Quest Journals
Journal of Research in Environmental and Earth Sciences
Volume 10 ~ Issue 12 (2024) pp: 10-24
ISSN(Online) :2348-2532
www.questjournals.org

Research Paper

Bioleaching of Uranium from Black Shale Using *Aspergillus Niger*

Nareman M. Harpy
Nuclear Materials Authority, Cairo, Egypt

Abstract
Black shale is significant due to its rich organic matter, serving as a primary source rock for hydrocarbons like oil and natural gas. Over time, its organic material undergoes thermal maturation, forming fossil fuels. The presence of kerogen also plays a crucial role in petroleum generation, making black shale vital in petroleum geology. This study investigates the uranium bioleaching efficiency by Aspergillus niger as a potent bioleaching agent of black shale ore samples (SH-1, SH-2, and SH-3) under varying conditions, including ore concentration, temperature, pH, and time duration. The research focuses on the uranium leaching rate, the final pH of the media, and the dry weight of the biomass. Key findings reveal that higher ore concentrations generally result in decreased uranium bioleaching efficiency, where the highest leaching observed at lower concentrations. An inverse relationship was noted between the final pH and uranium leaching, while biomass accumulation varied significantly among samples. Temperature optimization indicated that 30-35°C was the most effective, with all samples achieving over 74% uranium leachability. The initial pH also played a crucial role, extremely acidic or alkaline conditions (pH 1 or 10) significantly lowered leaching rates. Regarding time duration, the bioleaching process peaked in efficiency between 5 and 7 days, after which uranium leaching declined. The highest leaching rates were observed at 7 days for SH-1 and SH-2 samples. The final pH decreased over time but stabilized after 9 days. This study underscores the potential of optimizing bioleaching conditions to enhance uranium leaching from ores using fungal-mediated processes. Among the factors examined, temperature and pH were identified as critical for maximizing efficiency. Further research could refine these conditions, improving the commercial viability of bioleaching for uranium leaching.

Keywords*: Fungi, Bioleaching of uranium, Black shale, Low-grade ores.*

R*eceived 5 Nov., 2024;* ***R****evised 08 Dec., 2024;* ***A****ccepted 10 Dec., 2024*

I. Introduction

As the world intensifies efforts to reduce carbon emissions, nuclear power is increasingly recognized as a vital component of the global energy mix due to its high efficiency and low environmental impact. This growing demand for nuclear energy has significantly spurred the development of uranium mining, given uranium's strategic importance as the primary fuel for nuclear reactors. Ensuring a reliable supply of uranium is crucial for maintaining energy security and reducing dependence on fossil fuels, especially as global efforts to achieve energy independence intensify.

Bioleaching is an environmentally friendly and economically feasible technology that has gained considerable attention for its efficiency in resource leaching. This method has been successfully applied in extracting various metals, including copper, gold, cobalt, and uranium (Abdollahi et al., 2021; Li et al., 2020 and Martins & Leao, 2020). Recent studies have demonstrated that certain organic acids can dissolve minerals (Lazo et al., 2017 and Wang et al., 2013) and exhibit strong complexation abilities with metal ions (Kimuro et al., 2019; Sun et al., 2022a and Xu et al., 2022). For instance, uranyl ions (UO_2^{2+}) can form highly soluble complexes with organic ligands such as citric and oxalic acids (Jagetiya & Sharma, 2012 and Sun et al., 2022b).

Studies on the interactions between radionuclides and microorganisms have focused on the removal, leaching, and detoxification of radionuclide pollutants. These interactions, which include oxidation, reduction, chelation, biosorption, complexation, precipitation, and bioleaching, facilitate the biotransfer of radionuclides between environmental compartments and microorganisms (Francis, 1998; Gadd, 2000 and Gadd, 2002).

Fungal bioleaching, the process of solubilizing metals using fungi, is emerging as a sustainable alternative to conventional mining techniques. Filamentous fungi, particularly those from the *Aspergillus* and

Penicillium genera, have demonstrated the ability to solubilize metals through the secretion of organic acids, even in high metal concentrations (Burgstaller & Schinner, 1993 and Gadd, 1999). Metals are solubilized via bioleaching reactions such as acidolysis, redoxolysis, bioaccumulation, and chelate formation (Asghari et al., 2013; Borja et al., 2016 and Le et al., 2006).

Acidophiles produce various acids during their metabolic processes, which contribute to the dissolution of metal ores and, consequently, decrease the availability of metals. In contrast, chemolithotrophs facilitate the oxidation and reduction of sulfur compounds to generate energy, which further enhances acid production and increases metal solubilization. The bioleaching processes performed by both acidophiles and chemolithotrophs are eco-friendly and can be conducted at lower temperatures, offering additional benefits such as energy savings. (Adetunji et al., 2023 and Tang et al., 2024).

The bioleaching mechanism employed by fungi involves a combination of biomechanical forces exerted by fungal hyphae and the complexation effects of organic acids, leading to the dissolution of SiO_2 and other minerals on the ore surface. This process enhances uranium leaching by improving the ore's internal structure, increasing its porosity, and facilitating the transfer of uranium from the solid to the liquid phase. While organic acids play a primary role, the contribution of fungal hyphae is also significant (Wang et al., 2024).

The advancement of civilization and technology has significantly increased the consumption of radionuclides, creating challenges for industries as many high-grade mineral deposits are becoming scarce. Consequently, the demand for radionuclides now relies more heavily on low-grade ores (Bhatti et al., 1989 and Anjum et al., 2012). Among the naturally occurring actinides, uranium is the most abundant, with an average concentration of 2.8 mg kg^{-1} in the Earth's crust (Rudnick & Gao, 2014 and Escareño-Juárez et al., 2019).

Black shale is geologically significant due to its rich organic content, making it a key source of hydrocarbons, particularly shale oil and gas. Its sedimentary characteristics also offer insights into past environmental and climatic conditions. Studies of black shale help reconstruct ancient depositional environments, track changes in ocean oxygen levels, and understand tectonic events. Recent research highlights its economic importance, especially with its role in the shale gas boom, such as in China's Fuling gas field, which is a major producer (Li et al. 2023)

Black shale, a fine-grained sedimentary rock rich in organic material, often contains valuable metals such as copper, nickel, zinc, and uranium. Traditional methods of metal leaching from black shale can be costly and environmentally harmful, making bioleaching a more attractive option (Gadd, 2000). Bioleaching utilizes acidophilic microorganisms to oxidize sulfide minerals in black shale, converting them into soluble metal sulfates, which can then be recovered through hydrometallurgical processes (Schippers & Sand, 1999).

Aspergillus niger is particularly effective in this context, producing organic acids like citric and oxalic acids, which lower the pH and dissolve metal-bearing minerals. This fungal bioleaching process mobilizes metals from the rock matrix, facilitating their leaching and reducing the environmental impact of traditional mining methods (Gadd, 2007 and Brierley & Brierley, 2013). Recent advancements in microbial biotechnology and geochemical understanding have further enhanced the efficiency of bioleaching processes, especially in regions rich in black shale deposits (Brierley & Brierley, 2022). Tezyapar Kara et al., 2023 recommend that more pilot studies and collaborative research involving chemists, biologists, and metallurgists working together should be conducted to scale up bioleaching for industrial use.

In response to the challenges posed by population growth in Egypt, such as excessive electricity consumption, acute energy shortages, and the high costs and environmental impact of fossil fuel combustion, there is an urgent need for alternative energy sources. To address these issues, Egypt has recently signed an agreement to build its first nuclear power plant, aimed at mitigating electricity shortages. Consequently, the efficient, safe, and cost-effective leaching of uranium has become critically important.

To meet this demand, the present study focused on exploring the potential for effective microbial leaching of uranium from rock samples from Sinai, Egypt. The study tested the bioleaching capabilities of several native fungi using direct techniques and aimed to optimize uranium bioleaching efficiency by evaluating the impact of various factors on the bioleaching process.

Experimental Sections

Sampling and Samples Preparation

Three radioactive geologic samples were collected from the Budaa region in southwestern Sinai, Egypt, belonging to a geologic rock type known as black shale. The samples, designated SH-1, SH-2, and SH-3, are described as follows: SH-1 is grey and brown shale, SH-2 is carbonaceous shale rich in kerogen, and SH-3 is black shale, characterized as dark and friable. The samples were stored in sterile polyethylene packets, then crushed, quartered, and ground for further investigation.

Chemical Analysis of Samples

After the samples were crushed and ground, each underwent digestion using an acid attack method. In a Teflon beaker, 0.5g of each sample was treated with a mixture of 10 ml concentrated hydrofluoric acid (HF), 5 ml concentrated nitric acid (HNO_3), and 5 ml perchloric acid ($HClO_4$). The mixture was then heated at 220°C until completely dry. Following this, 5 ml of 1:1 diluted hydrochloric acid (HCl) was added, and the solution was diluted with distilled water to a final volume of 50 ml, following the procedure described by Shapiro, 1962.

