9).

Table 1: Application of the studied optimum conditions on uranium solubilization from 3% waste concentrations of samples W1, W2, W3 and W.4 using [*Asp.Lentulus*] grown on Dox liquid medium at 30 ^{o}C. Under Ph =4 for 7 days of incubation**.**

Waste samples	Uranium (ppm)	Uranium bioleaching efficiency (%)	Final pH
W.1	210	77	7.05
W.2	262	80.2	7.6
W.3	181	75.4	7.9
W.4	161	76.1	7.7
W.6	302	82	6.7

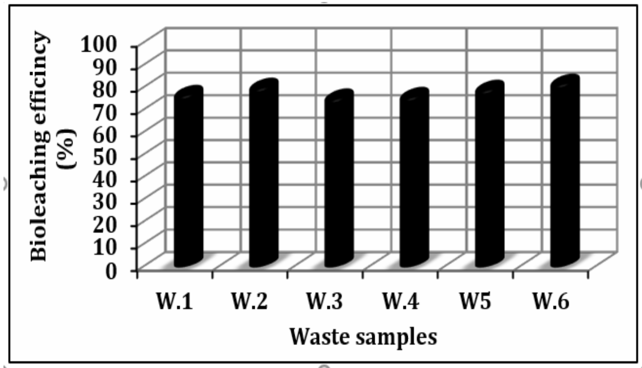

Fig.9: Uranium bioleaching efficiency (%) in different waste samples using optimum conditions for *Asp.Lentulus.*

It is noted that the greater the content of uranium in the waste samples, the greater the amount of uranium leaching and this may be attributed to the impact of the amount of uranium on the vitality of fungi and stimulate the production of a larger quantity of organic acids, which help more uranium to dissolve.

The high bioleaching efficiency in all waste samples is mainly attributed to the presence of high sulphate contents in these samples which converted by the assistance of microorganisms into sulphuric acids and so, increase the bioleaching efficiency. Microbial activity could be effectively eliminated from waste samples due to sulphuric acid leaching of the original ore which eventually leads to microbes killing with leaching of cellular compounds by the effect of acids and gamma-sterilization. This decreases the adsorption capacity of the waste samples and leads to enhanced uranium remobilization [57, 58].
It can be said that using *A. lentulus* for dissolving of radionuclides helps in maximizing the exploitation for radioactive waste before burying which is in particular of valuable significant effect on the economic and environmental domain [36].

3.5 Role of organic acids produced by fungal strains

The application of bioleaching techniques for radionuclides is accompanied by the production of some acids as an outcome from microbial activity, which acts to minimize the consuming rate of acid at uranium industrial plants [59]. The generated acids from microbial activity, as oxalic, citric, gluconic and malic acid help in the bioleaching of metals by the mechanism of Acidolysis.

Many fungi are able to produce organic acids such as citric and oxalic acids, and these metabolites can be used to extract uranium from ores, as organic acids can form complexes with uranium. Mixed organic acids produced by fungi are cheaper than those pure organic acids which purchased from the market, it is preferred for dissolution of uranium [4].

Organic acids have two main functions that are of a great significant role in bioleaching. First, they can facilitate the dissolute metals ions from the leached materials through chelation of the metals released in solution and also destabilize the bonds between the metal surface and bulk leaching materials [60]. Second, they can decrease the counter effect of metal ions assesses microorganisms through chelation or complexation [61].

The identification of organic acids produced by the tested fungal strain in our study was achieved using Aglient-1100 High-Performance Liquid Chromatography (HPLC), Table (2) clarify the data obtained by this technique.

From the obtained results, it appears that *A.lentulus* produced some organic acids like acetic, ascorbic, oxalic and citric. It is clearly apparent that acetic acid is superior among other metabolites generated by *A.lentulus*, its concentration increases from 3.3 $x10^{-2}$ M for control to the optimum 1.6 $x10^{-2}$ M at a pulp density of 3% then reach at 7% ore concentration to 2.7 x10-2 M indicating a slight increase in concentration.

