

Simultaneous lithium bioleaching and bioaccumulation from lepidolite using microscopic fungus *Aspergillus niger*

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Abstract

The leaching of lithium from lepidolite using the filamentous fungus *Aspergillus niger* was examined. Two mechanisms were suggested – biochemical using citric acid as the main bioleaching agent and biomechanical through hyphae penetration confirmed by XRD and SEM analyses. The bioleaching processes were conducted at various glucose concentrations (5 – 20 g.L⁻¹). The higher glucose concentration was, the higher Li bioleaching was observed. Li accumulation by fungal biomass played an important role in Li solubilisation from lepidolite. Totally, 11.5 mg of lithium were recovered from 1 kg of lepidolite by combination of bioleaching and bioaccumulation processes. As a result of bioleaching, the formation of new silicate phase of SiO₂ was detected. According to the results, fungal bioleaching of Li from lepidolite can be a perspective way of Li recovery from hard-rock ores.

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Introduction

Lithium, the lightest alkaline metal, becomes one of the most important elements in the earth due to its growing application in various industries such as energy sector, pharmaceuticals, medicine or ceramics (Zhang *et al.* 2018). Expanding market for lithium caused huge increase in demand for this metal. Several studies predict we face a shortage of lithium in the near future (Heydarian *et al.* 2018). Due to these reasons there is a growing pressure on the development of technologies for lithium extraction from all possible sources including hard-rock ores (Swain 2017).

Recently, many research groups are focused on developing environmentally sustainable as well

as cost effective technology (Meshram *et al.* 2014; Onalbaeva *et al.* 2016). Among them very promising are biotechnological methods using bioleaching as this method overcome the operational and technical problems associated with lithium recovery. Traditionally acidophilic bacteria are widely applied, however, their usage is mostly oriented on metal recovery from sulphidic ores or metal-bearing waste materials, but their utilisation in aluminosilicates is limited (Sedláková-Kaduková *et al.* 2017). The most abundant lithium containing rocks are pegmatites with various Li minerals (Zhang *et al.* 2018). The most interesting Li mineral for industry is spodumene due to its high Li content, large deposits and low content of other impurities

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(metals). Other minerals are petalite, lepidolite amblygonite, zinnwaldite and eucryptite. Lepidolite is the most abundant Li containing mineral, however, containing less Li than spodumene (Tadesse *et al.* 2019). The mineral composition of lepidolite and its aluminosilicate lattice was the reason why heterotrophic fungi have been selected for Li bioleaching from this mineral. Heterotrophic fungi have a great potential to degrade aluminosilicates resulting in metal extraction from these ores because in nature, fungi significantly contribute to the silicate bearing rocks (including mica, and iron and manganese bearing rocks) weathering (Gadd 2010). They use two, often, synergistic actions to degrade mineral substrates, namely biomechanical and biochemical processes. However, the microbial activity is considered to have much more significant impact on the degradation of these substrates than mechanical actions. In biomechanical weathering process fungal hyphae may directly penetrate into intact mineral material or by producing extracellular mucilaginous substances they can indirectly deteriorate the mineral firstly by acidic and metal-chelating metabolites excreted to mucilaginous slime but also by forming a mechanical pressure to the mineral by shrinking and swelling of biofilms (Gadd 2007). Biochemical action involves several mechanisms, such as acidolysis, complexolysis, reductive mobilization and the function of mycelium to bound the soluble metal species (Fomina *et al.* 2005). Silicate biodegradation may be also accelerated by carbonic acid attack due to the CO₂ released during fungal respiration (Sterflinger 2000).

Among the most bioleaching active fungal species been isolated and used in metal bioleaching belong species from the *Aspergillus* genus, with the most efficient *Aspergillus niger* (Šimonovičová *et al.* 2012). It was found that *A. niger* can degrade dunite, serpentine, olivine, muscovite, feldspar, kaolin, spodumene and nepheline (Gadd 2007), however its ability to deteriorate lepidolite was not previously reported.

According to our previous experience as well

as published studies dealing with the utilization of *Aspergillus* for metal bioleaching (Dursun *et al.* 2003; Littera *et al.* 2011; Marcinčáková *et al.* 2014) we decided to study not just the lithium bioleaching but also consequent lithium bioaccumulation in the *Aspergillus* biomass. Therefore, this work was aimed to investigate the ability of microscopic fungus *A. niger* to solubilize and subsequently accumulate Li from lepidolite. For this purpose, not just changes in pH, lithium concentration in media but also concentration of Li accumulated in biomass were determined experimentally. Besides, biomechanical process of lepidolite degradation resulting in Li dissolution was examined using XRD and SEM analyses.

