



# Costly effective bioleaching of valuable metals from low grade ore using *Aspergillus nidulans*

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## Abstract

This research investigated the feasibility of employing organic acids, like citric acid, produced by *Aspergillus nidulans* MT355567 in a bioleaching process to recover uranium (U) from a low-grade rock sample. The optimal conditions for fungal growth and maximum citric acid (CA) synthesis across three distinct media were determined. The maximum citric acid concentration was produced on medium made from wheat bran (83%) and tea waste (77%). An investigation was carried out to see how citric acid and, by consequence, uranium bioleaching affinity, were affected by varying carbon sources, nitrogen sources, pH, temperature, incubation period, ore particle size, and the solid–liquid ratio. At 25 °C and a pH of 5.0, media containing 100 g/L of sucrose as a carbon source and peptone as a nitrogen source made the highest yield of citric acid and U bioleaching. Higher U bioleaching was achieved with ore particles 0.075 mm at a ratio of 2 g/L after only 30 min of contact with the fungal filtrate. Iron interference has a negative impact on uranium extraction. Interestingly, none of the conditions applied to enhance CA synthesis and U-bioleaching caused iron (Fe) dissolution. Based on these findings, it appears that bioleaching using *A. nidulans* MT355567 metabolic products is a promising economic and ecofriendly technology for extracting uranium from low-grade ore that might be adopted on a pilot scale.

## Graphical abstract

summarizing the experimental workflow for bioleaching of uranium from low-grade ore using citric acid produced by *Aspergillus nidulans*. The process involved optimizing *A. nidulans* growth and citric acid biosynthesis, evaluating factors influencing bioleaching activity of the acid metabolite solutions, and finally applying the optimized conditions to bioleach uranium

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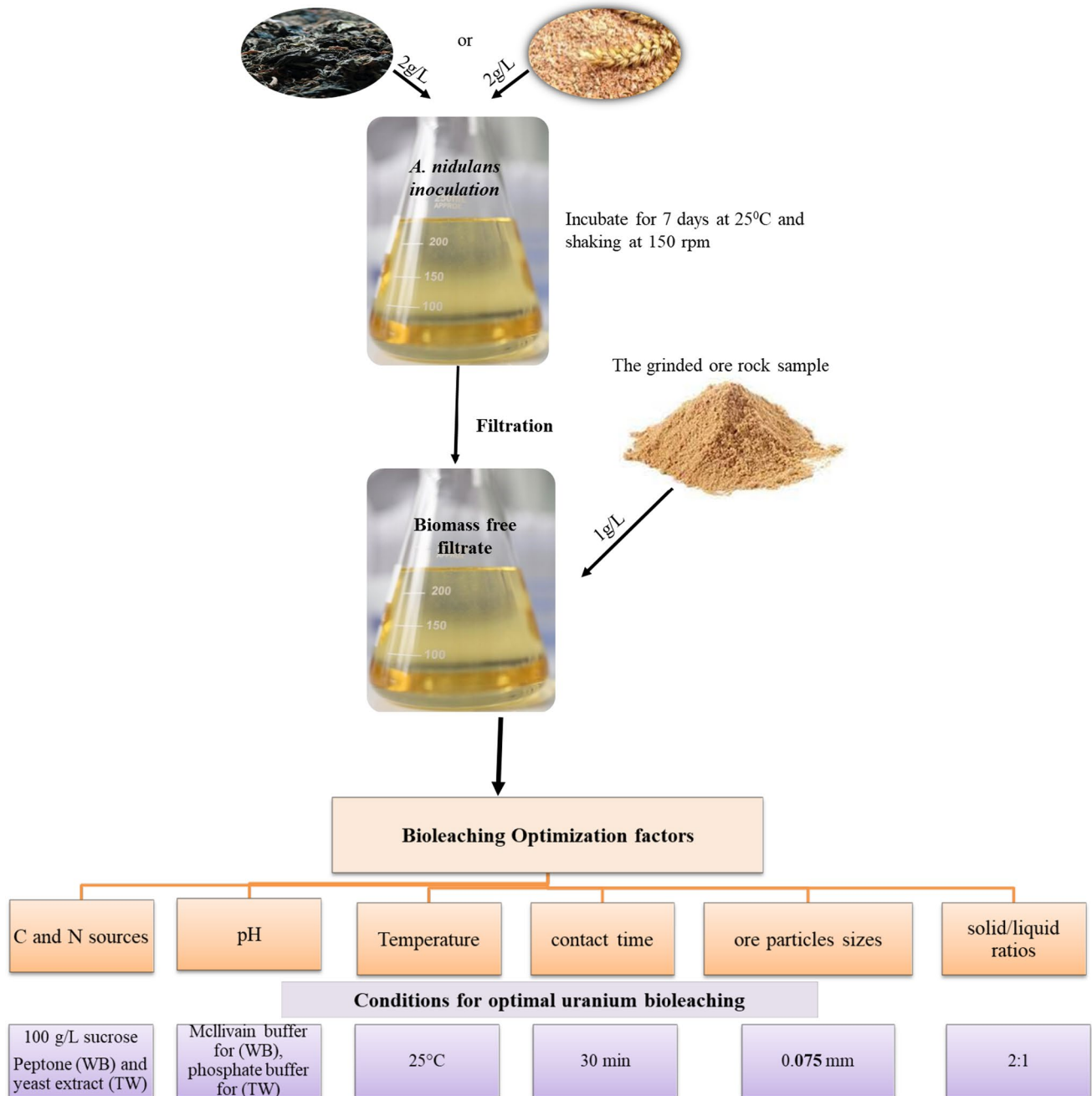
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from the ore sample. The schematic illustrates the key steps and the optimal condition for *Aspergillus nidulans* growth medium preparation using agricultural wastes, downstream application of metabolite synthesized for uranium bioleaching



**Keywords** Agriculture waste · Microorganisms · Citric acid · Tea waste · Uranium ore · Wheat bran

### Abbreviations

C Carbon  
CA Citric acid  
LOI Loss on ignition  
TW Tea waste



U Uranium  
WB Wheat bran

## Introduction

Historically, uranium has served as a source of atomic energy in the defense and power industries, as well as a source of alternative energy in the maritime industry. Growing uranium consumption has resulted to the eventual depletion of tall review and easily leachable ore deposits (Abostate et al. 2018; Sun et al. 2020). A progressive depletion of high-grade uranium reserves has resulted from the increased demand for nuclear energy. New technology for extracting low-grade and refractory uranium ore must be developed in order to meet the rising demand for this scarce material (Zhou et al. 2019). Uranium is a naturally occurring element with an average concentration of 2.8 parts per million in the Earth's crust. Traces of it occur almost everywhere.

