The Influence of Spore Age of *Aspergillus Niger* on Lithium Dissolution from Lepidolite

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Summary

Lithium and its compound have several commercial applications uses, including metal refining, organic synthesis and polymerization, manufacture of pharmaceuticals, glass, ceramics and batteries. Nowadays, lithium is becoming more and more interesting and attractive as a constituent of batteries for electric and hybrid vehicles. In nature lithium is the most frequently occurring metal; however, in very low concentration. The conventional processing of pegmatites containing lithium bearing aluminosilicates is time, energy and cost intensive. Biohydrometallurgical approaches are generally considered as technologies with low-cost and low-energy requirement. Some species of heterotrophic microorganisms such as Aspergillus and Penicillium have shown a great potential for metal bioleaching from ores and various waste materials such as fly ash, spent catalysts and electrical waste. Heterotrophic microorganisms of genera from Aspergillus exhibit a good potential in producing of organic acids, mainly oxalic, citric and gluconic acids, effective for metal extraction from low-grade ores and waste. This present study examines the influence of spore age of Aspergillus niger on lithium extraction from aluminosilicates. Spores or conidia, used for the experiment, were cultured 4 and 12 days. The metal bioleaching experiments were carried out in low nutrient media at ambient temperature. For the first time lithium was present in the solution on day 26 in both cases in the amount of 60 μg/l and 26 μg/l using 4-day and 12-day old spores, respectively. Since A. niger is characterized by a high ability to accumulate various metals lithium was also determined in the biomass. Results revealed that much more biomass (fungal mycelium) was generated by long-term cultured spores than short-term ones. Lithium concentrations accumulated in the biomass produced by 4-day and 12-day old spores were found to be 121μg/l and 545μg/l, respectively. In spite of rather low pH values, about pH=3, in both leaching systems a higher Li bioleaching efficiency was achieved using long-term cultured fungi. The results of a scanning electron micrograph (SEM) examination of the mineral before and after the bioleaching process pointed out the structural changes of the mineral surface after the attack by A. niger. X-ray analysis also confirmed the changes in crystalline structure of the mineral before and after the bioleaching process.

Keywords: bioleaching, aluminosilicate, Aspergillus niger, Li recovery

Introduction

Lithium is of the importance for a number of uses, including metal refining, organic synthesis and polymerization, manufacture of pharmaceuticals, glass, ceramics and batteries. Worldwide, rechargeable lithium batteries power about 60% of cellular telephones and about 90% of laptop computers (Smith, 2010). Lithium is even becoming more and more interesting and attractive as a constituent of batteries for electric and hybrid vehicles. According to the USGS, lithium demand for ion-battery usage is forecast to grow 20% per annum, while overall demand is expected to grow 4 - 5% every year (Goonan, 2012). Prognoses indicate that a demand for lithium is expected to increase. The concern of European Commission is that the demand for the metal could increase to the point at which a shortage of these metals will occur. According to the European Commission report, lithium is listed among the critical raw materials for European Union (Report of EC, 2010).

In nature lithium is present in a variety of aluminosilicates and continental brines. Lithium minerals of economic importance are mainly pegmatites, which include spodumene $LiAlSi_2O_6$, petalite $LiAlSi_4O_{10}$, lepidolite $K(Li,Al)_3(SiAl)_4O_{10}(F,OH)_2$ with the lithium presence ranging of 1.37–3.6% Li. In brines and lakes lithium presence is only about 0.02-0.15% (Siame, 2011). The conventional processing of lithium aluminosilicates is energy and capital intensive process therefore there is of real interest to find new alternative methods for its extraction. Biohydrometallurgical approaches are generally considered as "green technologies" with low-cost and low-energy requirement (Luptakova et al., 2002, Wu and Ting, 2006, Kusnierova et al., 2011, Qu et al., 2013, Willner and Fornalczyk, 2013). Some species of heterotrophic microorganisms such as *Aspergillus* and *Penicillium* have shown a great potential for metal bioleaching from ores and various waste materials such as fly ash, spent catalysts and electrical waste (Šimonovičová et al., 2013, Qu et al., 2013). Due to the strong adaptability, high metabolic activity and high production of organic acids, amino acids and other metabolites, *Aspergillus niger* becomes the one of the most widely used fungi in