Experimental

Ore samples

The raw lepidolite sample was obtained from prof. Rowson (University of Birmingham, UK). The sample origin is in the mine Beauvoir (France). The mineralogical composition of lepidolite used in experiments is in Table 1.

Microorganisms

The fresh culture of *Aspergillus niger* obtained from Dpt. of Soil Science, Faculty of Natural Sciences in Bratislava, Slovakia was maintained at 4 °C on a solid Sabourad Dextrose Agar (HiMedia Laboratories) slant. Stock cultures were subcultured every month.

Bioleaching experiment

Based on our former research (Marcinčáková *et al.* 2014) we conducted the pre-cultivation phase for 8 d. Obtained biomass was further used in the experiment.

Bioleaching media were composed of 5 g.L⁻¹ glucose and 0.5 g.L⁻¹ (NH₄)₂SO₄. Both, medium and mineral were sterilised by autoclaving for

Table 1. Mineralogical composition of lepidolite [%].

SiO ₂	Al ₂ O ₃	K ₂ O	Li ₂ O	Fe ₂ O ₃	TiO ₂	CaO	MgO	Na ₂ O
51.0	26.0	7.75	3.79	0.50	0.03	0.05	0.05	0.38

20 min at 120 °C. Initial pH value was adjusted to 5.1. 2 g of crushed mineral and 5 mL of cultivated conidia (biomass) were added into 200 mL of media. The experiment was carried out under 21 °C without shaking. At day 4, 11, 18, 25, 33 and 41 samples for the measurement of Li content in medium were withdrawn. After collection, the samples were filtered through the 0.45 µm-pore-size membrane filter. At the end of the experiments biomass was separated from the medium and Li content was analysed separately in the bioleaching residue, medium and biomass to calculate Li recovery efficiency. The experiment was conducted in duplicate.

To study the effect of glucose on bioleaching, assays were conducted in the medium with different variants of glucose concentrations in media, namely 5, 10 and 20 g.L⁻¹.

Biomass and residue processing after the experiments

At the end of experiment the biomass was removed and washed three times with distilled water. The bioleaching residue was obtained after the filtering the rest of the medium and washed with distilled water. Air-drying of the biomass and residue samples for 24 h was used prior mineralisation in oven for 4 h at 500 °C. To determine accumulated lithium the biomass was digested by the 2 M HCl. The amount of biomass was calculated per 1 L of media.

Analytical and characterisation methods

Li concentration was measured by Atomic Absorption Spectrophotometer (Perkin Elmer 3100). The initial sample and final leaching residues were also mounted with silver paste on aluminium stubs, then coated with 300 – 400 Å Au/Pd in a sputtering unit and finally examined in a JEOL scanning electron microscope. Mineral composition before and after the bioleaching process was determined by a diffractometer Bruker D2 Phaser (Bruker AXS, GmbH, Germany) in Bragg-Brentano geometry (configuration Theta-2Theta), CuKα radiation.

Results and Discussion

Rapid decrease of pH value in bioleaching media due to an organic acid production was observed within first 7 d (Fig. 1) followed by the slow pH decrease phase up to 42th d. Acid concentration and biomass dry weight increases (Fig. 1) indicated the active growth phase of *Aspergillus niger* (Aung and Ting 2005; Amiri et al. 2011).

The beginning of the slow pH decrease in media indicated the end of the active growth phase of the fungus (observed after 10 d of incubation), and has been associated with the accumulation of secondary metabolites and beginning of the stationary phase (Qu et al. 2013). A small increase in pH (from 5.2 to 5.6) was observed at the end of the growth phase in a control medium as a result of the carbon deficit essential