Uranium is a trace naturally occurring element with an average concentration in the Earth's crust of 2.8 parts per million. The Nuclear Energy Agency in Paris estimates that Egypt has less than 0.01% of the world's identifiable uranium reserves, which is insufficient to generate commercial quantities that can be exported profitably. Most uranium deposits in Egypt contain low-grade uranium ores that are amenable to leach (Abdel-Monem et al. 1996; Nuclear Energy Agency (NEA) 2014).

Most of the uranium in rock was consumed by conventional leaching methods; therefore, it is necessary to add additional resources or re-extract uranium from waste to safeguard the environment from its toxicity and use it in the energy sector.

To offset the negative effects of metal resource extraction, the global community is promoting environmentally friendly mining technology (Krishnamoorthy et al. 2021; Dusengemungu et al. 2021). Bioleaching is being investigated as a viable alternative to hydro- and pyrometallurgical metal recovery techniques since it is a cost-effective process. The process can utilize shake flasks, batch reactors, continuous reactors, columns, and heaps. Bioleaching has been identified as a method for recovering these resilient uranium metals in a productive, temperate, and ecologically friendly manner (Rasoulnia et al. 2021). Typically, heterotrophic bacteria, autotrophic bacteria, and fungal species are used for bioleaching. *Aspergillus* species are the most efficient bioleaching fungi. These fungi create organic acids, and the dissociation of organic acids

leads to the elimination of protons. Compared to  $H_2SO_4$  and other strong acids, the organic acids produced by microbes are largely non-corrosive and therefore ecofriendly. Iron and sulphur oxidizing bacteria are frequently used in the bioleaching of sulphide ores because they use the ore's iron and sulphur as a substrate and carbon dioxide as a carbon source for their growth. Metal dissolution can occur in a variety of ways, depending on the type of microorganism and the type of mineral (Krishnamoorthy et al. 2021).

Under leaching conditions, both soluble and insoluble organic-uranium complexes exist, such as citric-uranium complexes and oxalic-uranium complexes, respectively. Oxalic acid has a greater degree of hydrogen liberation than other organic acids and a stronger chemical affinity in the aqueous phase, both of which facilitate uranium leaching (Wang et al. 2015). Extraction of metals from nonsulfidic minerals can be catalyzed by organisms, yeasts, or heterotrophic microorganisms that can frequently tolerate high pH values. These microorganisms produce vast quantities of natural acids, including citric, lactic, gluconic, and tartaric acids, among others; these metabolites can liberate and mobilize metals from nonsulfidic solids, resulting in metal dissolution. *Aspergillus niger's* production of oxalic acid is dependent on the pH of the medium and the carbon source. Since the activity of oxaloacetate acetylhydrolase (OAH), the protein thought to be responsible for oxalate arrangement, is at its peak at pH 6, its production can proceed, whereas below pH 2 no oxalic acid is produced (Sawant et al. 2018). Acid-producing fungi, including *Aspergillus*, *Penicillium*, *Phanerochaete*, and *Chrysosporium*, have leached metals such as copper, nickel, aluminum, uranium, etc. (Das et al. 2019; Dusengemungu et al. 2021; El-Bondkly and El-Gendy 2022).

Bioleaching of metals with microorganisms can be “one-step” when microorganism growth and metal dissolution occur together. Otherwise, “two-step”, the microorganism is allowed to grow then the metal is included to medium containing metabolites generated already by the microorganism. The “two-step” bioleaching method is the most effective for a number of reasons: first, the production of the microorganism's metabolites is increased when it is grown in optimal conditions without the presence of metal. Second, the optimal conditions for microorganism growth and metal leaching are distinct. The handling of each of these steps can subsequently be optimized. Besides, microorganism development is usually initiated in neutral pH and utilizes natural substrate in sterile environment, sterilizing of mineral in industry is not conceivable. Some species such as heterotrophic microscopic organisms may impact



contagious development and change the anticipated way, they work (Gerayeli et al. 2013).

Citric acid (CA), a natural Krebs cycle metabolic intermediate, is commonly regarded as safe, nontoxic, and biodegradable in addition to its involvement in bioleaching. Due to these features, it has extensive application in numerous fields, including the food, dairy, pharmaceutical, cosmetic, agricultural, and biochemical industries. Its antioxidant properties make it useful in a wide range of healthcare products. There is an increasing demand for CA in the biomedical industry, where it is used as an active ingredient in the creation of a wide variety of biopolymers with applications ranging from nanomedicines and drug delivery systems to the cultivation of a wide variety of human cell lines and the solubilization of metals (Max et al. 2010; Behera et al. 2021).

The goal of this study is to develop a low-cost alternative growth medium for *A. nidulans* using agricultural residues (tea waste and wheat bran) to be employed in U bioleaching. The presence of ferrous iron ( $\text{Fe}^{+2}$ ) and metallic iron ( $\text{Fe}^0$ ) reduces U(VI) to the insoluble form U(IV), with uranium adsorbed on the iron surface (Dublet et al. 2017). Therefore, the dissolution of U without Fe is a valuable target. Here, we evaluated the ability to bioleach U without simultaneously dissolving Fe, which increases the purity of the bioleached U. An optimization study investigated the most influential variables on U bioleaching and CA production, including carbon source, nitrogen source, pH, temperature, incubation period, ore particle size, and solid–liquid ratio.

## Materials and methods

### Materials

#### The uranium ore sample

The ore material containing the uranium element that used in the current study was collected from Abu Zarab in Sinai, Egypt. The composition of uranium and other element oxides in the used ore sample was detected as mentioned below in the methods section and tabulated in Table 1.

### Microorganism and growth media

*A. nidulans* was isolated from monazite mineral collected from Egypt's Rosetta region on the Mediterranean coast. By sequencing the ITS region of the rRNA-encoding gene, the fungal isolate was identified. The sequence was submitted to the NCBI gene bank with the accession number “MT355567” (Abozaid et al. 2021).

Potato dextrose agar plates were used for the routine maintenance of the fungal culture. Sabouraud dextrose (Guinea et al. 2005), wheat bran (WB) and tea waste (TW) were used to determine the optimal culture medium for better uranium bioleaching. Wheat bran flour (70% extraction) was obtained from the local market and used directly without refining.

