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Biological Solubilization and Sorption of Uranium from ore Sample at Abu Thor area, Southwestern Sinai, Egypt using *Aspergillus Nidulans*

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Abstract: Now the solubilization process of heavy metals by microorganisms is an established biological technology technique, as biological solubilization, is a promising technology economically and ecofriendly alternative process to the traditional ones for treating ores. This work aimed to investigate the biological solubilization of uranium from its ore sample collected from Abu Thor, southwestern Sinai, Egypt by using a native isolated fungus from the rock sample. At the same time, evaluate the uranium biological sorption during the biological solubilization process.The radiometric measurements were estimated by using NaI (Tl) detector. The solubilization process of uranium was carried out for the studied ore sample using the native isolated fungus, which was identified morphologically as *Aspergillus nidulans*. It was leading to a high biological solubilization efficiency of uranium under optimized conditions of pH 3, 3% pulp density, 7 days incubation time, and 30^{o}C incubation temperatures. Furthermore, the biological sorption process was studied during the biological solobilization process. The results showed that the optimum biological sorption conditions were: pH value equals 9, 5% pulp density, 9 days incubation time, and 35°C incubation temperatures, which exhibited the maximum biological sorption. Finally, the biological recovery of uranium from one kg of the sample was achieved 83%.
Keywords: Uranium, *Aspergillus nidulans*, biological solubilization, biological sorption, biological recovery

1 Introduction

Wadi Abu Thor area is located about 40 km east of Abu Zeneima town in the southwestern part of the Sinai Peninsula. It is bounded by Latitudes 29o 02′ and 29o 05′ N and Longitudes 33o 21 ′ and 33o 23′ E (Fig.1). The basement and Paleozoic sedimentary rocks was covered the studied area. Gibbsite mineralization is dominant in the Um Bogma area. This mineralization is exposed definitely at W. Abu Thor, W. Naseib, and Talet Seleim. Gibbsite veins and pockets in Um Bogma area, especially in the dolomite of the Um Bogma Formation and the sandstone at the base of the Abu Thora Formation was reported [1]. The gibbsite is very porous cryptocrystalline and partially cemented with Fe-Mn oxides was emphasized [2]. Um Bogma gibbsite occurs in the form of cryptocrystalline material containing minute grains of Fe and Mn oxy-hydroxides was recognized and recorded several associated minerals such as incite, celestite, barite, monazite, plumbogummite, halite, atacamite, paratacamite and zircon [3]. The bacteria and fungi are the main micro-organisms contained within the studied gibbsite were revealed [4]. During this era of technology, biological technology plays a significant role within existing mining technologies.

Fig. 1: Landsat image showing the location of the studied area.

The biological technology, biological remediation, considered as a method that can be used to remediate polluted environments according to the increasing of industrial effluents, agriculture and household activities and also, economize the cost of transportation by uses safe microorganisms to remove the chemical contamination. Consequently, it is a possible method for soil and