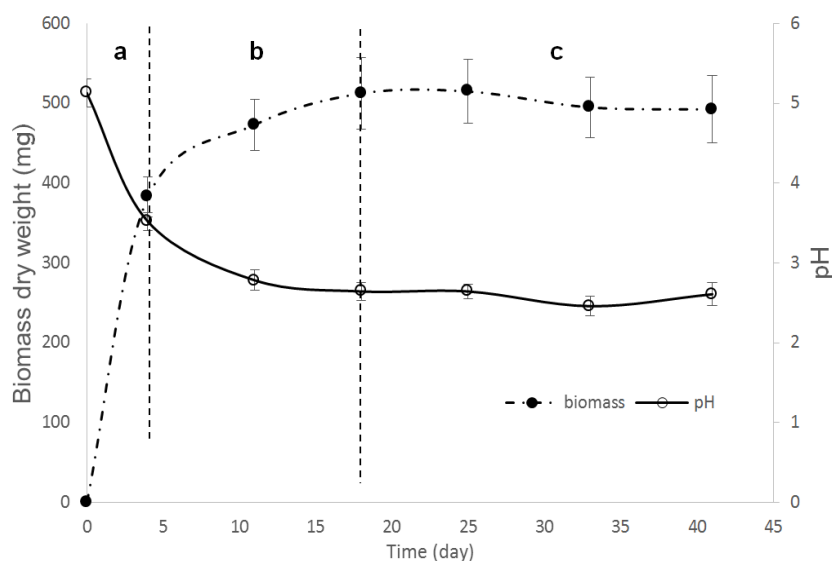
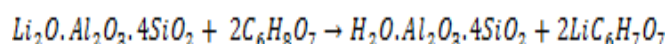


Fig. 1. Relationship between the changes of pH and *A. niger* biomass production during Li bioleaching from lepidolite (a – exponential growth phase, b – reduced growth phase and c – stationary phase). Glucose concentration 5 g.L⁻¹, pH 5.1, temperature 21 °C. Data represent Mean ± Error ($n = 3$).

for microbial growth and indicated no production of organic acids. In contrast, significant drop of pH values has been associated with accumulation of organic acids in the media as the result of high metabolic activity of the *A. niger* cells. According to various authors (Rezza *et al.* 2001; Aung and Ting 2005; Amiri *et al.* 2011; Gadd *et al.* 2014; Bahaloo-Horeh *et al.* 2016) malic, citric, gluconic and oxalic acids were produced by the microscopic fungus *A. niger*. The predominant organic acid secreted by *A. niger* in glucose medium is citric acid (Anjum *et al.* 2010). At the beginning of the incubation at pH 5.1, however, gluconic acid was probably produced since glucose oxidase is the most active at pH of 4.5 – 6.5 and inactive at pH levels below 3 (Ruijter *et al.* 2002; Wu and Ting 2006). The citric acid production by *A. niger* starts at a pH below 3 and proceeds at pH 2 (Amiri *et al.* 2011). The pH decrease below 3 was observed after the 14th d of the incubation. According to Amiri *et al.* (2011) the lower pH values resulted in decreasing the gluconic acid production and increasing of the citric acid concentration. Oxalic acid biosynthesis under optimal production conditions occurs in the pH range of 5 – 8 (Gadd *et al.* 2014; Bahaloo-Horeh *et al.* 2016).

The malic acid does not play as important role in bioleaching as citric and gluconic acids because it is produced in lower amounts and under both anaerobic and aerobic conditions it is easily degraded (Bahaloo-Horeh and Mousavi 2017). Since pH of the bioleaching medium was between

5 – 2, citric acid was probably the most important bioleaching agent in the process. The pK_a values of three carboxylic functional groups of citric acid were found at pH 3.1, 4.7 and 6.4 (Max *et al.* 2010). On the basis of dissociation constant values at pH = 2.8 it can be assumed that only one carboxylic group of citric acid was dissociated resulting in the formation of lithium citrate (Ilgar *et al.* 1993). The reaction can be expressed as follows:



Lithium was found in the solution after 25th d of incubation for the first time, its concentration was 27 µg.L⁻¹ (Fig. 2). Subsequently, rapid increase in lithium concentration was observed. However, part of the solubilised lithium was accumulated into the biomass that was confirmed by the biomass analysis. It is possible that simultaneous lithium bioaccumulation could significantly contribute to Li solubilisation from mineral since rapid decrease of Li⁺ cations concentration caused by their accumulation in the mycelium shifted the equilibrium and resulted in the increased rate of the Li dissolution. At the end of the experiment Li amount in biomass was 110 µg.g⁻¹ of dry weight. A total of 9.5 mg of Li was recovered from 1 kg of lepidolite.