The major elements, in their oxide forms, were measured using wet analysis techniques. The concentrations of SiO_2, Al_2O_3, TiO_2, and P_2O_5 were determined calorimetrically using a spectrophotometer (LABOMED, model SPECTRO UVD, USA). Total oxides, including Fe_2O_3, MgO, and CaO, were evaluated using traditional titration techniques. Additionally, the contents of Na and K were determined using flame photometry (JENWAY, model PEP7, UK). The loss on ignition (L.O.I.) was calculated gravimetrically.

For uranium content analysis, the studied samples were measured according to Farag et al., 2015 using a titration method. Both ore samples and bioleaching liquors were titrated against standard ammonium metavanadate (NH_4VO_3), with the endpoint indicated by the appearance of a purplish color. The uranium concentration was calculated using the following equation:

$$\mathbf{U\ (mg/L) = (T \times V_1 \times 10^3)\ /\ V\ ppm}$$

Where: T is the titration intensity of the NH_4VO_3 solution, V_1 is the volume of NH_4VO_3 solution consumed, V is the volume of the measured sample.

Microbiological Studies

The native fungal strains isolated from the studied sample, having undergone prolonged exposure to heavy metals in the soil, may develop physiological adaptations or significant shifts in their microbial populations. These changes can affect the strains' activity and potentially alter their capacity and behavior in interacting with metals. *Aspergillus niger* which was used in the bioprocess in the present work.

Fungal Growth, Isolation, Purification, and Identification: The direct plating technique was utilized to cultivate fungi from the samples. Fine powder from each sample was evenly spread onto Sabouraud agar plates and incubated for 7 days to promote fungal growth, following the methods described by Gilman, 1957 and Pitt, 1979. Individual fungal colonies that developed were carefully selected, and hyphal tips were extracted and transferred onto fresh Sabouraud agar plates to obtain pure fungal isolates. These isolates were routinely examined under a microscope to ensure the absence of contamination. The identification of the purified fungal strains was identified based on macroscopic and microscopic characteristics according to (Kilch, 2002; Watanabe, 2002 and Humber, 1997) up to species level.

The medium used in this work was Dox agar medium of composition (gl^{-1}): $NaNO_3$, 2; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.5H_2O$ trace; sucrose, 30; agar, 15.5g, yeast extract was added to initiate fungal growth. The pH value of the media was adjusted to be 6.5 before autoclaving at 1.5 atm for 20 minutes.

Investigation of factors affecting uranium bioleaching

To determine the optimal conditions for uranium solubilization, various factors were investigated, including the activity of different fungal strains, ore concentrations, initial pH, incubation periods, and incubation temperatures. The impact of these factors on both the growth of the isolated fungal strains and the efficiency of uranium solubilization was thoroughly assessed.

Preparation of the Leach Liquor:

One hundred milliliters of Dox liquid medium were placed in 250 ml Erlenmeyer flasks, which were then supplemented with varying ore concentrations [0%, 1%, 3%, 5%, and 7% (w/v)]. The flasks were autoclaved at 1.5 atm for 20 minutes. After cooling, 0.5 ml of spore suspension was inoculated into each flask, and the flasks were incubated at room temperature for 7 days on an orbital shaker set at 100 rpm. At the end of the incubation period, the mycelial mats were harvested, thoroughly washed with distilled water, dried at 85°C for 24 hours, and their dry weight was determined. The filtrates were retained for subsequent uranium analysis.

The steps involved in preparing the Dox liquid medium, supplementing it with ore, autoclaving, spore inoculation, processing mycelia, and determining uranium content were repeated for all subsequent experiments. The flasks were incubated for various periods (3, 5, 7, 9, and 10 days). Triplicate sets of flasks were incubated at temperatures of 20°C, 30°C, 35°C, 40°C, and 45°C, and the initial pH was adjusted to 1, 2, 5, 7, 9, and 10. Uranium content in the fungal filtrate was determined as described previously. Additionally, the mycelial mats were harvested, dried, and their dry weight was measured for all the different factors investigated.

II. Results and discussion

Table 1 and Figure 1 shows the chemical composition of the three samples (SH-1, SH-2, and SH-3), revealing varying concentrations of key oxides and uranium (U) content. SiO_2 is a major component in all samples, with SH-1 and SH-3 having higher levels, indicating a more siliceous nature, while SH-2 contains significantly less. Al_2O_3 is notably present, with SH-1 having the highest concentration at 17.05%. CaO is present in moderate amounts, while MgO varies significantly, reaching its highest concentration in SH-3. Loss on Ignition (L.O.I.)

reflects the weight loss upon heating due to the release of volatile substances like water and CO_2. SH-2 shows a significantly higher L.O.I., which may indicate a higher content of volatile materials or organic matter.

Table 1: Results of major oxides (wt%) of the studied samples.

	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	P_2O_5	L.O.I	Total	U (ppm)
SH-1	53.75	1.1	17.05	0.045	4.45	3	1.1	1.05	0.045	17.45	99.04	205
SH-2	57.3	0.5	5.1	0.033	1.7	2.4	0.3	0.3	0.04	31.1	98.773	100
SH-3	51.5	1	12.6	0.1	4.9	5.77	1.03	0.87	0.02	23.1	100.89	165

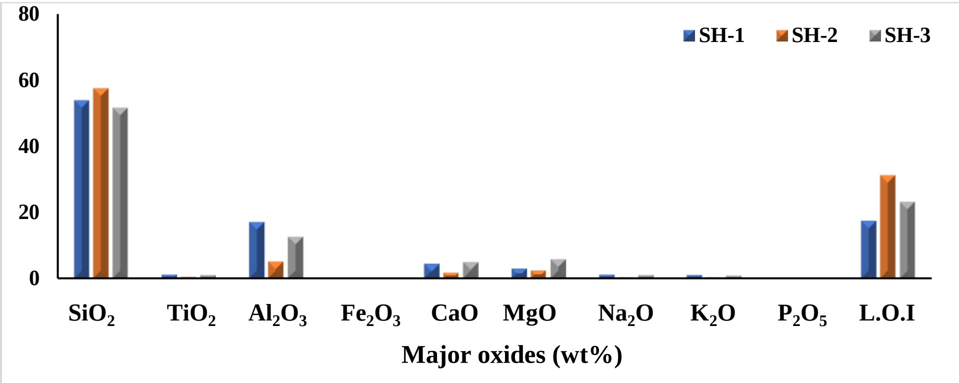

Figure 1: Percentage of major oxides in the studied sample.

Microorganisms' isolation and identification

Four fungal species were successfully isolated from the tested samples, all of which belong to the genus *Aspergillus*. These fungi were characterized by their septate mycelium, which features a vesicle at the end of the sporophore and bears catenate conidiospores. Additionally, they produce ascospores. The cultures of these isolates on Czapek's agar plates. The identified species include, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ficuum and Aspergillus fumigatus.*

Direct Bioleaching Experiment (Contact Leaching)

The direct microbial leaching studies on the SH-1, SH-2, and SH-3 indigenous fungal isolates demonstrated varying levels of uranium bioleaching efficiency using direct techniques. The bioleaching capabilities differed among the tested isolates. All the fungi were able to grow in the presence of a 1% ore concentration of the studied samples, which were incubated at 30°C for 7 days. The leaching efficiencies, presented in Fig. 2, show that *Aspergillus niger* exhibited the highest bioleaching efficiency at 71.28%, followed by *Aspergillus flavus* at 66.20% for the SH-1 sample. Therefore, *A. niger* demonstrated superior uranium bioleaching efficiency compared to the other fungi and was thus selected for use in the bioprocess in the present study.

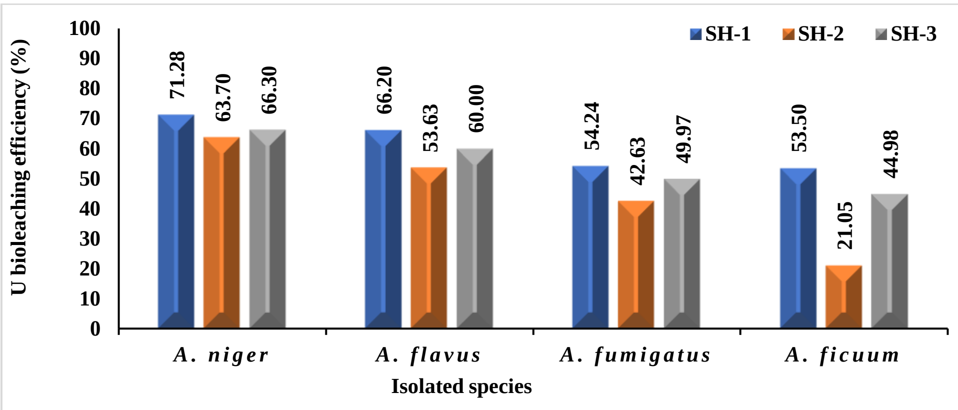

Figure 2: Leaching efficiencies of the isolated species from the studied samples.