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groundwater treatments synchronously. The biological remediation expression is divided into two words: “bio” denotes life, and “remediation” expresses solving a problem essentially by using living organisms to repair the environment [5]. The microorganism, under its favorable conditions success in the biological remediation process, which is achieved interaction within their ability [6], "bioremediation techniques include bio-stimulation, bio-augmentation, bio-accumulation, bio-sorption, and the use of bio-films" [7]. For the extraction methods of metal, conventional methods as hydrometallurgy and pyrometallurgy, are confronted with difficulties like environmental contamination, low recovery efficiencies, and high operational costs. [8]. Therefore, the biological method of leaching, sometimes called “biological solubilization or biological hydrometallurgy or biological mining”, utilizes microorganisms for solubilizing metal oxides and sulfides with simple method and low cost [9]. Bioleaching is a specified technology for bioremediation where plays a significant role in the process of harmful metal extraction to harmless metal ions, where the bioleaching process has occurred naturally since many thousands of years [10]. The bioleaching process mainly depends upon the enzymatical activity of microorganisms [11], which is in charge of changing the substance toxicity by different microorganisms like bacteria and fungi [12] and higher plants [13]. The bioleaching process of metal ions is depend upon several parameters like the pH value, size of particles, pulp density, frequency of stirring (rpm), the temperature of the reaction, microorganisms nutrient concentration, an oxygen content, and reaction contact time [14,15]. These factors greatly effect on the overall success of the metals solubilization process and it is essential to study their impacts to obtain the highest recovery of metals [16, 17]. As a branch of biological technology, the metabolic an independent process and the physic-chemical process are highly important to the environment as a biological sorption processes, which based on the remove of an organic or inorganic substances from the solution including living or dead microorganisms. According to the simplicity of biological sorption process, it is utilized as an alternative technique to traditional ion exchange methods [18]. As a biological remediation technique, biological sorption process is depending on the elements capability to bind onto the functional groups as active sites that are existing on the surface of microorganisms. This process is a complicated process that is influenced by several parameters like pH, reaction temperature, the contact time of reaction, concentration of contaminant, and the structure of the cell wall [19]. Biological techniques have focused upon increasing awareness to use as a method of solubilizing. On the other hand, the biological process utilized for removing and recovery of radionuclides and heavy metals according to their high achievement. Radionuclides like uranium and thorium are naturally distributed to nuclear power production, as well as to a number of human activities (e.g., mining, production, and use of phosphate fertilizers, copper metallurgy, and military activities) involving in the environment as more harmful pollutants [20]. It exists naturally in the ecosphere more abundant than silver or gold as a mineral and the nuclear industry waste product, with an atomic number of 92, deemed as heavy metal, and it has been exploited both for nuclear power and for nuclear weapons. It can be existed naturally with low levels as; 0.1–5 μg/l in water, 0.01–2 μg in food, and 0.1–2 mg/kg in soil and rocks. There are 19 different isotopes for uranium with half-lives in the range from 1 μs (^{222}U) to 4.47 × 109 years (^{238}U) with an atomic mass in the range from 218 to 242 g/mol., three isotopes of them are naturally found as: ^{238}U (T1/2 4.47 × 109 years), ^{235}U (T1/2 7.038 × 108 years), and ^{234}U (T1/2 2.446 × 105 years), with an approximate abundances 99.275%, 0.72%, and 0.054%, respectively. UO_2 or other forms of uranium can be extracted chemically from its ores, which are used in the nuclear industrial field [21].

The present work aimed to examine the biological solubilility of uranium from its high-grade sample of uranium collected from Abu Thor, southwestern Sinai, Egypt by using an isolated fungus from the rock sample. At the same time, evaluate the uranium biological sorption during the biological solubilization process.

2 Mechanism of Fungal Solubilization

The biological solubilization mechanism of fungi is correlating to the production of metabolites with low molecular weight known as organic acids. The role of secreted organic acids is to act as bio-lixiviants for metals during the solubilization process. This role can be carried out through several mechanisms separately or simultaneously [22]. The main potential fungal mechanisms of biological solubilization process (Fig. 2) can be occurred by the principle of i) acidolysis, ii) complexolysis, iii) redoxolysis, iiii) biological sorption, and v) biological accumulation [23]. The most important mechanisms for metals mobilization are acidolysis and complexolysis, where the fungal organic acids support the mobilization process of metals by supplying with protons and anions through liberating metal-free cations by protonation. [24]. Another type of fungal metabolites known as amino acids can be secreted together with organic acids. Nevertheless, utilization of amino acids has not a highly significant role in metal solubilization due to the rare efflux of amino acid among filamentous fungi [23]. The mechanism of biological sorption is considered as a physicochemical passive process, which takes place through the biological components that are excreted by fungi or between metal ions and fungal surfaces allowing the metal ions to bind from solution, then it has to be recovered [25]. The cell wall of fungi act as a highly reactive surface were composed of various functional groups (e.g. amine, phosphate, carboxyl, and hydroxyl groups) [26]. In addition, it should be noted that the biological sorption can

carried out with living and dead biomass [27]. Consequently, nutrients have no role for nonliving biomass and can be applied this process in highly toxic environments [28].