The production of organic acids as the main metal leaching agents is significantly affected by the amount of sugars in the medium (Castro *et al.* 2000). The latter authors found that higher glucose concentration in media leads to higher citric acid

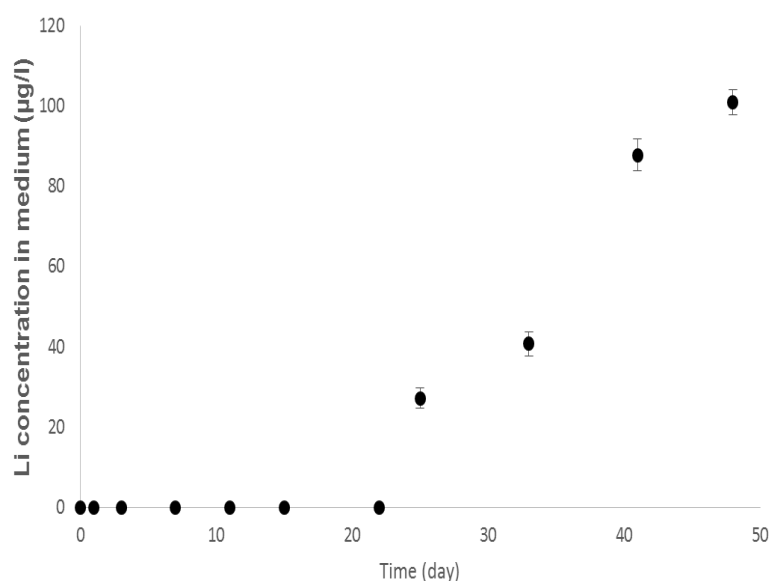


Fig. 2. Changes of Li concentration in media during Li bioleaching by *A. niger*. Glucose concentration 5 mg.L⁻¹, pH 5.1, temperature 21 °C. Data represent Mean ± Error (n = 3).

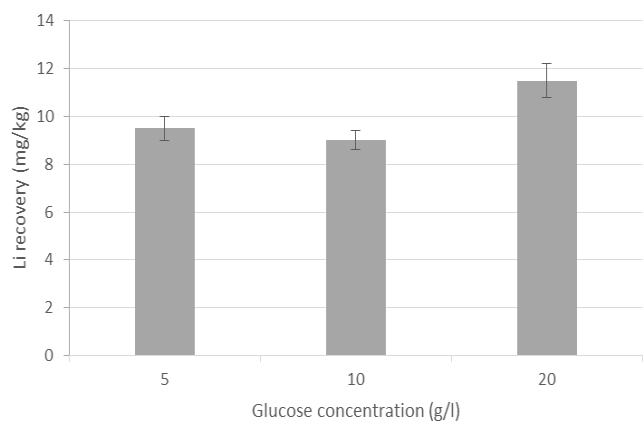


Fig. 3. Final recovery of Li from lepidolite by *A. niger* bioleaching under various glucose concentrations. Initial pH 5.1, temperature 21 °C. Data represent Mean \pm Error ($n = 3$).

production. Previous studies (Wu and Ting 2006; Bahaloo-Horeh and Mousavi 2017) showed that the most important organic acid in bioleaching by *A. niger* is citric acid, while Ilgar *et al.* (1993) even recognized citric acid as responsible for lithium solubilisation from β -spodumene.

To optimize the bioleaching efficiency, we studied the influence of various glucose concentration in medium to enhance the lithium bioleaching efficiency from lepidolite. Low-nutrient medium with initial glucose concentration 5, 10, and 20 g.L⁻¹ was used for the experiment. No significant changes in media pH were observed among experimental variants. Fast pH decrease was recorded in all three media from 5.1 to 2.6 up to 19th d of the bioleaching with no changes till the end of experiment. However, higher glucose concentration in media significantly affected the amount of *A. niger* biomass produced in media.