Effect of ore concentrations

The concentration of ores significantly impacts bioleaching efficiency. Higher concentrations lead to increase metal ion release, which can be toxic to the microorganisms involved in bioleaching (Zhang et al., 2023). Optimizing ore concentration is essential to balance metal leaching with maintaining microbial health (Tezyapar Kara et al., 2023).

Trace amounts of metal ions are crucial for microbial growth and metabolic activity, serving as enzyme co-factors (Alshiyab et al., 2008). However, high concentrations become cytotoxic, disrupting various cellular processes. The cytotoxic effects of metal ions on acidophilic microorganisms (APM) manifest in several ways: (1) Metal ions can displace essential metals from protein binding sites; (2) Metal ions disrupt cell membrane integrity, damage organelles, and ultimately induce apoptosis (Bruins et al., 2000 and Zhang et al., 2023); (3) Metal ions interfere with the synthesis of intracellular macromolecules, affecting transcription and translation (Bruins et al., 2000). For instance, copper ions alter protein conformation by covalently modifying cysteine and histidine groups, impairing protein function (Fan et al., 2004).

Figure 3 present data examining the effects of varying ore concentrations (1% to 7%) on bioleaching performance. Key parameters measured include the percentage of uranium leached, the final pH of the medium after incubation, and the dry biomass weight per 100 ml of medium. A control sample using *Aspergillus niger* is also included.

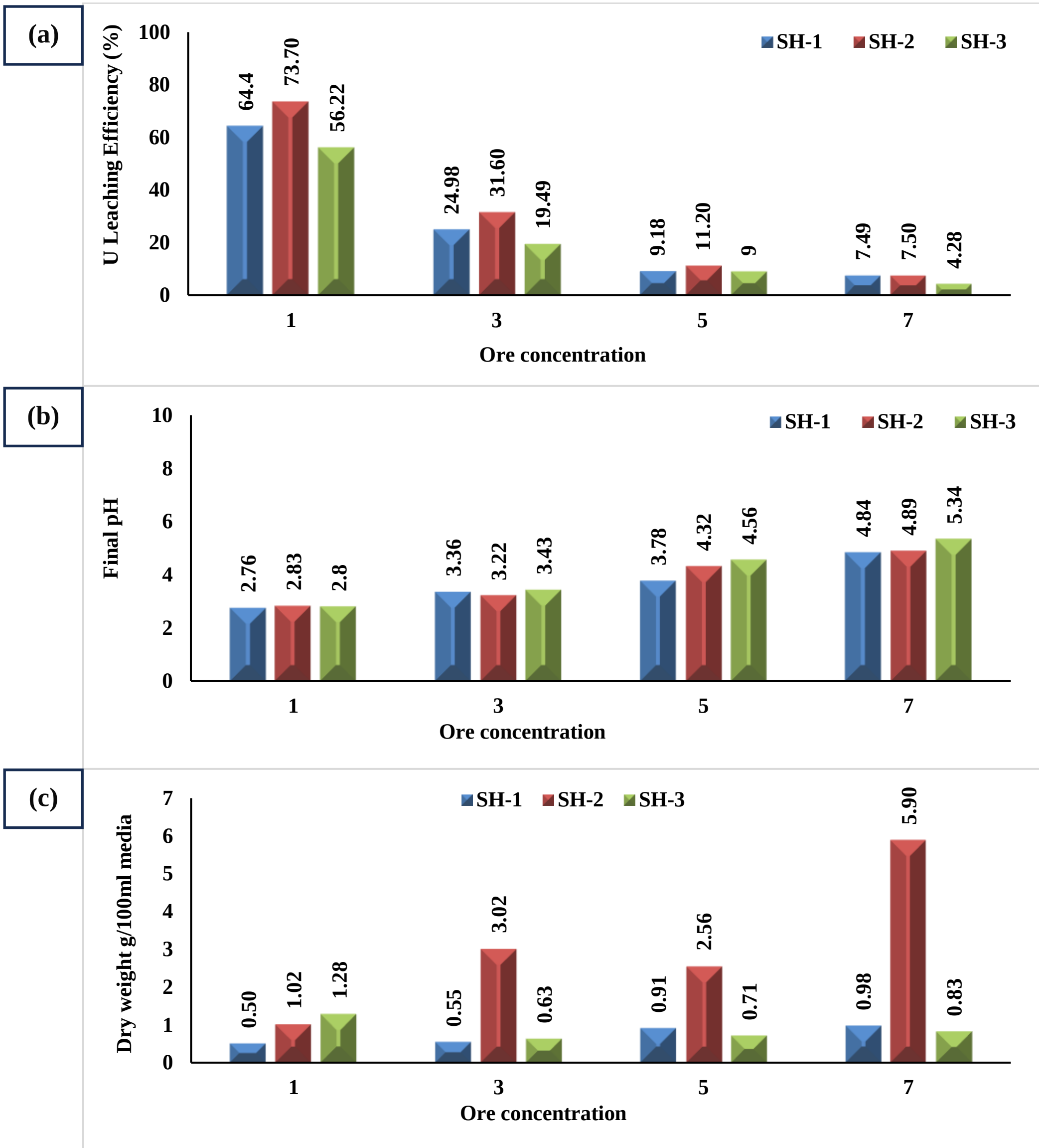

Figure 3: Effect of different ore concentrations on (a) uranium bioleaching percentage, (b) final pH, and (c) dry weight (g/100 ml of media).

In SH-1, uranium leaching decreases as ore concentration increases, starting from 64.4% at 1% ore concentration and dropping to 7.49% at 7%. A similar trend is observed in SH-2, where uranium leaching declines from 73.70% at 1% ore concentration to 7.50% at 7%. In SH-3, uranium leaching also decreases, starting at 56.22% at 1% and reducing to 4.28% at 7% ore concentration. Across all samples, the bioleaching effectiveness diminishes as ore concentration increases. SH-2 achieves the lowest uranium percentage at the highest ore concentration, suggesting it might be the most effective under these conditions.

The decrease in uranium bioleaching efficiency with increasing ore concentration can be attributed to several factors affecting the bioleaching environment. Higher ore concentrations introduce more metal ions, which can be toxic to the microorganisms involved in bioleaching. This toxicity can inhibit microbial growth, metabolic activity, and the production of organic acids or other compounds essential for uranium leaching (Kour et al., 2021).

A review on the cytotoxic mechanisms of metal ions in bioleaching techniques highlights several ways in which metal ions induce cytotoxicity in microbial cells. These mechanisms include the replacement of essential metals at protein binding sites, disruption of cellular membrane integrity, and interference with the transcription and translation of genetic information, leading to apoptosis. This aligns with earlier findings that metal ions, such as copper, can modify protein structures and disrupt their normal biological functions (Sarkodie et al., 2022).

Final pH values for the samples show an increasing trend with higher ore concentrations. For SH-1, pH rises from 2.76 at 1% ore to 4.84 at 9% ore. In SH-2, pH increases from 2.83 at 1% ore to 4.45 at 9% ore. Similarly, for SH-3, pH increases from 2.8 at 1% ore to 3.96 at 9% ore. This consistent increase in pH across all samples suggests that higher ore concentrations may lead to greater neutralization of acidic components. SH-1 exhibits the highest final pH, indicating a more effective neutralization process compared to SH-2 and SH-3.

The pH increases, due to the neutralization of acids by the ore's buffering components, the solubilization of uranium significantly decreases. This results in lower leaching rates, as uranium is less likely to remain in a soluble, bioavailable form (Sarkodie et al., 2022)

Another study from 2021 confirmed that maintaining an optimal acidic pH is crucial for efficient uranium bioleaching, and any shift toward neutral or alkaline pH conditions can severely impair the process by reducing uranium solubility and microbial activity (Kour et al., 2021).

Dry weight measurements reveal varying responses to ore concentration among the samples. In SH-1, the dry weight increases from 0.50 g at 1% ore to 0.98 g at 7% ore. SH-2 shows a significant rise in dry weight, increasing from 1.02 g at 1% ore to 5.9 g at 7% ore. SH-3 exhibits a more modest increase, with dry weight rising from 1.28 g at 1% ore to 0.83 g at 7% ore.

These changes in dry weight suggest that biomass responses to ore concentration vary among the samples. Generally, dry weight increases with higher ore concentrations in SH-1, SH-2, and SH-3, indicating potential biomass growth or accumulation of other materials. This increase may be due to microorganism proliferation driven by the availability of nutrients released from the ore. As microorganisms grow, they might accumulate more metals within their cells or produce extracellular polymeric substances, contributing to the rise in dry weight. Additionally, microorganisms could be accumulating stress-related compounds or other cellular materials in response to higher metal concentrations, leading to increased biomass.