### Chemicals

All chemicals used for bioleaching experiments were analytical-grade, Merck, life science, industrial and lab chemicals products (<https://www.merckmillipore.com/INTL/en>). The aqueous solutions were prepared with distilled water.

### Instrumentation

The concentration of uranium and other major oxides was measured in ore sample using Spectrometer T80 UV/VIS Spectrometer (PG Instruments Ltd). All pH measurements were measured by using a calibrated pH meter (Jenway 3510) equipped with a Standard Calomel Electrode (SCE). Electromagnetic Sieve Shaker- HAVER EML 200 digital T-Haver and Boecker was used to obtain different grain sizes (ranged from 0.25 to 0.025 mm).

### Biochemical and chemical methods

#### Determination of citric acid production and protein contents

Citric acid (CA) is one of the most common components of fungal metabolites and involved in uranium leaching from ore sample (Xu et al. 2019). Therefore, the citric acid

**Table 1** Major elemental composition of the investigated low-grade uranium ore sample

Major oxides	SiO <sub>2</sub>	TiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	MgO	CaO	Na <sub>2</sub> O	K <sub>2</sub> O <sub>2</sub>	P <sub>2</sub> O <sub>5</sub>	LOI*	(U)
Wt (%)	49.1	0.83	16.7	12.78	0.3	2.94	1.29	1.38	0.96	14.62	0.0404

\*Loss on ignition

concentration was evaluated as an indicator for uranium leaching efficiency. It was determined titrimetrically in the filtrate by using 0.1N NaOH and phenolphthalein as indicator and calculated as % according to the following formula (AOAC 2002).

$$\%CA = \frac{\text{Normality of NaOH} \times \text{volume of NaOH} \times \text{Equiv.wt. of CA}}{\text{Weight of sample} \times 100}$$

The fungal extracellular proteins were determined according to (Gregor et al. 1977; Dawnay StJ. et al. 1991).

### Determination of uranium and major oxides concentrations in the ore sample

The uranium (U) concentration in the leach liquor was quantified using volumetric titration with ammonium vanadate, following the method of Davies and Gray (1964). The concentrations of the major oxide compounds in the leach liquor were determined using wet chemical procedures according to the method described by Shapiro and Brannock (1962).

### The bioleaching experiment

Bioleaching experiments were performed using a batch system. The experiments were carried out using seven days old *A. nidulans* biomass-free filtrate mixed with sterilized uranium-containing ore sample in ratio (1 g/L). The mixture was mixed for maximum 3 h contact time at room temperature in an orbital shaker at 150 rpm. Wheat bran (2 g/L) or tea waste (2 g/L) media was used for the bioleaching optimization experiments as they afford the best fungal growth and uranium bioleaching.

### Bioleaching optimization factors

The uranium bioleaching process was optimized using various factors such as various carbon sources and doses, various nitrogen sources, different buffer types, various time periods, different temperatures, different grain sizes and various solid liquid ratios. The experiment components were shaken at 150 rpm and the concentration of uranium was measured every time to select the optimized conditions for uranium leaching (see Graphical abstract).

**Effect of carbon source and dose** Glucose, sucrose, and starch were used as different carbon sources. Experiments were carried out in 250 ml sterilized conical flasks containing 50 ml

of 2 g/L wheat bran or tea waste media in addition to (50 g/L) of the corresponding carbon source. The effect of different sucrose concentrations (50, 100, and 150 g/L) was also investigated under the above-mentioned experimental condition, as it was the best carbon source.

**Effect of different nitrogen sources** Different nitrogen sources such as Peptone, yeast extract, beef extract and urea were evaluated for the maximum uranium bioleaching. Ten grams of each corresponding source was provided separately to one liter of the media.

**Effect of different buffer types** Different buffers were employed to alter the medium pH, and the buffer solutions (acetate (pH 5.6), phosphate (pH 5.6) and Macclivian (pH 5) were used.

**Effect of different temperatures** Seven days old *A. nidulans* biomass-free filtrate was mixed with sterilized uranium-containing ore sample in ratio (1 g/L). The mixture was mixed for 3 h contact time at different temperature 15, 20, 25, 30 and 35 °C) in an orbital shaker at 150 rpm.

**Effect of different contact time** The effect of fungal filtrate contact time with the ore sample on the U bioleaching efficiency was also assessed at different time periods (15, 30, 45, 90, 120, and 180 min).

**Effect of different grain (ore particles) sizes** The ore sample was grinded to a fine powder using mortar and pestle. The powder was sieved using a sieve set with pore size ranging from (0.25 to 0.025) mm. The collected particles from each sieve were used separately in each experiment.

**Effect of different solid/liquid ratios** Different ratios of ore particles (solid) to fungal filtrate (liquid) were used to assess the influence on U bioleaching. The following ratios (1: 1, 2:1, 3:1, 4:1, and 5:1 g/L) were used at the best measured conditions.

## Results and discussion

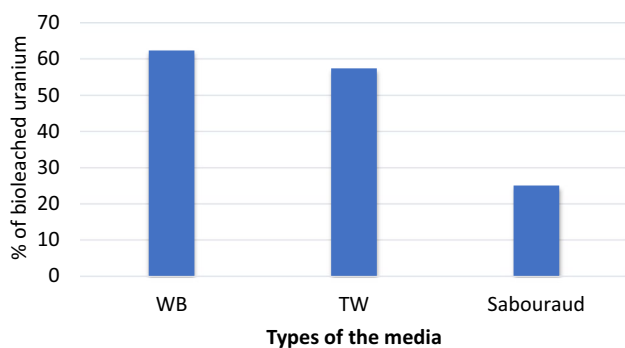
### Cost-effective substrates for *A. nidulans* CA production and U bioleaching

In the current study, wheat bran or tea waste were used as a substrate for *A. nidulans* growth in comparison to the routine