Fig. 2: Schematic diagram represent biological solubilizatin and mineralization mechanisms of uranium.

The biological accumulation is a specific type of biological sorption wherein metal ions incorporation take place in the living biomass as a protection way against the toxicity of metals (Fig. 3), this uptake is based on an active process in the fungal cell membrane, but a passive one can also be involved [27].

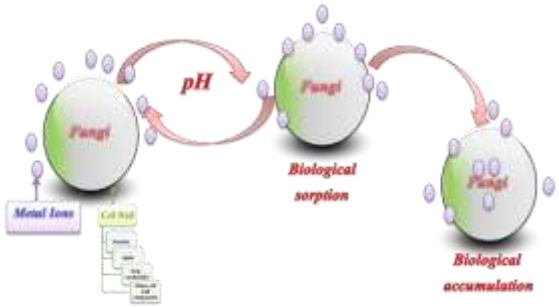

Fig.3: The biological sorption and accumulation mechanisms of metal ions.

In the Direct mechanism; a physical contact between the microorganism cells and the ore surface. Oxidatio reactions occurs through several catalyzed enzymes causing electrons transferred from elements such as Fe or S to O_2 (Fig. 4). The released electrons from the oxidation reaction are moved through the cell membrane protein in the microorganism forming water molecules. The majority of uranium ores containing iron, or addithon of iron salt, that is act as a catalyst oxidized to iron (III) sulphate according to the following reactions (Eq.1, 2), and the biological solubilization can be desceibed as in Eq. (3).

$$4FeS_2 + 14O_2 + 4\ H_2O \rightarrow 4FeSO_4 + 4\ H_2SO_4 \quad (1)$$

$$4FeSO_4 + O_2 + 4\ H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2\ H_2O \quad (2)$$

$$2UO_2 + O_2 + 2\ H_2SO_4 \rightarrow 2UO_2SO_4 + 2\ H_2O \quad (3)$$

Microorganisms bind to specific sites on the surface of the ore, causing dissolution of uranium through electrochemical interactions. [29].

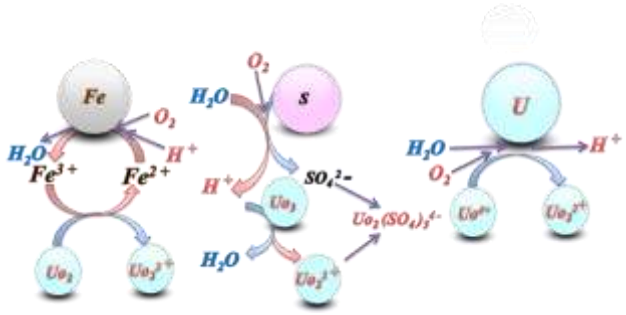

Fig.4: Diagram showing the roles of iron, sulfur, and uranium oxidizing microorganisms in the biological solubilization process.

3 Experimental Techniques

The studied sample has been collected during a field trip to Abu Thor area, which is located in southwestern Sinai, Egypt. The sample was ground to -200 mesh size and has been well mixed to attain homogeneity.

3.1 Characterization of the Studied Sample

The collected sample was chemically analyzed to determine its composition, especially major and trace elements. Major elements were determined using the traditional wet chemical techniques [30]. SiO_2, Al_2O_3, TiO_2 and P_2O_5 were determined spectrophotometrically at relevant wavelengths, while both Na and K were determined by the flame photometric technique. On the other hand, Fe_2O_3, CaO, and MgO were evaluated using the titration method while the loss on ignition (L.O.I) was gravimetrically estimated (± 1%). The trace elements content were measured with atomic absorption, uranium was analyzed in the sample as well as in the biological mass by titration against NH_4VO_3 [30]. The practical work was done in the Nuclear Materials Authority laboratories, Cairo, Egypt.