Bioaccumulation involves the uptake of metals from a bioleaching medium through the outer membranes of microorganisms (Trivedi et al., 2022). In this interaction, soluble metals are transported intracellularly via the cell membrane, resulting in metal ion accumulation in the cell vacuole (Dusengemungu et al., 2021). Functional groups such as phosphate, amine, carboxyl, and hydroxyl on the cell wall facilitate metal solubilization from a solid matrix. Metal ions act as cation exchangers and bind to these functional groups. Metal ion solubilization by fungal mycelium occurs through both active biosynthetic reactions and passive adsorption (Dusengemungu et al., 2021 and Adetunji et al., 2023).

In present experiments, 1% ore concentration was found to be optimal for fungal growth and uranium solubilization. At low to moderate concentrations, fungal growth is supported by the availability of essential nutrients and the manageable level of metal ions for bioleaching. However, at higher concentrations, metal toxicity, oxidative stress, and changes in pH can inhibit fungal growth, reduce bioleaching efficiency, and slow down biomass production. Understanding the optimal ore concentration for fungal growth is essential to balancing nutrient availability and minimizing toxicity for effective bioleaching.

Effect of Initial pH

Initial pH is a crucial factor influencing fungal activity in metal leaching processes. Optimizing the initial pH is essential to create a favorable environment that supports both fungal growth and its leaching efficiency.

Organic acids produced by fungal strains play a crucial role in bioleaching, particularly in the leaching of metals such as uranium. These acids, typically including citric, oxalic, and gluconic acids, are secreted by fungi as part of their metabolic processes. The key functions of these organic acids in bioleaching include **Metal Solubilization:** Organic acids can chelate metal ions, making them more soluble. In the context of uranium, the acids bind to uranium compounds, converting them into soluble forms that can be more easily extracted from ores or contaminated environments (Gadd, 2007 and Fomina & Gadd, 2014). **pH Reduction:** The production of organic acids lowers the pH of the surrounding environment, which can enhance the solubility of metals like uranium. A lower pH environment also helps in breaking down the mineral matrix, releasing trapped uranium (Diels et al., 2002 and Gadd, 2017). **Complexation:** Organic acids form stable complexes with metal ions. This complexation helps in mobilizing uranium from solid phases into the solution, facilitating its leaching in the bioleaching process (Li et al., 2021 and Rhee et al., 2012 **Enhanced Leaching Efficiency:** The presence of organic acids can improve the overall efficiency of the bioleaching process by increasing the availability of uranium and other metals for leaching. This is particularly important in low-grade ores where conventional leaching methods might be less effective (Tsekova, and Ganeva, 2004 and Hamidpour et al., 2020).

The experiments were conducted at six different initial pH values: 1, 2, 5, 7, 9, and 10. For SH-1, SH-2, and SH-3, uranium bioleaching percentage, final pH, and dry weight were measured. A control sample was used as a baseline for comparison.

Figure 4 shows that in SH-1, the uranium bioleaching percentage peaks at pH 5 (76.53%) and decreases as the pH rises to 10. SH-2 follows a similar trend, with a peak uranium percentage of 74.74% at pH 5, followed by a decline. SH-3 also peaks at pH 5 (66.36%) but experiences a more significant drop at pH 9 and 10. Across all samples, uranium retention or leaching is highest at pH 5, suggesting this pH level is optimal for uranium interaction due to its chemical properties.

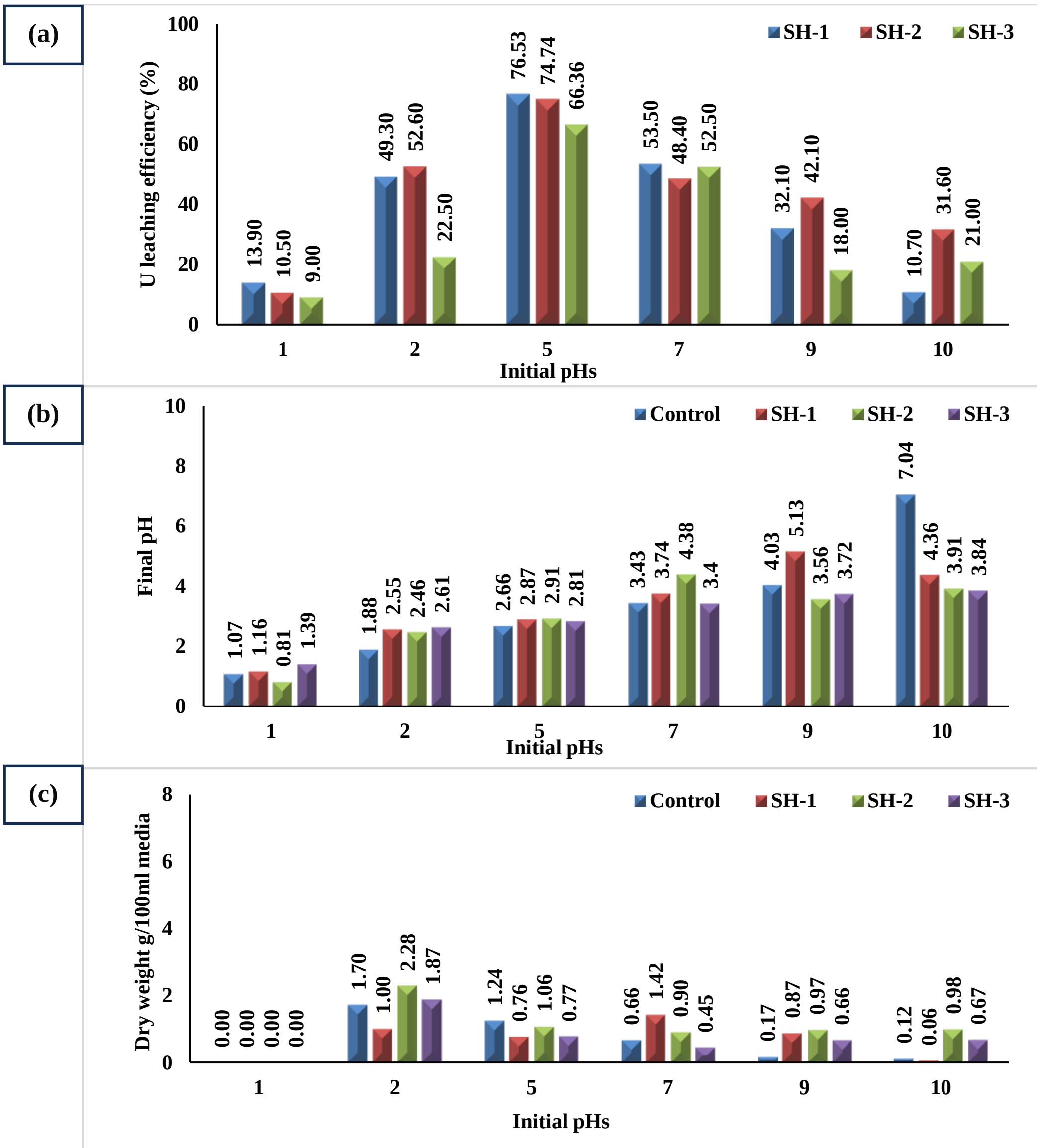

Figure 4: Effect of different initial pHs on (a) uranium bioleaching percentage, (b) final pH, and (c) dry weight (g/100 ml of media).

For final pH, SH-1 shows an increase with the initial pH but does not reach the initial levels, indicating buffering or interaction that prevents the final pH from matching the initial pH. SH-2 also exhibits a rise in final pH with the initial pH, with slight variations compared to SH-1. SH-3's final pH stabilizes around 3.40-3.84 for initial pH levels of 7 and above. The control sample's final pH increases more linearly with the initial pH, particularly reaching a higher final pH of 7.04 at an initial pH of 10. The final pH values are generally lower than the initial pH, indicating that the media has some buffering capacity or that the sample materials interact with the pH. The control sample's final pH is closer to the initial pH, reflecting less interaction.

The initial pH of the growth medium plays a critical role in determining the growth of fungi, including *Aspergillus niger*. pH influences fungal metabolism, enzyme activity, nutrient availability, and the overall environment in which the fungus grows.

In terms of dry weight, SH-1 shows a significant decrease at higher pH levels, with a notably low value of 0.06 g/100 ml at pH 10. SH-2 experiences a less drastic decrease, with higher dry weight values at pH 9 and 10. SH-3 exhibits generally lower dry weight across all pH levels, particularly at higher pH levels. The control sample also shows a consistent decrease in dry weight with increasing pH, reaching its lowest at pH 10. The decrease in dry weight across all samples, especially at pH 10, could be due to degradation of sample material or loss of mass resulting from chemical reactions at higher pH levels.