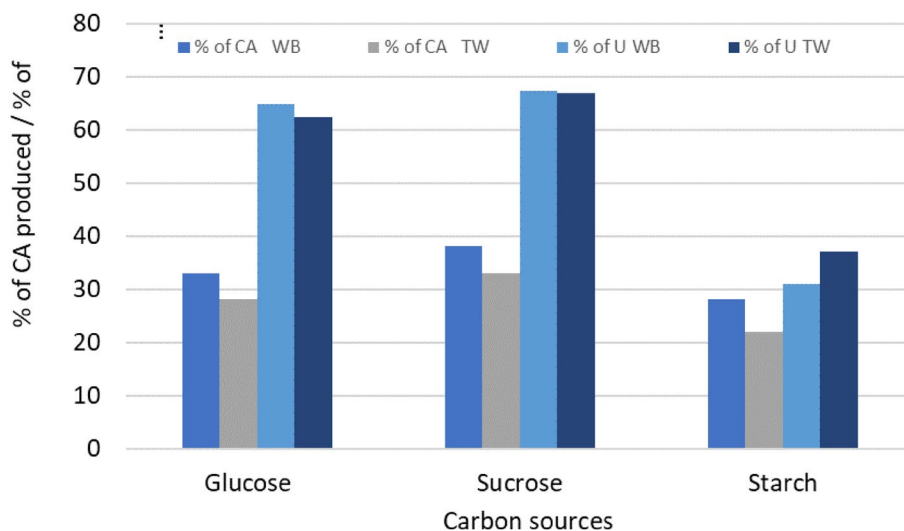
used media (Sabouraud dextrose medium). Both natural substrates provide better growth as indicated by the fungal protein content which reaches up to maximum of 3.3 and 3.1 g/l respectively. Also, both substrates provide a superior CA production and U bioleaching affinity, although, using media containing wheat bran provides the highest values. Therefore, wheat bran and tea waste containing media were used for the whole study (Fig. 1).

Wheat grains and wheat bran were the best substrates for the growth of *Morchella spongiosa* (MR17) with high lignocellulolytic activity. Wheat grains yielded a protein content = 17.4 mg/ml, while wheat bran yielded only 11.2 mg/ml. (Reddy and Kanwal 2022).



**Fig. 1** Percentage of uranium bioleached from low-grade ore using citric acid produced by *Aspergillus nidulans* grown on different substrates. The fungus was cultured on media prepared using wheat bran (WB), tea waste (TW), or Sabouraud dextrose broth

**Fig. 2** Effect of different carbon sources on citric acid (CA) production and uranium (U) bioleaching activity by *Aspergillus nidulans*. The fungus was cultured in wheat bran (WB) and tea waste (TW) media supplemented with different carbon sources: glucose, sucrose, or starch. Citric acid concentrations in the culture filtrates were quantified. The bioleaching activity was evaluated by determining the percent of uranium extracted after treating the low-grade uranium ore sample for 180 min contact time at 25 °C



## Chemical composition of the used ore sample

*A. nidulans* affinity to bioleach U from low grade ore sample was assessed, the chemical analysis of ore sample has showed that the  $\text{SiO}_2$  was the predominant metal oxide of 49.1% with trace amounts of 0.3% MgO was present of the total composition.  $\text{Al}_2\text{O}_3$  and  $\text{Fe}_2\text{O}_3$  are, respectively, the second and third most abundant metal oxides in the ore sample (Table 1).

Wang et al. (2015) bioleached uranium from a sample of uranium ore from a uranium deposit in West China. The chemical composition was like our ore sample, but with different proportions: 85.44 percent of their ore sample was  $\text{SiO}_2$ , followed by 6.56 percent  $\text{Al}_2\text{O}_3$  and 1.49 percent  $\text{Fe}_2\text{O}_3$ . U constitutes 0.04% of our sample, but 0.118% of the ore sample from west China; hence, the ore is a readily leachable uranium ore.

## Effect of carbon sources on uranium bioleaching

In the current study, 50 g/L of glucose, sucrose or starch were used to select the best carbon source for uranium bioleaching. The results showed that the sucrose was the best for uranium bioleaching reached 67.32 and 66.83% in comparison to 30.94 and 37.12% for starch, in both substrates: wheat bran and tea waste medium respectively (Fig. 2).

The pH of the media was decreased from 5.6 to 4.5 and 4 for wheat bran and tea waste media respectively, indicating citric acid production. Citric acid (CA) is one of the most common components of fungal metabolites and has a role in uranium leaching from ore sample (Xu et al. 2019). With

measuring citric acid levels, we found an increase of up to 38 and 33% for fungi cultured on wheat bran and tea waste, respectively, accompanied by an increase in protein concentration of 1.1 and 1 mg/ml. The type of carbon source has a substantial effect on the growth and citric acid generation capability of fungi (Das et al. 2019). Due to the existence of extracellular-bound invertase that is active at low pH and rapidly hydrates sucrose into glucose and fructose, sucrose is favored over glucose and fructose (Sati and Bisht 2006; Shah et al. 2020). As the conversion of polysaccharides into simple sugar involves additional steps, they are not favored as a carbon source. It takes a substantial amount of time for the decomposition process to attain the sugar catabolism rate required for citric acid production. The increased protein content of the fungus cultured on a medium containing sucrose may represent the fungus' ability to create extra enzymes necessary for citric acid synthesis. The acidity of the medium facilitates metal leaching from any substance. Reduced enzymatic activity influences the pH of the fermentation medium, which slows the rate of polysaccharide breakdown (Das et al. 2019).

Increasing the sucrose concentration to 100 g/L increased the fungus protein content in wheat bran and tea waste containing medium to 1.5 and 1.2 mg/ml, citric acid production to 45 and 42%, and bioleaching ability to 71.78 and 69.31%, respectively. A slight difference was observed between 100 and 150 g/L of sucrose (Fig. 3). Previously, a correlation between sugar concentration and citric acid production by *Aspergillus niger* was identified. Two date varieties with varying sugar contents were added to a medium inoculated with *A. niger* for the production of citric acid. The GHARS variety with a higher sugar content produced 0.32 g/g citric acid, while the MECH DEGLA variety with a lower sugar content produced 0.28 g/g citric acid (Chergui et al. 2021). In a different study, date syrup with a higher sugar concentration

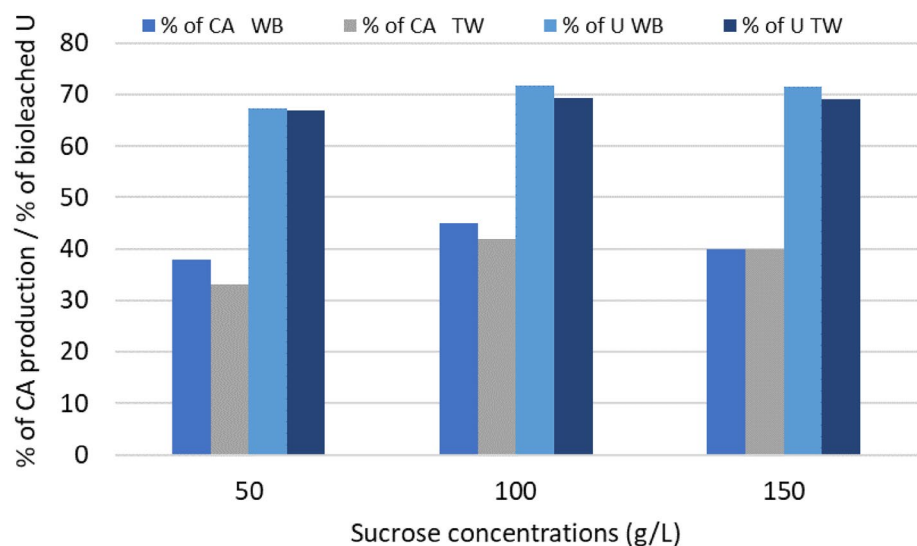
produced an even greater amount of CA, 80.6 g/L (Mostafa and Alamri 2012).