Tab. 1 Mineralogical composition of the lepidolite

Tab. 1 Skład mineralogiczny lepidolitu

SiO_2	Al_2O_3	K_2O	Li_2O	Fe_2O_3	TiO_2	CaO	MgO	Na_2O
51 %	26.03 %	7.75 %	3.79 %	0.50 %	0.03 %	0.05 %	0.05 %	0.38%

bioleaching (Aung and Ting, 2005, Ren et al., 2009, Qu et al., 2013). These metabolites dissolve metals from minerals by displacement of metal ion from the ore or soil matrix by hydrogen ions or by the formation of soluble metal complexes and chelates (Burgstaller and Schinner, 1993, Ren et al., 2009). Although the process of lithium extraction from minerals using *A. niger* looks promising, only few studies have been performed. Ilgar at al. 1993 investigated kinetics of lithium extraction from alpha- and beta – spodumene. Rezza et al. 1997 and Rezza et al. 2001 also examined lithium recovery from spodumene and tried to understand the mechanisms involved in the attack of the fungus to the mineral. The purpose of this investigation was to examine the influence of spore age of *Aspergillus niger* on lithium extraction from lepidolite. Since *A. niger* is capable of producing more organic acids in the medium with low glucose content (Rezza et al. 1997) as the bioleaching medium the low nutrient medium was used.

Materials and methods

Ore samples

The crushed lepidolite used in this work was obtained from Dr. Rowson (University of Birmingham, UK). The mineralogical deposit of the ore is situated in Beauvoir (France). The composition of this mineral is shown in Table 1.

Microorganisms

Heterotrophic microorganism of Aspergillus niger was obtained from Department of Soil Science, Faculty of Natural Sciences in Bratislava and maintained at 4 °C on a solid Sabourad Dextrose Agar slant. Stock cultures were subcultured every month.

Bioleaching experiment

The experiments were carried out in 250 ml Erlenmeyer flasks containing 200 ml of liquid bioleaching media composed of glucose – 5 g/l and (NH4)2SO4 – 0.5 g/l with the initial pH value of 5.1. To the bioleaching media 2 g crushed mineral and 2 ml of 5-day and 12-day old spores or conidia, respectively, were added. The flasks were sealed with removable cotton and the experiment was carried out at at 21 °C. Prior to leaching the medium and mineral were sterilized by autoclaving for 20 min at 120 °C before spores added. Each experiment was conducted in duplicate.

For elemental analysis the samples were collected by disposable sterilized pipettes and filtered through the 0.45 µm-pore-size membrane filter. At the end of the experiments the biomass was easily removed and washed with distilled water. The biomass and residue samples were air-dried for 24 h. Thereafter, the biomass was digested by the hydrochloric acid method to determine lithium accumulated in the biomass. Li concentration was measured by Atomic Absorption Spectrophotometer (Perkin Elmer 3100) at 670 nm. The initial sample and final leaching residues were also mounted with silver paste on aluminium stubs, then coated with 300 – 400 A 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

Lithium concentration in leach liquor achieved during bioleaching by Aspergillus niger of different spore age is shown in Fig. 1. As it can be seen lithium was present in the solution for the first time on day 26 in both cases in the amount of 60 µg/l and 26 µg/l using 4-day and 12-day old spores, respectively. During bioleaching using short-term cultured spores, the highest amount of Li (98 µg/l) in the solution was found on day 33. Consequent lithium concentration loss observed on the following days may be explained by Li accumulation into the biomass (fungal mycelium).

In the case of Li bioleaching using long-term cultured spores, gradual Li release into the solution was observed throughout the bioleaching process and at the end of the experiment 101 µg/l Li were found in the solution.

The pH changes over the time during lithium bioleaching with A. niger of different spore age are plotted in Fig. 2. As it can be seen, in the first 12 days of bioleaching in both systems a pH rapidly decreased up to value of 2.8 and 3.3 using 4-day and 12-day old spores, respectively. Afterwards the pH remained stable around pH 2.8 using 4-day old spores. In the case of 12-day old spores, pH slightly decreased up to 2.35 reached on day 40. Based on the results it is evident that a little higher hydrogen consumption in the leaching system occurred. The reaction of the acidolysis (1) can be explained as follows:

$$(\text{M-mineral}) + \text{HL} \rightarrow (\text{H-minerals}) + \text{ML} \qquad (1)$$

where M is a monovalent metal such as lithium and HL is an organic acid. The extent of this reaction depends on the proton activity of the organic acid (Ilgar et al. 1993).

Since not only acidolysis but also complexolysis is involved in degradation of aluminosilicates as Ilgar et al., 1993 reported, it may be assumed that metabolic oxalic and citric acids as chelating agents solubilized the minerals by forming metal-organic coordination complexes or chelates (2) as follows:

$$(\text{M}_m\text{-mineral}) + n\text{HL} \rightarrow (\text{H}_m\text{-mineral}) + \text{H}_{n-m}(\text{M}_m\text{L}_n) \qquad (2)$$