The initial pH significantly affects the growth of *Aspergillus niger* by influencing enzyme activity, nutrient availability, organic acid production, cell membrane integrity, and competition with other microorganisms. In these experiments, an initial pH of around 5 was found to be optimal for fungal growth and uranium solubilization, as it provided the best conditions for enzyme function, acid production, and nutrient uptake. At pH levels outside this optimal range, growth and bioleaching efficiency were reduced due to less favorable conditions for metabolic activity and nutrient availability.

Effect of Incubation periods

The duration of incubation affects fungal growth and uranium bioleaching efficiency. Initially, shorter incubation periods enable fungi to establish and begin growing. As the period extends, fungal growth typically increases, peaking under optimal conditions. However, extended incubation may lead to growth plateau or decline due to nutrient depletion and byproduct accumulation.

In figure 5 the dry weight of *Aspergillus niger* fungal biomass was measured at various time intervals to evaluate its growth progression. The recorded dry weights are presented as follows: day 3: 0.17, day 5: 0.31, day 7: 0.35, day 9: 0.94 and day 10: 1.01 g/100ml. The data indicates a steady increase in biomass over time, with a significant acceleration in growth between days 7 and 9. The growth rate seems to plateau between days 9 and 10, suggesting the fungus is approaching its peak growth.

The growth rate of *Aspergillus niger* between different intervals was calculated using the formula:

Growth Rate= (Dry Weight at Day X−Dry Weight at Day X_1 Days) / Days Interval

The growth rates of *Aspergillus niger* were assessed at various time intervals: from day 3 to 5, the growth rate was 0.07 g/day; from day 5 to 7, it was 0.02 g/day; from day 7 to 9, it increased to 0.29 g/day; and from day 9 to 10, it decreased to 0.07 g/day. The highest growth rate occurred between days 7 and 9, indicating this period is critical for maximizing biomass yield. After day 9, the growth rate significantly declines, suggesting that the fungus is entering a stationary phase, likely due to nutrient depletion or other limiting factors.

The final pH of the fungal growth medium was monitored at the same intervals: day 3 (pH 2.87), day 5 (pH 3.38), day 7 (pH 2.39), day 9 (pH 3.17), and day 10 (pH 4.50). The pH fluctuates over time, initially increasing slightly from day 3 to day 5, then dropping on day 7, and rising consistently towards day 10. The final pH reached its highest value of 4.5 on day 10.

The data suggests a correlation between fungal growth and pH changes in the media. The decrease in pH observed on day 7 coincides with the slower growth rate, while the subsequent increase in pH aligns with the growth plateau observed between days 9 and 10. These pH fluctuations likely reflect the metabolic activities of the fungus, such as organic acid production or nutrient consumption, which impact the medium's pH. understanding this relationship can aid in optimizing culture conditions for maximum biomass production.

Over time, fungal growth can affect the pH of the medium due to the release of organic acids or consumption of nutrients. In this data, pH levels fluctuated throughout the incubation, with the sharpest changes occurring after the stationary phase (7–9 days). These pH changes may further influence fungal activity, with more acidic conditions supporting fungal growth in earlier stages, and higher pH levels (towards the end of incubation) inhibiting growth.

In summary, *Aspergillus niger* exhibits the most rapid growth between days 7 and 9, with a significant growth plateau after day 9. The fluctuation in pH during this period suggests a link between metabolic activity and growth rate, underscoring the importance of monitoring and controlling pH to optimize fungal biomass production.

The data presented in figure 5 illustrates the effects of incubation time on uranium removal, final pH, and the dry weight of *Aspergillus niger* biomass in different samples (SH-1, SH-2, SH-3) and a control.

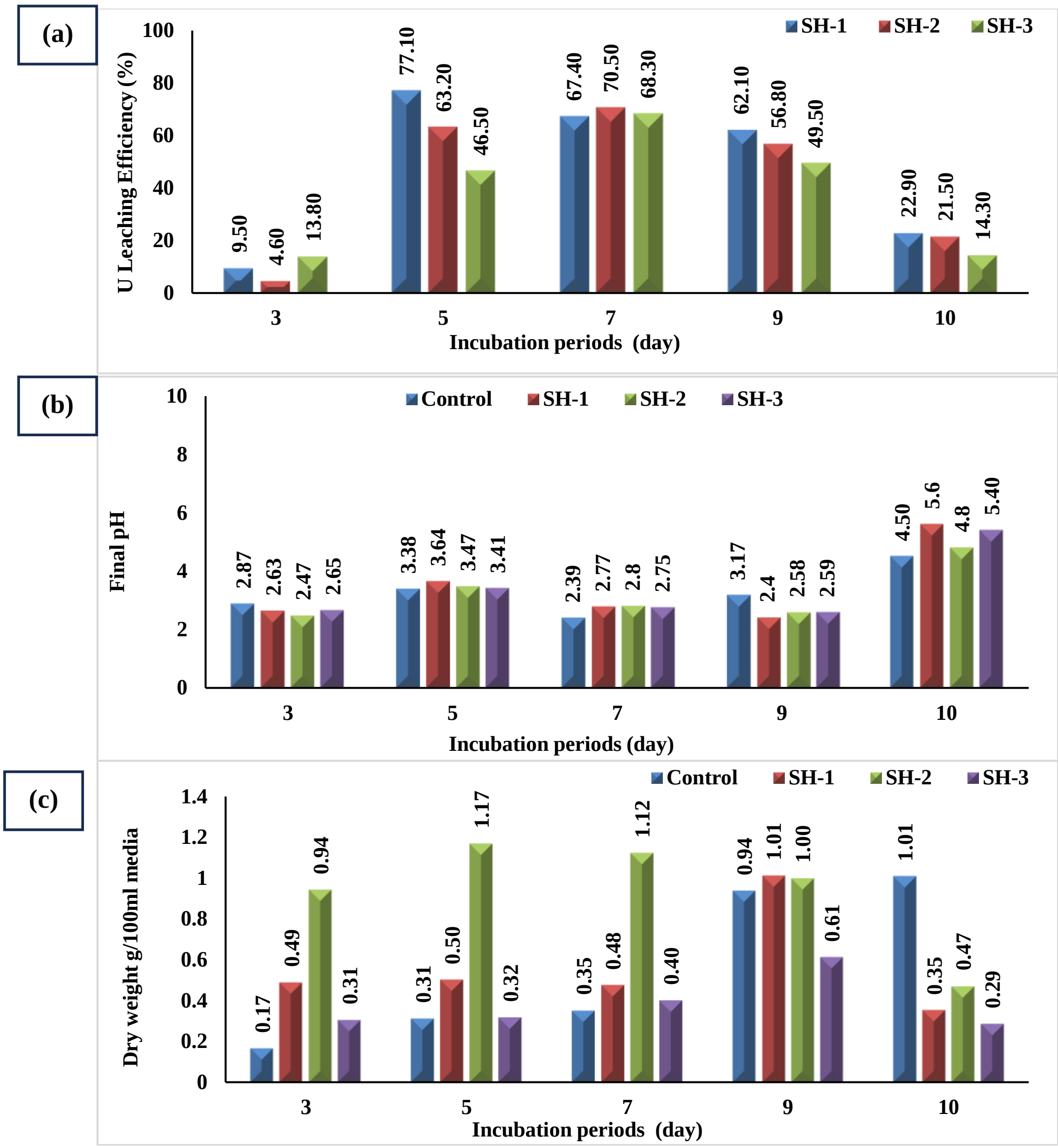

Figure 5: Effect of different Incubation periods on (a) uranium bioleaching percentage, (b) final pH, and (c) dry weight (g/100 ml of media).

For SH-1, the uranium removal percentage peaks at 77.10% after 5 days and then declines steadily, reaching 22.9% by 10 days. This suggests that uranium uptake is most effective early in the incubation period. Similarly, SH-2 shows the highest uranium removal at 70.50% after 7 days, which drops to 21.5% by 10 days. SH-3 also peaks at 68.30% after 7 days, with a decline to 14.30% by 10 days. Overall, uranium removal is most effective between 5 and 7 days of incubation, with efficiency decreasing thereafter. This decline may be due to factors such as saturation of binding sites on the fungal biomass or changes in environmental conditions, such as pH, that reduce uranium uptake.

Fungi like *Aspergillus niger* produce secondary metabolites, including organic acids, during growth, which are crucial for bioleaching. During the exponential growth phase (around days 5–7), the fungus produces the highest levels of these metabolites, enhancing metal solubilization. However, as incubation continues, metabolic activity slows down, and less acid is produced, reducing bioleaching efficiency.