### Effect of nitrogen sources

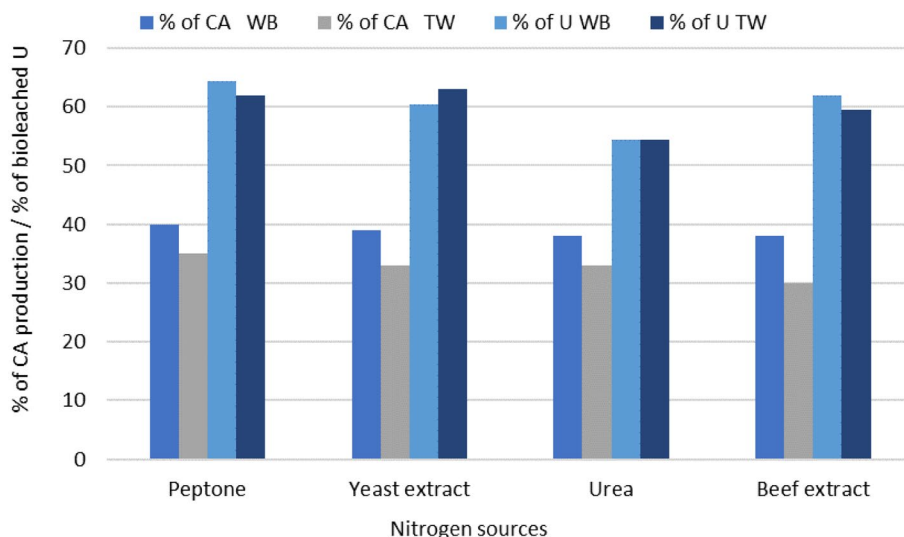
Different nitrogen sources such as Peptone, yeast extract, beef extract and urea were evaluated for the improved uranium bioleaching. The best nitrogen source for wheat bran medium was peptone with CA production level of 40%, and the uranium leaching was 64.35%. While yeast extract was the best for tea waste medium with 33% citric acid production level and 63.11% uranium leaching efficiency, (Fig. 4).

Nitrogen has a significant impact on citric acid production, as it is not only essential for cellular metabolism but also a fundamental component of cell proteins. In CA production, ammonium salts such as ammonium nitrate and sulphate, urea, peptone, malt extract, etc. are utilized (Behera et al. 2021). Because the consumption of acid ammonium compounds results in a decrease in pH, which is essential for citric fermentation, these compounds are preferred. In the oleaginous yeast *Yarrowia lipolytica*, growth on a complex nitrogen source such as peptone enables rapid growth with limited accumulation of lipids droplets, whereas growth on a simple nitrogen source such as ammonium promotes accumulation of lipids in large lipid droplets (Pomraning et al. 2017). *Aspergillus niger* isolated from the leaf litter soil of Sathuragiri Hills produced different amounts of citric acid using various nitrogen sources. Various concentrations of citric acid were produced ( $10.22 \pm 0.05$ ,  $9.13 \pm 0.07$ ,  $6.36 \pm 0.04$  g/L) for ammonium chloride, ammonium sulphate and urea respectively which were used as nitrogen sources (Shankar and Sivakumar 2016). Depending on the nitrogen source, the production of citric and gluconic acid by *Aspergillus niger*, *Penicillium bilaii*, *P. simplicissimum*, and *Paxillus involutus* resulted in different solubilization activities

**Fig. 3** Effect of different sucrose concentrations on citric acid (CA) production and uranium (U) bioleaching activity by *Aspergillus nidulans*. The fungus was cultured in wheat bran (WB) and tea waste (TW) media supplemented with different concentrations of sucrose (50, 100 or 150 g/L). Citric acid concentrations in the culture filtrates were quantified. The bioleaching activity was evaluated by determining the percent of uranium extracted after treating the low-grade uranium ore sample for 180 min contact time at 25 °C



**Fig. 4** Effect of different nitrogen sources on citric acid (CA) production and uranium (U) bioleaching activity by *Aspergillus nidulans*. The fungus was cultured in wheat bran (WB) and tea waste (TW) media supplemented with 100g/L sucrose as a carbon source and different nitrogen sources: peptone, yeast extract, urea, or beef extract. Citric acid concentrations in the culture filtrates were quantified. The bioleaching activity was evaluated by determining the percent of uranium extracted after treating the low-grade uranium ore sample for 180 min contact time at 25 °C



for natural gypsum (Gharieb and Gadd 1999). Enzymes play a crucial role in the formation of CA. Inhibition of some enzymes during glycolysis causes high flux of citric acid that results in its accumulation by this study. Nitrogen deficiency inhibits the anabolism of the fungus, resulting in protein degradation and an increase in intracellular  $\text{NH}_4^+$  concentration. This high intracellular  $\text{NH}_4^+$  concentration inhibits an essential enzyme in the glycolysis pathway, resulting in the accumulation of carbonic anhydrase (CA), which increases the bioleaching activity of the fungus (Behera et al. 2021).

### Effect of different buffer types

The pH level has a significant effect on fungal growth and citric acid production. A direct correlation was observed between the ability of *Aspergillus niger* to produce citric acid and the pH value of the medium (Shankar and Sivakumar 2016).