Citric acid is primal in leaching of heavy metals whether in ore materials or solid wastes. Ren *et al.,* dissolve zinc from industrial filters [62]. The leach liquor content of citric acid was $2.5x10^{-2}$, $1.4x10^{-2}$, and $1.33x10^{-2}$ M for control, 3% and 7% ore concentration, respectively. On the other hand, secretion of oxalic acid by *A.lentulus* was plausibly affected by raising ore content in growing media, where it starts by $2.16x10^{-2}$ M for control then decrease to $5.6x10^{-3}$ M and ends up with $3.8x10^{-3}$ M.

Table 2: Concentration of organic acids in molar (M) excreted by *A.lentulus.*

	Oxalic	Citric	Acetic	Ascorbic
Control	$2.16x10^{-2}$	$2.5x10^{-2}$	0.033	$2.17x10^{-4}$
3% ore concentration	$5.6x10^{-3}$	$1.4x10^{-2}$	0.016	$1.08x10^{-5}$
7% ore concentration	$3.8x10^{-3}$	$1.33x10^{-2}$	0.027	$1.01x10^{-5}$

For ascorbic acid which is essential acid in biological systems, excretion by *A.lentulus* was $2.17x10^{-4}$ M for the control sample and decrease to 1.08 $x10^{-5}$ M at 3% and reach to 1.01 $x10^{-5}$ M at 7% ore concentration.

In general, all measured organic acids secreted in bioleach liquor by *A.lentulus* behave nearly in a corresponding way as they have high concentration in the control sample, by adding ore to solution (growing media), its concentration decrease gradually by increasing ore content (Fig. 10), as they consumed in leaching process which confirms that some organic acid is of primal implicating effect on dissolving and exploitation of metals.

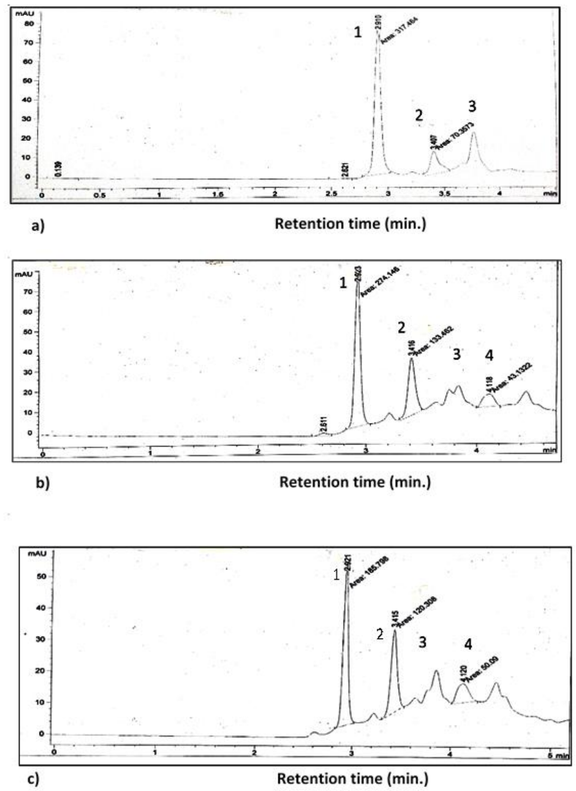

Fig.10: Typical organic acids chromatograph for *A.lentulus grew* on Dox liquid medium:

1: Oxalic acid 2: Citric acid 3: Acetic acid 4: Ascorbic acid
A: Control.
B: 3% sample W6 concentration.
C: 7% sample W6 concentration.

4 Conclusions

Some uranium tailings have valuable amounts of radioactive elements and economic metals beside the toxic ones (especially for the studied samples). Remediation or extraction of such elements is required for the future utilization. Bioleaching using some fungal species are widely used in such cases. It is sensitive to metals concentration and the composition of sample, so it is preferred to characterize samples before starting the operation; such in case for samples having high organic matter content. Disposing of the organic matter prior to leaching process is required for higher leaching efficiency. Bioleaching of U is also affected by incubation temperature, incubating time and growing media pH. By applying the optimum conditions (3% pulp density, 30^0C incubation temperature, 5 days incubating time and pH of 4) obtained in different waste samples, the leachability of U reached to 82%. So, waste samples could be managed to utilize its uranium content and decrease its total radioactivity. Bioleaching is proved to be an economic and friendly environmental technique**.**

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