In SH-1, SH-2, and SH-3, the final pH generally increases between 5 and 7 days but decreases at 9 days. By 10 days, the pH rises again, particularly in SH-1 and SH-3. These pH changes could be linked to the metabolic activities of the fungi, such as the production of organic acids or nutrient consumption, which impact uranium uptake. The increase in pH in the later stages (9-10 days) might correlate with reduced uranium removal, as a higher pH could decrease uranium solubility, making it less available for uptake.

For the control sample, dry weight shows some variability but generally increases over time. In SH-1, dry weight consistently increases, with a notable peak at 9 days (1.01 g/100 ml). SH-2 shows an increasing trend up to 5 and 7 days, then stabilizes or slightly decreases. SH-3 also demonstrates an increase in dry weight over time, peaking at 9 days (0.61 g/100 ml). The increase in dry weight over time indicates fungal growth. The peak dry weight at 9 days in SH-1 and SH-3 suggests that fungal biomass was most abundant at this point, possibly leading to saturation of uranium binding sites, which could explain the reduction in uranium removal efficiency after this time. The slight decrease in dry weight in SH-2 after 7 days may indicate a plateau or decline in growth, possibly due to nutrient depletion or toxic effects of accumulated uranium.

Overall Interpretation the data suggest that *Aspergillus niger* is most effective in removing uranium from the media within the first 5 to 7 days. The observed decrease in uranium removal efficiency beyond this period is likely due to factors such as saturation of biomass, changes in pH, and the metabolic state of the fungus. The variations in final pH and dry weight further support these observations, highlighting the importance of environmental conditions and fungal growth dynamics in the bioleaching process. For optimal uranium removal, shorter incubation times (around 5-7 days) may be more effective, and controlling pH could enhance uranium uptake by the fungal biomass.

Effect of incubation temperature

Temperature affects bioleaching processes through two competing factors **Enhanced Reaction Rate:** Higher temperatures generally speed up the reaction rate, improving bioleaching efficiency by increasing the metabolic activity of microorganisms and accelerating metal solubilization and **Microbial Activity Fluctuations:** While temperature boosts microbial activity up to a certain level, excessive heat can stress or damage microorganisms, reducing their effectiveness and potentially hindering the bioleaching process.

For uranium solubilization, SH-1 showed a peak at 30°C, with 85.10% uranium leaching, but this dropped steadily as the temperature increased, reaching a low of 15.31% at 45°C. SH-2 displayed similar behavior, with the highest uranium leaching at 35°C (75.8%) and a decrease to 12.73% at 45°C. SH-3 followed a comparable trend, peaking at 35°C with 76.46% uranium leaching and dropping to 19.77% at 45°C. Across all samples, uranium leaching was most effective at moderate temperatures (30°C to 35°C) and declined significantly at higher temperatures (40°C and above). This suggests that moderate temperatures are optimal for uranium leaching, likely due to favorable microbial activity and environmental conditions. In contrast, the reduced leaching at higher temperatures could be due to sample degradation or changes in uranium chemistry.

Impact on Bioleaching Efficiency: The growth of fungi like *Aspergillus niger* is closely linked to their ability to produce organic acids (e.g., citric acid), which play a role in metal solubilization during bioleaching. At optimal temperatures, organic acid production is enhanced, leading to better bioleaching outcomes. However, as temperature rises beyond the optimal range, the fungus's ability to produce these acids may diminish, thereby reducing its capacity to solubilize metals like uranium.

Temperature also affects the medium's pH, which can further impact fungal growth. In the experiments, the pH tended to stabilize or rise at higher temperatures, which might inhibit fungal growth, as fungi like *Aspergillus niger* typically prefer acidic conditions for optimal activity.

In terms of final pH, SH-1 experienced a steady decrease from 20°C to 35°C, followed by a slight increase at 45°C, with the final pH reaching 4.95. SH-2 showed a similar pattern, with the pH dropping until 35°C and then rising at higher temperatures. SH-3 also followed this trend, with the pH decreasing up to 35°C before increasing to 5.01 at 45°C. The control group exhibited a similar response, with pH decreasing at first and then increasing at higher temperatures. Overall, the final pH decreased with temperature increases up to 35°C, followed by a rise at 45°C. This suggests that the media became more acidic at moderate temperatures, potentially due to microbial metabolic activity, with some stabilization or buffering at higher temperatures.

Regarding dry biomass weight, SH-1 showed a decrease in weight as temperature increased, with the highest weight recorded at 30°C (3.1099 g/100 ml) and the lowest at 45°C (0.0546 g/100 ml). SH-2 followed a similar trend, peaking at 30°C with a biomass of 1.9442 g/100 ml, then dropping to 0.2571 g/100 ml at 45°C. SH-3 also exhibited the same pattern, with a peak at 30°C (1.6602 g/100 ml) and the lowest biomass at 45°C (0.0462 g/100 ml). The control group mirrored this trend, with dry weight decreasing at higher temperatures. High temperature stress at temperatures (above 35°C), fungal growth tends to decline due to stress on cellular processes. High temperatures can denature enzymes, disrupt cellular membranes, and impair the fungus's ability to maintain internal homeostasis. This leads to a reduction in fungal activity, biomass growth, and its capacity to solubilize metals like uranium. For example, in our data, temperatures above 40°C resulted in a significant decrease in both biomass and uranium leaching efficiency, suggesting thermal stress that inhibits fungal metabolism.

Optimal temperature for metabolism of fungal growth typically follows a bell-shaped curve in response to temperature, with a specific range where growth is most efficient. For *Aspergillus niger*, moderate temperatures (around 30°C to 35°C) promote optimal metabolic activity, including the production of enzymes needed for nutrient breakdown and growth. Within this temperature range, the fungus can efficiently utilize available resources, leading to higher biomass production and more effective bioleaching.

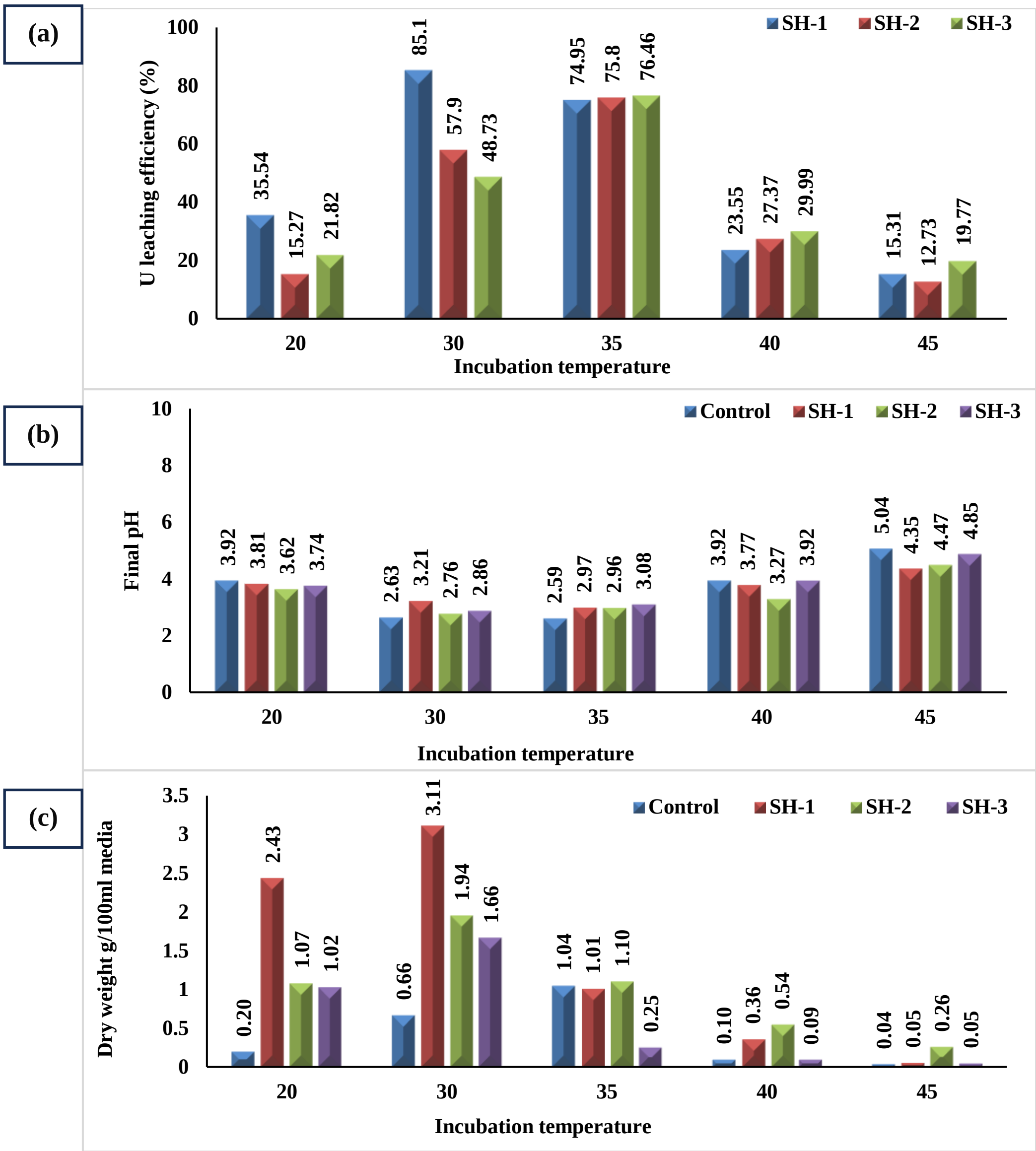

Figure 6.: Effect of varying Incubation temperatures on (a) uranium solubilization by SH-1, SH-2, and SH-3 using *Aspergillus niger*, (b) final pH of the media, and (c) fungal biomass growth.