The highest amount of biomass (1,080 mg.L⁻¹) was found in medium with highest glucose concentration. Favourable effect of higher saccharide concentration on *A. niger* growth and biomass production was confirmed by various authors (Castro *et al.* 2000; Wu and Ting 2004; Anjum *et al.* 2010; Qu *et al.* 2013). Here, higher fungal growth resulted in higher Li recovery. The highest lithium recovery 11.5 mg.kg⁻¹ of lepidolite (summarised from Li bioleaching into solution and Li bioaccumulation into biomass) was found at glucose concentration 20 mg.L⁻¹, while only 9.5 mg of Li was recovered from kg of lepidolite at the lowest glucose concentration in media (5 mg.L⁻¹) (Fig. 3). The direct connection between the metal recovery percentage and fungal growth was also observed by Amiri *et al.* (2011) in their study of metal dissolution from spent catalysts by *A. niger*. The authors suggested that higher bioleaching efficiency was caused by higher metabolite production.

SEM analysis showed high interpenetration activity of *A. niger* into the lepidolite (Fig. 4). It confirms that biomechanical process was also a part of Li bioleaching from lepidolite. However, according to literature (Gadd 2010) biomechanical processes are considered less important in mineral weathering in comparison with biochemical processes.

The micro-morphological changes support the observation that fungal carboxylic acids, e.g. citric and oxalic acids, with strong chelating properties disrupted the surface of the mineral due to chemical corrosive action and resulted in the Li mobilization to the solution confirming complexolysis as main

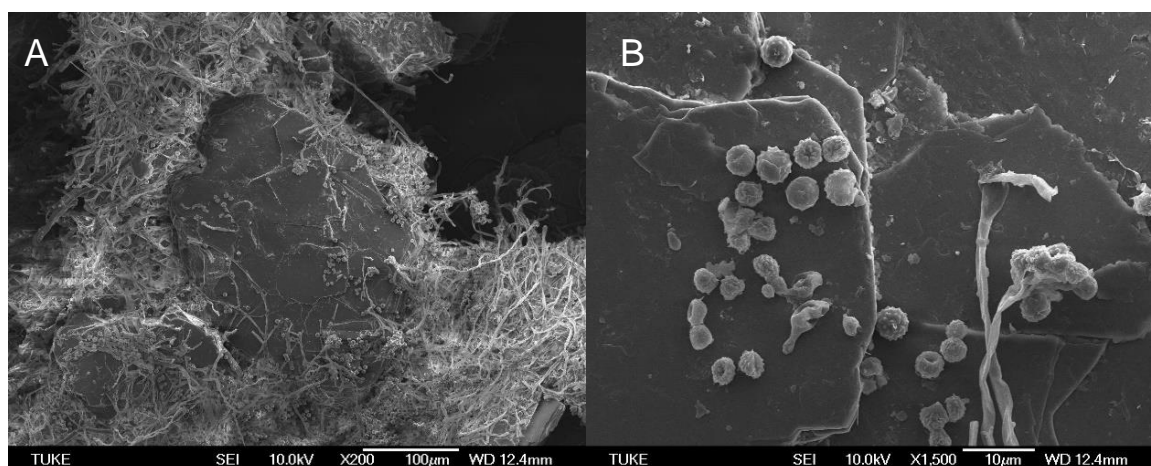


Fig. 4. Attachment of *A. niger* hyphae to lepidolite surface observed with SEM. Hyphae growing on the surface of lepidolite particles (A) and produced spores attached to the surface (B).