The best buffer for the fungus cultured in wheat bran was McIlvain which accompanied with high citric acid production of 63% and uranium leaching of 74%, while the best buffer for tea waste was phosphate buffer which accompanied with high citric acid production of 60% and uranium leaching of 71.78%. As shown in Fig. 5. The current outcome conforms to Shankar and Sivakumar (2016). During the sporulation and production phases, the pH of the fermentation medium is crucial. Microbes such as *Aspergillus*, *Rhizopus*, and *Penicillium* species can rapidly lower the pH to below 3, whereas *Sporotrichum* and *Pleurotus* species

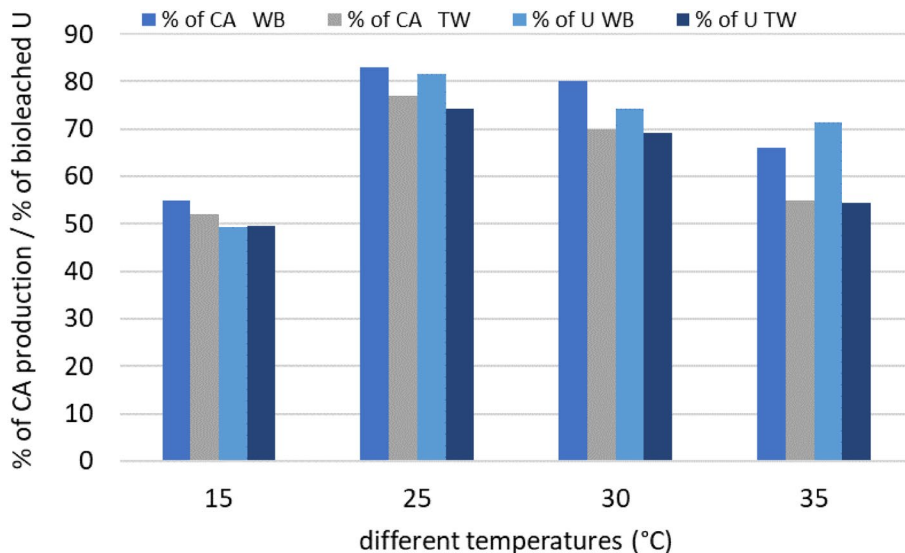
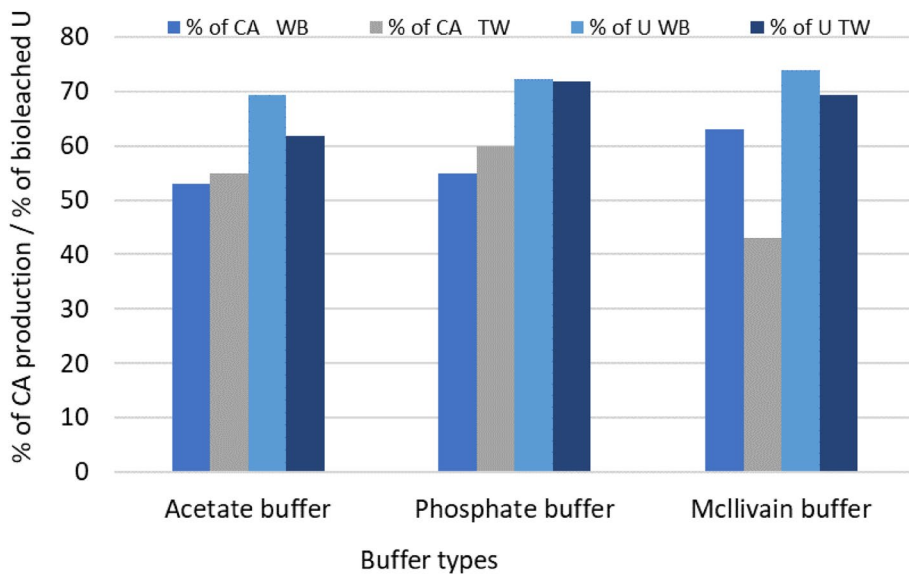
produce a more stable pH between 4 and 5 (Del Campo et al. 2006) Developing spores absorb ammonia and release protons during the germination phase, increasing the acidity of the medium and promoting citric acid synthesis. At a pH of less than 2, the formation of undesirable products such as oxalic and gluconic acid is inhibited, and the risk of contamination by other microorganisms is reduced, making citric acid recovery easier (Sawant et al. 2018). Protein production was 3 mg/ml for the fungus cultured on wheat bran and 2.4 mg/ml for tea waste cultured fungus after growth in McIlvain buffer.

It was observed that the concentration of citric acid increased with an acidic pH of 5.5 and then gradually decreased with the growth of fungi. At a pH of 5.5, the optimal citric acid concentration of 1.94 g/L was obtained from corn starch hydrolysate using *Aspergillus Niger* (Amenaghawon and Aisien 2012). The pH is significant in two ways. First, for spore germination, this is necessary for fermentation, a pH of 5 or higher is required. Second, when ammonia is absorbed by germinating spores, protons are released. This results in the release of hydrogen ions, which lowers the medium's pH. The low pH has the effect of increasing citric acid production and creating an environment that is close to sterile, thereby decreasing the risk of contamination. At pH5, the production of citric acid was 66.7% (Narayanamurthy et al. 2008; Shankar and Sivakumar 2016).





**Fig. 5** Effect of different buffer types on citric acid (CA) production and uranium (U) bioleaching activity by *Aspergillus nidulans*. The fungus was cultured in wheat bran (WB) and tea waste (TW) media supplemented with 100 g/L sucrose as a carbon source, peptone for WB, yeast extract for TW as a nitrogen source. Citric acid concentrations in the culture filtrates were quantified. The bioleaching activity was evaluated by determining the percent of uranium extracted after treating the low-grade uranium ore sample for 180 min contact time at 25 °C



**Fig. 6** Effect of different temperatures on citric acid (CA) production and uranium (U) bioleaching activity by *Aspergillus nidulans*. The fungus was cultured in wheat bran (WB) and tea waste (TW) media supplemented with 100 g/L sucrose as a carbon source, peptone for WB, yeast extract for TW as a nitrogen source, McIlvain buffer for

WB, phosphate buffer for TW, at different temperatures. Citric acid concentrations in the culture filtrates were quantified. The bioleaching activity was evaluated by determining the percent of uranium extracted after treating the low-grade uranium ore sample for 180 min contact time at (15–25 °C)

**Effect of different temperature**

The highest citric acid production by *Aspergillus nidulans* was observed at 25 °C, with 83 and 77% citric acid production for wheat bran and tea waste-based culture mediums, respectively, and 81.6 and 74.25% uranium leached for

wheat bran and tea waste. In contrast, citric acid production and uranium leaching were lowest at 35 °C (Fig. 6).