3.2 Radiometric Analysis

The used technique for radiometric measurements is called

the narrow beam transmission method in which utilize a 3"x3" NaI (Tl) gamma ray scintillation detector. The detector is shielded against induced X-rays by 0.6 cm thickness of cylindrical copper for protection and a chamber of lead bricks for isolation from the environmental radiations and also, 5 cm thickness of lead cover protecting the detector. The detector is connected with Tennelec high voltage power supply with HV digital display and nuclear Enterprises main shaping amplifier. Moreover, It is connected with Nuclease PCA- 8000 computer based, 8192 multichannel analysis unit with high level technical operation features and color graphic display of spectra.

3.2 Biological Techniques

The biological investigations comprise the fungal isolation from the studied rock sample and the optimization of the biological solubilization factors as follows:

3.3.1 Culture Medium Preparation and Sterilization

The preparation of Sabouraud agar media g/L was as follows: 10 g/L peptone, 40 g/L dextrose, and 20 g/L agar. The medium pH value was adjusted to a value of 5.6. The preparation of biological leach liquor was 250 ml Erlenmeyer flask has100 ml of peptone broth, and then was supplemented with concentration of 1% from a high-grade uranium. The flasks were autoclaved at 120 °C and 1.5 atm for 20 minutes, inoculated with 1 ml of spore suspension after cooling, and finally were incubated at 30 °C for 7 days.

3.3.2 Isolation of Fungi

After incubation, the culture was filtered several times and the filtrate was used for chemical analyses. The direct plating technique was utilized in the isolation of fungi from the sample by directly spreading a fine powder from a sample upon the agar media surface under sterilization. The agar plates were incubated at 28 °C ± 2 until clearly grew of the fungal colonies then examined with a microscope to identify any contamination [31,32].

3.3.3 Biological Solubilization Experiment

Several parameters were evaluated for the purpose of achieving high efficiency of uranium solubilization. These appropriate parameters include initial pH value (1, 3, 5, 7, 9, 11), pulp density (1, 3, 5, 7, 9)%, incubation time (1, 3, 5, 7, 9, 11) days, and temperature (25, 30, 35, 40, 45) °C. 100 ml of peptone broth in 250 ml Erlenmeyer flasks with ore sample concentrations then autoclaved at 1.5 atm for 20 min, and inoculated with 1ml spore suspension after cooling. After exceeding the incubation time, the medium was filtered and centrifuged at 4000 rpm, repeated for each factor. The filtrate was preserved for chemical determination. Three vial series have been prepared for each experiment.

4 Results and Discussion

4.1 Characterization of the Studied Sample

The studied ore sample was identified geologically as a gibbsite bearing sediment with a chemical characterization as in Table (1).

Table 1: Complete chemical analysis of the studied sample.

Major oxides	*Wt.(%)*	*Trace elements*	Ppm
SiO_2	51.61	***Ni***	70
Al_2O_2	16.71	***Cu***	2163
TiO_2	0.95	***Zn***	393
FeO_2	14	***Zr***	2707
CaO	2.53	***Rb***	33
MgO	0.61	***Y***	93
P_2O_3	0.95	***Ba***	1890
Na_2O	2.53	***Pb***	110
K_2O	2.53	***Sr***	409
L.O.I	8.5	***Ga***	17
Total	102.5	***V***	736
		Nb	76
		Th	246
		U	1450

The minerals that are identified in Abu Thor are generally represented by REEs mainly Nd adsorbed on clay and gypsum, aurorite and monazite [33].

3.1 Radiometric Analysis

The studied sample was measured radiometrically by utilizing the NaI (Tl) scintillation detector [34]. The recorded results were 1587 and 1650 ppm for uranium and radium, respectively, and under the limit of detection for thorium and potassium.

4.2 Fungal Identification

The fungal isolate from the studied sample that has a long-time exposure of soil to heavy metals can lead to physiological adaptation or considerable modification of

their microbial populations, reducing their activity and their number and such changes may be associated with increased metal sorption capacity. It was identified morphologically as *Aspergillus nidulans (A. nidulans)*, which was used in the biological processes in the present work. It has septate hyphae with a woolly texture colony with green colour due to pigmentation of the spores and white mycelia [35, 36].