III. Conclusions

In the context of unconventional gas resources, black shale is a primary target for shale gas exploration. The natural gas trapped within the shale matrix has become an important energy resource, especially in regions like North America. The studied optimum conditions of the uranium bioleaching controlling factors from three black shale samples (SH-1, SH-2, SH-3) using *Aspergillus niger* was achieved at 7 days as the incubation period, 30-35 ºC as the incubation temperature, pH 5, and ore concentration 1 % pulp intensity of the core samples. At low to moderate concentrations, fungal growth is supported by the availability of essential nutrients and the manageable level of metal ions for bioleaching. However, at higher concentrations, metal toxicity, oxidative stress, and changes in pH can inhibit fungal growth, reduce bioleaching efficiency, and slow down biomass production. In our experiments, an initial pH of around 5 was found to be optimal for fungal growth and uranium solubilization, as it provided the best conditions for enzyme function, acid production, and nutrient uptake. At pH levels outside this optimal range, growth and bioleaching efficiency were reduced due to less favorable conditions for metabolic activity and nutrient availability. The relationship between incubation time and fungal growth follows a typical microbial growth curve, where the initial rapid growth gives way to slower growth and eventual decline. *Aspergillus niger* grows most efficiently between 5 and 7 days of incubation, after which nutrient depletion and environmental stress cause a decline in growth and bioleaching activity. conditions for optimal activity. Incubation temperature directly affects the growth of fungi by influencing metabolic activity, enzyme stability, and nutrient uptake. Moderate temperatures (around 30°C to 35°C) are generally most favorable for fungal growth and bioleaching, while higher temperatures can induce stress, reduce biomass production, and impair the bioleaching process.

ACKNOWLEDGEMENTS

The author extends heartfelt gratitude to the Chief Editor and reviewers for their invaluable feedback and constructive suggestions. Special appreciation is given to Prof. Dr. Ibrahim El Aassey for his insightful discussions and fieldwork support, and to Dr. Marwa M. Abdel-Azeem for her continuous support.

Conflict of Interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

References

[1]. Abdollahi, H., Saneie, R., Shafaei, S.Z., Mirmohammadi, M., Mohammadzadeh, A., and Tuovinen, O.H., 2021. Bioleaching of cobalt from magnetite-rich cobaltite-bearing ore. Hydrometallurgy, 204, p.105727. https://doi.org/10.1016/j.hydromet.2021.105727

[2]. Adetunji, A.I., Oberholster, P.J., and Erasmus, M., 2023. Bioleaching of metals from e-waste using microorganisms: A review. Minerals, 13(6), p.828. https://doi.org/10.3390/min13060828

[3]. Alshiyab, H., Kalil, M.S., Hamid, A.A., and Wan Yusoff, W.M., 2008. Effect of salts addition on hydrogen production by C. acetobutylicum. Pakistan Journal of Biological Sciences, 11(18), pp.2193-2200. https://doi.org/10.3923/pjbs.2008.2193.2200

[4]. Anjum, F., Shahid, M., and Akcil, A., 2012. Biohydrometallurgy techniques of low-grade ores: A review on black shale. Hydrometallurgy, 117-118, pp.1-12. https://doi.org/10.1016/j.hydromet.2012.01.007

[5]. Asghari, I., Mousavi, S.M., Amiri, F., and Tavassoli, S., 2013. Bioleaching of spent refinery catalysts: A review. Journal of Industrial and Engineering Chemistry, 19, pp.1069-1081. https://doi.org/10.1016/j.jiec.2012.12.005

[6]. Bhatti, T.M., Malik, K.A., and Khalid, A.M., 1989. Microbial leaching of low-grade sandstone uranium ores: Column leaching studies. In Malik, K.A., Naqvi, S.H.M. and Aleem, M.I.H. (eds.) Biotechnology for Energy, Faisalabad, Pakistan: Nuclear Institute for Agriculture and Biology (NIAB) and National Institute for Biotechnology and Genetic Engineering, pp.329-340.

[7]. Borja, D., Nguyen, K.A., Silva, R.A., Park, J.H., Gupta, V., Han, Y., Lee, Y., and Kim, H., 2016. Experiences and future challenges of bioleaching research in South Korea. Minerals, 6(4), p.128. https://doi.org/10.3390/min6040128

[8]. Brierley, C.L., and Brierley, J.A., 2013. Bioleaching: A microbial process for metal leaching. In Biohydrometallurgy: Fundamentals and applications, CRC Press, pp.78-94.

[9]. Brierley, C.L., and Brierley, J.A., 2022. Bioleaching: Recent advances and future perspectives. Springer.

[10]. Bruins, M.R., Kapil, S., and Oehme, F.W., 2000. Microbial resistance to metals in the environment. Ecotoxicology and Environmental Safety, 45(3), pp.198-207. https://doi.org/10.1006/eesa.1999.1860

[11]. Burgstaller, W., and Schinner, F., 1993. Leaching of metals with fungi. Journal of Biotechnology, 27(1), pp.91-116. https://doi.org/10.1016/0168-1656(93)90101-R

[12]. Diels, L., van der Lelie, N., and Bastiaens, L., 2002. New developments in treatment of heavy metal contaminated soils. Revue de Métallurgie, 99(10), pp.925-930. https://doi.org/10.1051/metal/20029910925

[13]. Dusengemungu, L., Kasali, G., Gwanama, C., and Mubemba, B., 2021. Overview of fungal bioleaching of metals. Environmental Advances, 3, p.100083. https://doi.org/10.1016/j.envadv.2021.100083

[14]. Escareño-Juárez, E., Pardo, R., Gascó-Leonarte, C., Vega, M., Sánchez-Báscones, M.I., and Barrado-Olmedo, A.I., 2019. Determination of natural uranium by various analytical techniques in soils of Zacatecas State (Mexico). Journal of Radioanalytical and Nuclear Chemistry, 319(3), pp.1135–1144. https://doi.org/10.1007/s10967-019-06428-6

[15]. Fan, Y.J., Yang, H.Y., and Zi, J.C., 2004. The influence of the ions in solution on the growth of bioleaching engineering bacteria. Non-Ferrous Mining Metal, 20(2), pp.17–19. https://doi.org/10.3969/j.issn.1007-967X.2004.02.006 (in Chinese)

[16]. Farag, N.M., El-Sayed, G.O., Morsy, A.M.A., Taha, M.H., and Yousif, M.M., 2015. Modification of Davies & Gray method for uranium determination in phosphoric acid solutions. International Journal of Advanced Research, 3(12), pp.323–337.

[17]. Fomina, M., and Gadd, G.M., 2014. Biosorption: Current perspectives on concept, definition, and application. Bioresource Technology, 160, pp.3–14. https://doi.org/10.1016/j.biortech.2013.12.102

[18]. Francis, A.J., 1998. Biotransformation of uranium and other actinides in radioactive wastes. Journal of Alloys and Compounds, 271–273, pp.78–84.

[19]. Gadd, G.M., 1999. Fungal production of citric and oxalic acid: Importance in metal speciation, physiology and biogeochemical processes. Advances in Microbial Physiology. https://doi.org/10.1016/s0065-2911(08)60165-4

[20]. Gadd, G.M., 2000. Metals, minerals and microbes: Geomicrobiology and bioremediation. Microbiology, 146(1), pp.47–57. https://doi.org/10.1099/00221287-146-1-47

[21]. Gadd, G.M., 2002. Microbial interactions with metals/radionuclides: The basis of bioremediation. In Keith-Roach, M.J., and Livens, F.R. (eds.) Radioactivity in the environment, Vol. 2, Elsevier, pp.179–203.

[22]. Gadd, G.M., 2007. Geomicrobiology of metals. In Microbial mineral leaching, Springer, pp.123–145.