A similar effect of temperature was observed in a study, with citric acid production increasing between 25 and 30 °C, followed by a decline in production. The fermentation temperature is significant because cells cultured at temperatures below ideal exhibit signs of poor growth and metabolic

production (Dunya A Alhadithy 2020). When *Aspergillus niger* cells are incubated at low or high temperatures, citric acid synthesis is affected by slow fungal germination, slow metabolic activity, enzyme denaturation, and decreased cell viability. The ideal temperature found in this study is consistent with the fact that filamentous fungus, such as *A. niger*, are mesophilic, needing temperatures between 25 and 35 °C for optimal growth (Rinu and Pandey 2010).

The temperature of the fermentation medium is one of the most important parameters affecting citric acid synthesis. Upon growing the fungus at 25 °C, pH of both waste media decreased to 3 for wheat bran and 3.5 for tea waste medium. While, protein content was 3.2 and 3 mg/ml for the fungus cultured on wheat bran and tea waste media respectively.

CA production is dependent on the enzymes of glycolysis and the TCA cycle. These enzymes are temperature dependent. When the temperature of the medium was low, so was the enzyme activity, which had no effect on the production of citric acid. However, when the temperature of the medium exceeded 30 °C, citric acid biosynthesis decreased. It may be due to the accumulation of byproducts such as oxalic acid, while temperatures below 25 °C reduced the growth of the organism and fermentation rates (Arzumanov et al. 2000).

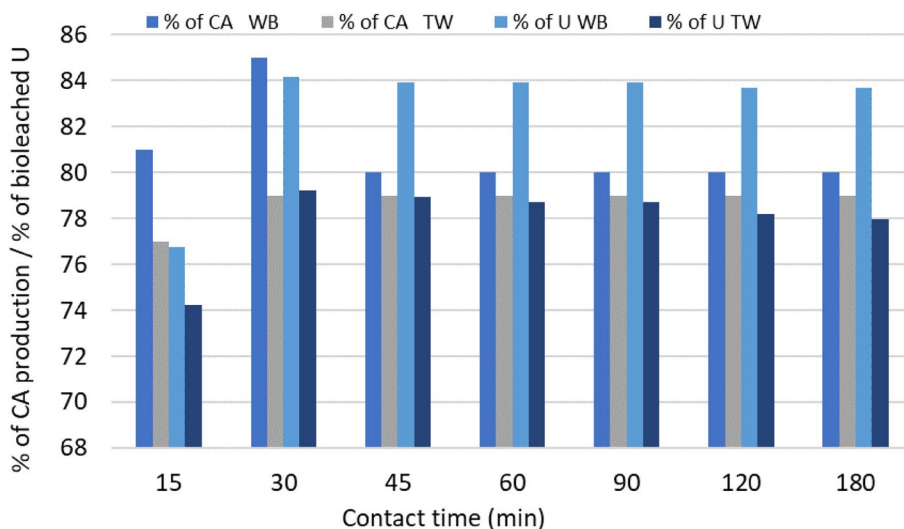
### Effect of different contact time

To determine the optimal time for uranium leaching, the contact time between the fungal filtrate and the ore sample

was varied from fifteen to one hundred and eighty minutes. The optimal contact time was thirty minutes, which improved uranium leaching by 84.16% for wheat bran fungal filtrate and 79.22% for tea waste (Fig. 7). After 30 min of experimentation, uranium leaching stabilizes, possibly due to the consumption of citric acid and other bioactive compounds required for the bioleaching process. However, observation showed this rapid response, which is dependent on the metabolites produced. This is due to the fact that the fungal filtrate was used, which already contains the necessary metabolites for U bioleaching. Using a one-step leaching strategy, Xu et al. (2019) determined that citric acid production and U leaching taking a long time reached up to 20 days.

### Effect of particle size

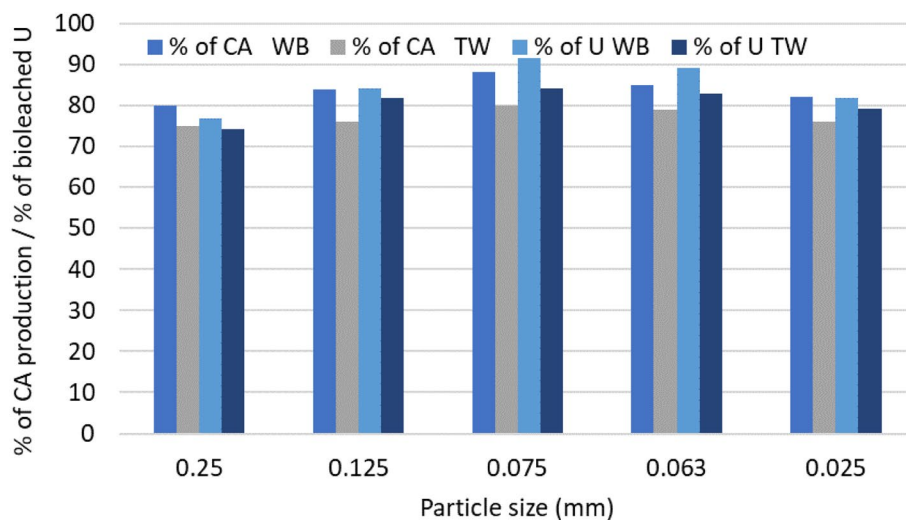
Using a series of particle sizes, the effect of ore particle size on uranium solubilization by fungal extract was studied (0.25–0.025 mm). In all mediums, WB and TW, a strong leaching ability for the fungal filtrate was identified for all particle sizes examined; however, WB performed somewhat better. The particle size with the maximum CA production and best U bioleaching ability was 0.075 mm, with bioleaching 91.58 and 84.15 percent for wheat bran and tea waste-based cultures, respectively (Fig. 8). The small particle size is desirable because it increases the surface area of the intact portion of the ore with the filtrate



**Fig. 7** Effect of different contact time (min) between ore sample and the fungal filtrate on citric acid (CA) production and uranium (U) bioleaching activity by *Aspergillus nidulans*. The fungus was cultured in wheat bran (WB) and tea waste (TW) media supplemented with 100 g/L sucrose as a carbon source, peptone for WB, yeast extract

for TW as a nitrogen source, McIlvain buffer for WB, phosphate buffer for TW. Citric acid concentrations in the culture filtrates were quantified. The bioleaching activity was evaluated by determining the percent of uranium extracted after treating the low-grade uranium ore sample for (15–180 min) contact time at 25 °C