4.3 Biological Process

The biological solubilization controlling factors and biological sorption controlling factors, which occurred during the biological slubilization process were determined as follows:

4.3.1 Effect of Initial pH

The pH factor has a significant role to affect the quantity and sort of the fungal metabolites that are produced and contributed to the solubilization process. The final pHs decreased by increasing of the fungal medium initial pH. The most suitable pH value was carried out by using the fungal strain A. nidulans at room temperature for 7 days on a peptone broth media recorded the highest leachability at 1% pulp density of the studied sample. The resuls showed that the optimum pH value equals 3 is obviously the highest biological slubilization capacity for uranium, which reached 82%. The decrease of pH value indicating that the metals in ore particles could promote the fungal growth and metabolism in the early stage of solubilization, but the increased pH value appeared to be correlated to the fungal activity as a result of nutrients deficiency, the accumulation of metabolites and uranium component, which the pH factor promote the fungal excreation of organic acids and secondery metabolites. Whereas, the optimized pH for the biological sorption process achieved at the value equals 9. Fig. (5) demonstrated that the quantity of uranium, which solubilized decreased according to increasing the initial pH value of the fungal medium and the uranium uptake was increased with increasing it up to the value equals 9 then decreased. The heavy metals uptake was inhibited at low pH and increased with the initial pH increase, the metal ions biological sorption depends on the pH of the solution that affects the electrostatic binding of ions to appropriate functional groups [37]. Results showed that the maximum fungal sorption capacity for uranium ions concentrations during the solubilization process was observed to be 15.5 mg/g. the sorption process of metal ions depends on the pH othe solution, which influnces electrostatic binding of the uranium ions the fungal active sites and functional groups as amino acids. The role of the existing functional groups are to serve as a matrix of ─CooH and ─NH_2 groups interact with the uranium ions by the phenomena of protonation of the cell wall functional groups that depends on the pH of the solution. In the case of increasing pH, these groups deprotonated and thus formed negatively charged sites. At pH values higher than 9.0, the sorption capacity was decreased. Consequently, uranium ions may be precipitated out because of the high concentrations of OH^- ions in the solution.

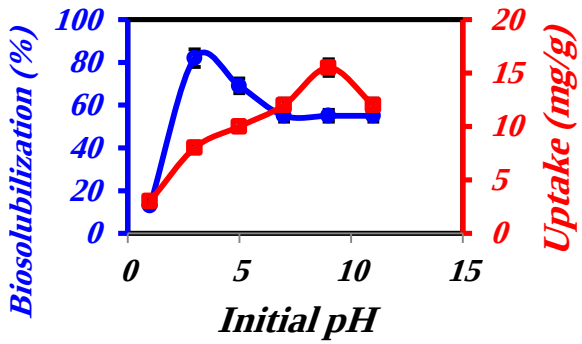

Fig. 5: Different Initial pHs vs. solubilization efficiency and uptake of uranium by *A. nidulans*.

4.3.2 Effect of Pulp Density

The initial metal concentration had a strong effect on the biological solubilization and sorption capacities. The solubilization process of uranium by fungal strain *A. nidulans* increased by increasing the pulp density in the fungal medium up to 3% for 7 days incubation period at the room temperature and a pH value equals 3 that accomplished the best percentage of uranium solubilization capacity, which reached 87% as in Fig. (6). The uranium solubilisation increased in relation with the increase in pulp density, while the uranium leaching rate underwent an opposite response. This phenomenon may be appeared at low pulp densities, as a result of the easily extraction o furanium minerals from the aqueous solution, which contributed to the high uranium leaching rate. At an optimum pulp density value of 3%, it seems that the leaching capacity of uranium decreases as pulp densities increase that due to lacking oxygen transfer may cause limited nutrition supplying to the microorganisms, or distortion of cells in the high solid concentrations, as mentioned by [38]. The negative effect upon the uranium biological solubilization process had been according to the presence of high pulp density. This can be attributed to the improvement of the shear force generated by the high density of the paste, which interfered with the immobilization of the microorganism on the uranium ore surface. Therefore it may be concluded that the decrease ine the solubilization efficiency may be due to lack of oxygen or improper mixing between the lixiviant and ore particle or both. On the other hand, the fungal biosorption capacity can be analyzed from the Fig.(6) trend, the uranium biological sorption best efficiency occurred at 5% pulp density of the studied sample that increased and reached the saturation value recorded 19.5 mg/g *by A. nidulans* . At a high concentration of metal ions, the number of uranium ions sorbed was higher than at a lower concentration of metals, where more bonding sites were available for interaction. The obtained result was in accordance with Amin et al., (2016), who noticed that when the metal ion concentration has increased, biological sorption capacities have also