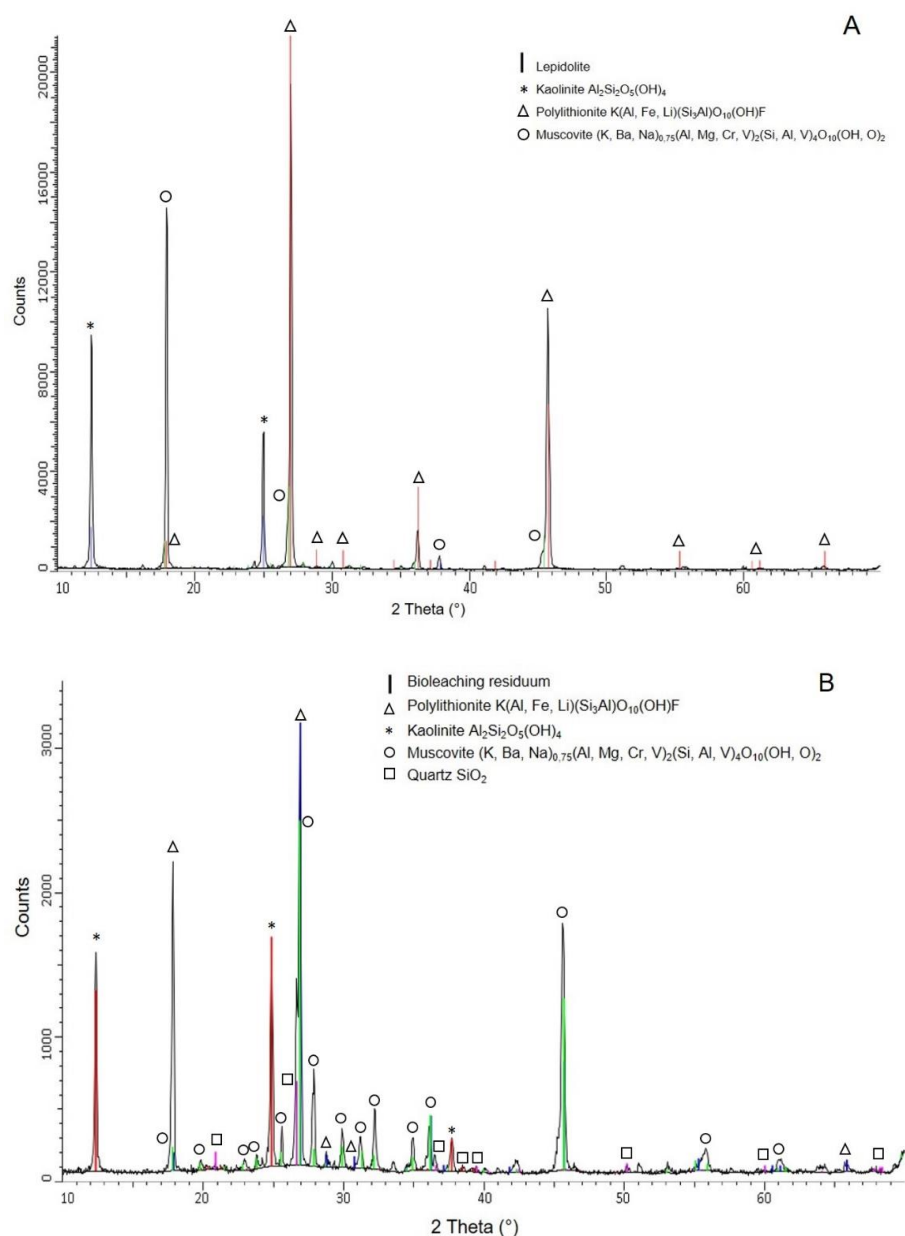


Fig. 5. X-ray diffraction of original ore (A) and bioleached residue (B) (Sedláková-Kaduková *et al.* 2020 – Supplementary information).

mechanisms of fungal bioleaching. The changes of the mineral caused by the fungal leaching were also studied by comparing the mineral phases prior to and after the bioleaching through XRD analysis (Fig. 5) (Sedláková-Kaduková *et al.* 2020 – Supplementary information).

Prior to bioleaching, the crystalline phase of polythionite, muscovite and kaolinite were clearly detected. After the bioleaching the phase changes with a dominance of muscovite were observed (Fig. 5). Appearance of new silicate phase of SiO_2 was detected. The formation of silicate phases was observed also during the aluminosilicate phlogopite bioleaching by bacteria *Acidithiobacillus ferrooxidans* (Tariq *et al.* 1993).

In the bioweathering of both silicate and aluminosilicate minerals the breakup of Si–O–Si (siloxane) as well as Al–O bonds or cation removal from the silicate crystal lattice can result in the collapse of the silicate lattice structure (Gadd 2010) contributing to Li release into the solution phase.

Conclusions

Microscopic fungus *Aspergillus niger* can solubilize Li from lepidolite through two main mechanisms – biochemical leaching and biomechanical deterioration. The biochemical process involved complexolysis by mostly citric

acid and consequent bioaccumulation of lithium inside the fungal biomass. Contribution of biomechanical process was confirmed by SEM analyses. Hyphal penetration along the crystal boundaries together with the erosion of mineral surface by organic acids was observed. The results also displayed significant differences in Li bioleaching efficiency when various glucose concentrations were used. The highest efficiency was found at the highest (20 g.L⁻¹) glucose concentration. These findings indicate that *A. niger* could be effective for Li bioleaching from hard-rock lithium ore lepidolite.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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