**Fig. 8** Effect of different particle size of the ore sample (mm) on citric acid (CA) production and uranium (U) bioleaching activity by *Aspergillus nidulans*. The fungus was cultured in wheat bran (WB) and tea waste (TW) media supplemented with 100 g/L sucrose as a carbon source, peptone for WB, yeast extract for TW as a nitrogen

source, McIlvain buffer for WB, phosphate buffer for TW. Citric acid concentrations in the culture filtrates were quantified. The bioleaching activity was evaluated by determining the percent of uranium extracted after treating the low-grade uranium ore sample (0.25–0.025 mm particles) for 30 min contact time at 25 °C

containing fungal metabolites, hence increasing the bioleaching capability. In the solution, particles less than 0.07 mm in diameter accumulated into a flat structure. This would lower the surface area between the metal and the fungal filtrate, hence decreasing the efficacy of bioleaching (Mezaguer et al. 2013; Abostate et al. 2018; Abozaid et al. 2021).

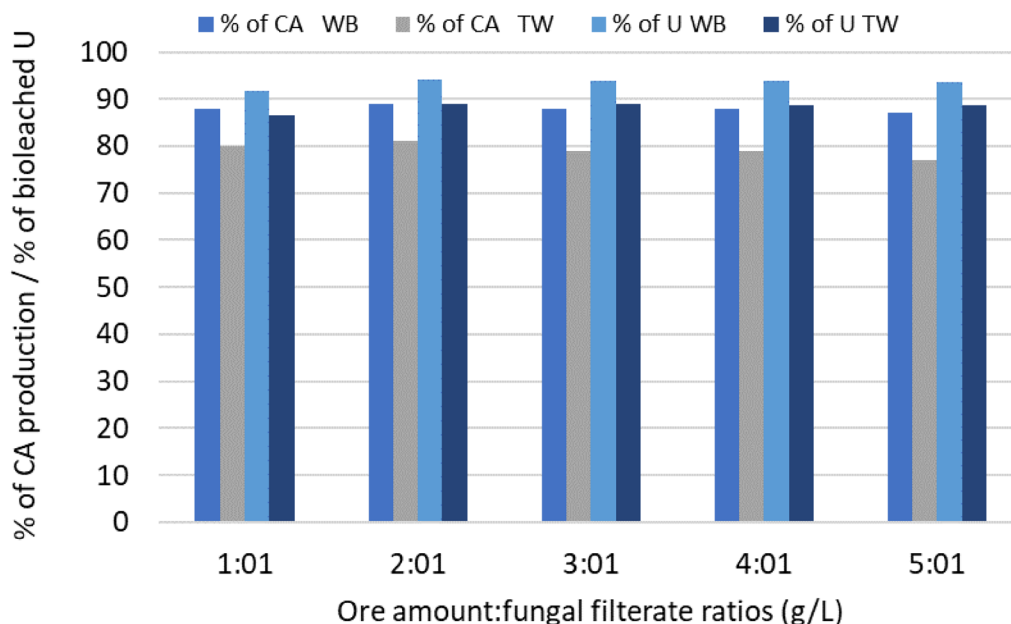
#### Effect of solid liquid ratio (S/L):

Using varying ranges (1:1–5:1 w/v), the influence of ore particle weight (S) on the fungal filtrate (L) employed in U bioleaching was examined. In both WB and TW, a substantial leaching ability was seen for the fungal filtrate at all investigated S/L ratios; however, WB performed slightly better. Wheat bran and tea waste-based cultures had bioleaching percentages of 94.05 and 89.11 percent, respectively, with an optimum S/L ratio of 2:1 g/L for maximum CA production and U bioleaching ability (Fig. 9). Higher S/L ratios demonstrate comparable CA generation and U bioleaching affinity. This could be due to the consumption of acid and other bioactive chemicals necessary for the bioleaching process with the requisite fungal culture, and any further ore particles would not find any acids in the reaction media to solubilize the extra U (Abostate et al. 2018; Abozaid et al. 2021).

## Conclusion

Using agricultural waste as a substrate for *A. nidulans* growth, such as wheat bran and tea waste, provides a superior alternative to the commonly used culture media, with superior growth and performance. The optimal growth and citric acid production of *A. nidulans* across different media compositions and process parameters. Notably, wheat bran and tea waste served as cost-effective substrates for the synthesis of high yield citric acid. Bioleaching of low-grade uranium ores with the metabolic products of *A. nidulans* is a promising and environmentally benign technique. Sucrose (100 g/L), peptone and yeast extract at 25 °C were the optimal carbon and nitrogen sources for maximum uranium extraction driven by citric acid. Rapid uranium extraction that reached 94% within 30 min was achieved from fine ore particles (0.075 mm) in a molar ratio of 2:1 (ore particle and fungal filtrate) highlighting the efficiency of the bioleaching process. In contrast to conventional acid leaching techniques, bioleaching by *A. nidulans* metabolite selectively recovered uranium without co-dissolving iron impurities. The amorphous nature of iron causes it to dissolve and precipitate with leached uranium, thereby inhibiting the leaching of pure uranium and reducing its purity. This represents the first application of uranium bioleaching that dissolves uranium without dissolving any associated uranium impurities. The economic viability and environmentally benign nature of this biotechnology highlights its potential to supplant conventional approaches. This





**Fig. 9** Effect of different solid: liquid ratio of the ore sample (g) to the fungal filtrate (L) on citric acid (CA) production and uranium (U) bioleaching activity by *Aspergillus nidulans*. The fungus was cultured in wheat bran (WB) and tea waste (TW) media supplemented with 100 g/L sucrose as a carbon source, peptone for WB, yeast extract for TW as a nitrogen source, McIlvain buffer for WB, phosphate

buffer for TW. Citric acid concentrations in the culture filtrates were quantified. The bioleaching activity was evaluated by determining the percent of uranium extracted after treating the low-grade uranium ore sample (0.075 mm particles, in 1:1–5:1 solid/liquid ratio) for 30 min contact time at 25 °C

innovative research developed a novel biological technique for uranium extraction from low-grade ores. The use of citric acid solutions derived from microorganisms for bioleaching has promising implications for environmentally responsible mining practices. Additional testing at the pilot scale can facilitate the industrial adoption of this revolutionary bioprocessing technology.

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**Data availability** The datasets generated and/or analyzed during the current study are present in the current manuscript.

## Declarations

**Conflict of interest** The authors declare that they have no competing interests.

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

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