increased [39]. The microorganisms couldn't be saturated by uranyl ions at low concentrations of uranium, as the uranyl ions amounts were less than the microorganism binding sites which exist on their surface [40]. On the contrary, after microorganisms active sites can be perfectly saturated with uranium ions from their high concentration solution. At the moment that full saturation was occurred, adsorption accessibility of uranium was reduced, that may explain the rapid adsorption at the initial step then slowed down when the saturation took place.

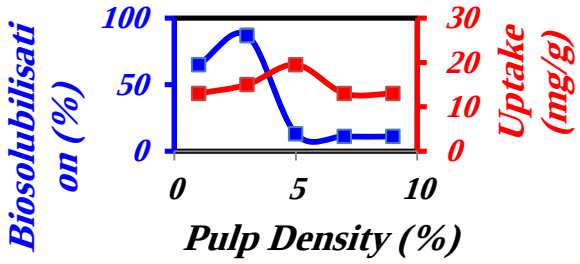

Fig. 6: Different pulp densities vs. solubilization efficiency and uptake of uranium by *A.nidulans*.

4.3.3 Effect of Incubation Periods

Solubilization efficiency of uranium by *A. nidulans* was strongly impacted with the reaction incubation period. The most effective solubilization capacity has happened at pH 3, 3% pulp density, and at room temperature; where the amount of uranium which solubilized from the sample was reached up to 86% in 7 days. After which the uranium-solubilization efficiency of the tested fungal isolate was partially reduced up to 11 days (Fig.7). These results are consistent with those achieved by El Sayed (2012) who demonstrated the optimum incubation periods were exhibited at 8 and 6 days for *A. niger* and *A. fumigatus* respectively to uranium and REEs solubilization from its gibbsite ore sample [41]. Furthermore, in the biological sorption process an saturation time was achieved at 9 days and after that no further increase in the amount of the sorbed uranium was observed. The initial rapid phase is probably due to the abundant of availability of active metal binding sites on the fungal surface and with the gradual occupancy of those sites. The sorption becomes less efficient in the slower stage as a result of competition for decreasing availability of active binding sites intensities by the metal ions remainingin solution. The contact time impact on the uranium biological sorption was investigated during the biological solubilization, indicating that the sorption happened as rapid adsorption upon the microorganism surface from 3 to7 days as a first stage and the second one was slow from 7 to10 days, maybe as a result of electrostatic interactions between the fungal active sites with metal ions [39]. As time, the efficiency of active sites decreased, which reduce the adsorption rate as indicated before [40].

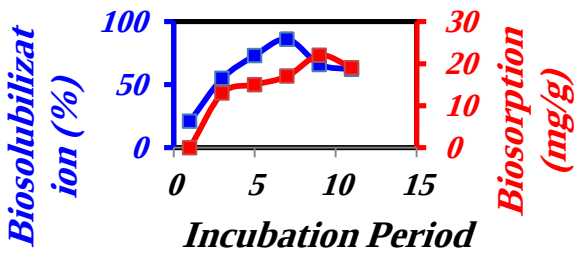

Fig.7: Different incubation periods vs. solubilization efficiency and uptake of uranium by *A.nidulans*.