[23]. Gadd, G.M., 2007. Geomycology: Biogeochemical transformations of rocks, minerals, metals, and radionuclides by fungi, biofilms, and bioweathering. Mycological Research, 111(1), pp.3–49. https://doi.org/10.1016/j.mycres.2006.12.001

[24]. Gadd, G.M., 2017. Geomicrobiology of the built environment. Nature Microbiology, 2, p.16275. https://doi.org/10.1038/nmicrobiol.2016.275

[25]. Gilman, J., 1957. A manual of soil fungi. Soil Science, 84(2), p.183.

[26]. Hamidpour, M., Kalbasi, M., Afyuni, M., and Shariatmadari, H., 2020. Adsorption of heavy metals by humic acid-coated fungal mycelium: Isotherm, kinetic, and thermodynamic studies. Journal of Environmental Management, 260, p.110080. https://doi.org/10.1016/j.jenvman.2020.110080

[27]. Humber, R.A., 1997. Chapter V-1 - Fungi: Identification. In: L.A. Lacey, ed. Manual of Techniques in Insect Pathology. London: Academic Press, pp.153-185.

[28]. Jagetiya, B., and Sharma, A., 2013. Optimization of chelators to enhance uranium uptake from tailings for phytoremediation. Chemosphere, 91(5), pp.692–696. https://doi.org/10.1016/j.chemosphere.2012.11.044
[29]. Kimuro, S., Kirishima, A., Kitatsuji, Y., Miyakawa, K., Akiyama, D., and Sato, N., 2019. Thermodynamic study of the complexation of humic acid by calorimetry. Journal of Chemical Thermodynamics, 132, pp.352-362. https://doi.org/10.1016/j.jct.2019.01.011
[30]. Klich, M.A., 2002. Identification of Common Aspergillus Species. Utrecht, Germany: Centraalbureau Voor Schimmelcultures.
[31]. Kour, G., Kothari, R., Mohan Singh, H., et al., 2021. Microbial leaching for valuable metals harvesting: Versatility for the bioeconomy. Environmental Sustainability, 4, pp.215-229. https://doi.org/10.1007/s42398-020-00143-9
[32]. Lazo, D.E., Dyer, L.G., Alorro, R., and Browner, R., 2017. Treatment of monazite by organic acids I: Solution conversion of rare earths. Hydrometallurgy, 174, pp.202–209. https://doi.org/10.1016/j.hydromet.2017.10.003
[33]. Le, L., Tang, J., Ryan, D., and Valix, M., 2006. Bioleaching of nickel laterite ores using multi-metal tolerant Aspergillus foetidus. Minerals Engineering, 19, pp.1259-1265. https://doi.org/10.1016/j.mineng.2006.02.006
[34]. Li, J., Kang, Z., Kang, Z., and Zhang, X., 2023. Geochemical characteristics and geological significance of black shale at the bottom of the Mufushan Formation in the Lower Cambrian, Lower Yangtze Platform, South China. Minerals, 13(8), p.1095. https://doi.org/10.3390/min13081095
[35]. Li, J., Li, C., and Sarkar, B., 2021. Effects of organic acids on metal adsorption onto andosols and their relationships with the surface properties of soils. Journal of Hazardous Materials, 408, p.124925. https://doi.org/10.1016/j.jhazmat.2020.124925
[36]. Li, J.Y., Wen, J.X., Guo, Y., An, N., Liang, C.J., and Ge, Z.Y., 2020. Bioleaching of gold from waste printed circuit boards by alkali-tolerant Pseudomonas fluorescens. Hydrometallurgy, 194, p.105260. https://doi.org/10.1016/j.hydromet.2020.105260
[37]. Li, X.D., Wu, B., Zhang, Q., Liu, Y.Q., Wang, J.Q., Li, F.S., Ma, F.J., and Gu, Q.B., 2020. Complexation of humic acid with Fe ions upon persulfate/ferrous oxidation: Further insight from spectral analysis. Journal of Hazardous Materials, 399, p.123071. https://doi.org/10.1016/j.jhazmat.2020.123071
[38]. Martins, F.L., and Leao, V.A., 2022. Chalcopyrite bioleaching in chloride media: A mini-review. Hydrometallurgy, 216, p.105995. https://doi.org/10.1016/j.hydromet.2022.105995
[39]. Pitt, J.I., 1979. The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces. Academic Press Inc. Ltd.
[40]. Rhee, Y.J., Hillier, S., and Gadd, G.M., 2012. Lead transformation to pyromorphite by fungi. Current Biology, 22(24), pp.2376-2382. https://doi.org/10.1016/j.cub.2012.10.034
[41]. Rudnick, R.L., and Gao, S., 2014. Composition of the continental crust. In: H.D. Holland and K.K. Turekian, eds. Treatise on Geochemistry. 2nd ed. Oxford: Elsevier, pp.1-51.
[42]. Sarkodie, E.K., Jiang, L., Li, K., Yang, J., Guo, Z., Shi, J., Deng, Y., Liu, H., Jiang, H., Liang, Y., Yin, H., and Liu, X., 2022. A review on the bioleaching of toxic metal(loid)s from contaminated soil: Insight into the mechanism of action and the role of influencing factors. Frontiers in Microbiology, 13, Article 1049277. https://doi.org/10.3389/fmicb.2022.1049277
[43]. Schippers, A., and Sand, W., 1999. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. Applied and Environmental Microbiology, 65(1), pp.319-321.
[44]. Shapiro, L., and Brannock, W.W., 1962. Rapid analysis of silicate, carbonate and phosphate rocks. U.S. Geological Survey Bulletin, pp.21-53. Available at: https://pubs.usgs.gov/bul/1144a/report.pdf
[45]. Sun, J., Li, Q., Li, T., Xu, K.L., Cui, Z., and Li, G.Y., 2022b. Insights into formation and dissolution mechanism of bio-ore pellets in the one-step uranium leaching process by Aspergillus niger. Minerals Engineering, 184, p.107672. https://doi.org/10.1016/j.mineng.2022.107672
[46]. Sun, X.L., Ma, X.S., Leng, L., and Fang, Y.C., 2022a. Optical properties of the Suwannee River fulvic acid complexation with thorium using 3D fluorescence spectroscopy. Spectroscopy, 37(8), pp.26-33. https://doi.org/10.56530/spectroscopy.xl4975e8
[47]. Tang, H., Xiang, G., Xiao, W., Yang, Z., and Zhao, B., 2024. Microbial-mediated remediation of heavy metals toxicity: Mechanisms and future prospects. Frontiers in Plant Science, 15, Article 1420408. https://doi.org/10.3389/fpls.2024.1420408
[48]. Tezyapar Kara, K., Kremser, S.T., Wagland, F., and Coulon, F., 2023. Bioleaching metal-bearing wastes and by-products for resource recovery: A review. Environmental Chemistry Letters, 21, pp.3329-3350. https://doi.org/10.1007/s10311-023-01611-4
[49]. Trivedi, A., Vishwakarma, A., Saawarn, B., and Mahanty, B., 2022. Fungal biotechnology for urban mining of metals from waste printed circuit boards: A review. Journal of Environmental Management, 323, p.116133. https://doi.org/10.1016/j.jenvman.2022.116133
[50]. Tsekova, K., and Ganeva, S., 2004. Influence of some physicochemical parameters on uranium biosorption by Rhizopus arrhizus. Journal of Applied Microbiology, 97(3), pp.455-461. https://doi.org/10.1111/j.1365-2672.2004.02308.x
[51]. Wang, Y., Wang, J., Ding, D., Li, G., Sun, J., Hu, N., Li, F., Ma, J., Zhang, H., Ding, Y., and Dai, Z., 2024. Hyphae and organic acids of Aspergillus niger promote uranium leaching by destroying the ore surface and increasing the porosity and permeability of ores. Nuclear Engineering and Technology, 56, pp.1880-1886. https://doi.org/10.1016/j.net.2023.12.049
[52]. Wang, Y.D., Li, G.Y., Ding, D.X., Zhou, Z.X., Deng, Q.W., Hu, N., and Tan, Y., 2013. Uranium leaching using mixed organic acids produced by Aspergillus niger. Journal of Radioanalytical and Nuclear Chemistry, 298(2), pp.769-773. https://doi.org/10.1007/s10967-013-2664-y
[53]. Watanabe, T., 2002. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. 2nd ed. CRC Press LLC.
[54]. Xu, H.S., Gong, G.Q., Zhang, Y.J., Yuan, F., and Zhang, Y.X., 2022. The spectroscopic characteristics of fulvic acid complexed with copper ion and the construction of the mechanism of action. Spectroscopy and Spectral Analysis, 42(4), pp.1010–1016. https://doi.org/10.3964/j.issn.1000-0593(2022)04-1010-07
[55]. Zhang, X., Shi, H., and Tan, N., 2023. Advances in bioleaching of waste lithium batteries under metal ion stress. Bioresource Bioprocessing, 10, p.19. https://doi.org/10.1186/s40643-023-00636-5