4.3.4 Effect of Incubation Temperatures

The effect of the incubation temperature was found to be significant upon the solubilization of uranium process from its ore; its maximum solubilization efficiency was exhibited at 30 °C as considered the most appropriate temperature for the growth and metabolisms of the fungi. Therefore, many organic acids were produced at this temperature, and more uranium ions were solubilized from the sample. When the temperature is not suitable, the growth and enzymatic activities of microorganisms is limited especially when it is too high, where *A. Nidulans* solubilized approximately 84% of the uranium of the studied sample with the previous optimum conditions; pH value equals 3, 3% pulp density, and 7 days as an incubation period (Fig. 8). The final pH value at this incubation temperature shifted towards acidity. the heavy metals maximum leaching achieved at 30 °C and 35 °C for *A. niger* and *A. fumigatus* respectively [41]. by temperatures increasing (40 °C and 45 °C), the fungal metabolic rate decreases and the microorganism can not even be live [37]. At the same time, the uptake of uranium from studied sample by the tested fungal biological mass was low at 25 °C for *A. nidulans* estimated 18 mg/g, and reached its maximum efficiency at 35℃ which recorded 24 mg/g. Above 35 °C the biological sorption efficiency of uranium was decreased by increasing the temperature due to weakened growth of the fungi. It is not recommended, from the economical point of view, to use high temperatures during any biological process [42]. From the obtained results can suggested a weak interaction between uranyl ions and binding sites of biomass could be due to Vander-waal's interactions, hydrogen bonding, etc., which are broken at high temperatures. This could be the reason, that at a high temperature, a steep decrease in uranium uptake was observed.

4.3.5 Scaling-up the Uranium Solubilization Process

By using *A. nidulans* and applying the uranium biological slubilization optimum conditions that obtained previously (pH value equals 3, 3% pulp density, 7 days incubation periods, and 30^0C incubation temperatures) on 1 kg of the studied sample, the efficiency of uranium to be recovered

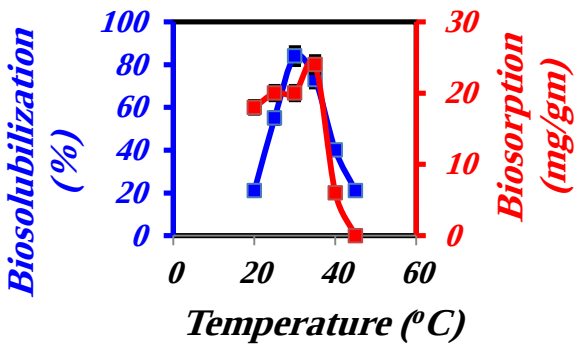

Fig. 8: Different incubation temperatures vs. solubilization efficiency and uptake of uranium by *A.nidulans*.

achieved a 83 %, which accomplished the highest biological subilization efficiency. The attained efficiency is close to that observed [43] as the maximum biological dissolved efficiency of uranium recovery reached 85.14% by *Acidithiobacillus ferrooxidans* bacteria. The maximum efficiency of uranium recovery 71% uranium was obtained with the *Cladosporium oxysporum* isolated fungal strain, from water samples obtained from uranium mines, whereas exhibited 59% and 50% by *Aspergillus flavus* and *Curvularia clavata* respectively [43, 44]. The recovery of uranium from low-grade ore using *Aspergillus niger* isolated from uraniferous rock sample accomplished 71.4% and asserted that the uranium is nearly free from other radionuclides present in the original sample without any subsequent purification processes, whereas could not achieved by the conventional process of uranium recovery [45].

5 Conclusions

In the present work, the direct biological solubilization mechanism was achieved by utilizing *A. nidulans*, which requires physical contact between the microorganism membrane cell surface and the ore. It was acquired an optimization at pH value equals 3, 3% pulp density, 7 days incubation time, and 30 °C incubation temperatures, which achieved a high biological solubilization efficiency. At the same time as the biological leaching process, the uptake efficiency was estimated as a dual process. The absorption accomplished optimum conditions where the pH value equals 9, 5% pulp density, 9 days incubation time, and 35 °C incubation temperatures. Furthermore, the biological recovery of uranium upon 1 kg of the ore sample using *A. nidulans* achieved 83%.

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