Since *A. niger* is characterized by a high ability to accu-

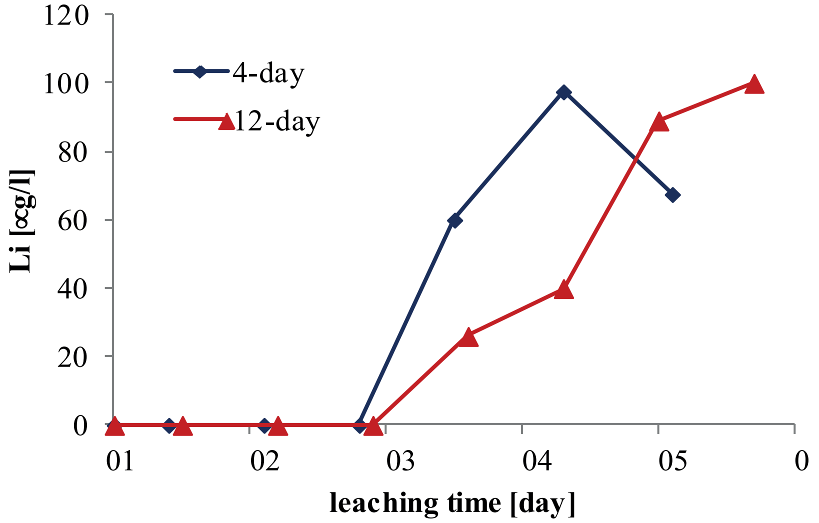

Fig. 1 Comparison of lithium bioleaching by *A. niger* of different age

Rys. 1 Porównanie bioługowania litu z użyciem *A. Niger* w różnym wieku

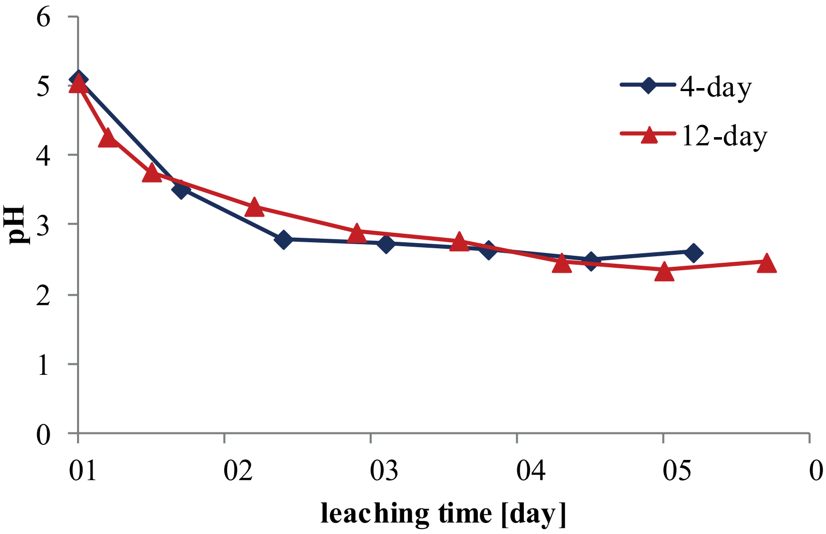

Fig. 2 pH changes over the time during Li bioleaching with *A. niger*

Rys. 2 Zmiany pH w czasie podczas bioługowania litu z użyciem *A. Niger*

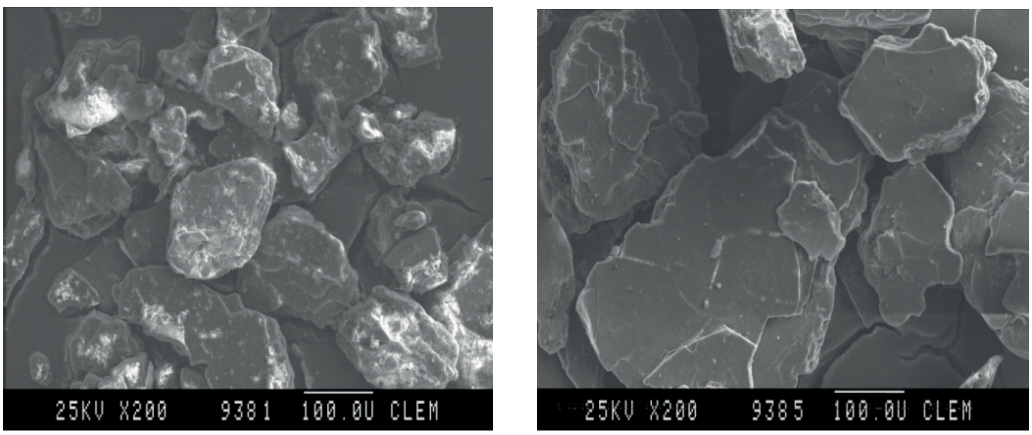

Fig. 3 SEM of the mineral before a) and after b) bioleaching with *A. niger*

Rys. 3 SEM minerału przed (a) i po (b) bioługowaniu z użyciem *A. Niger*

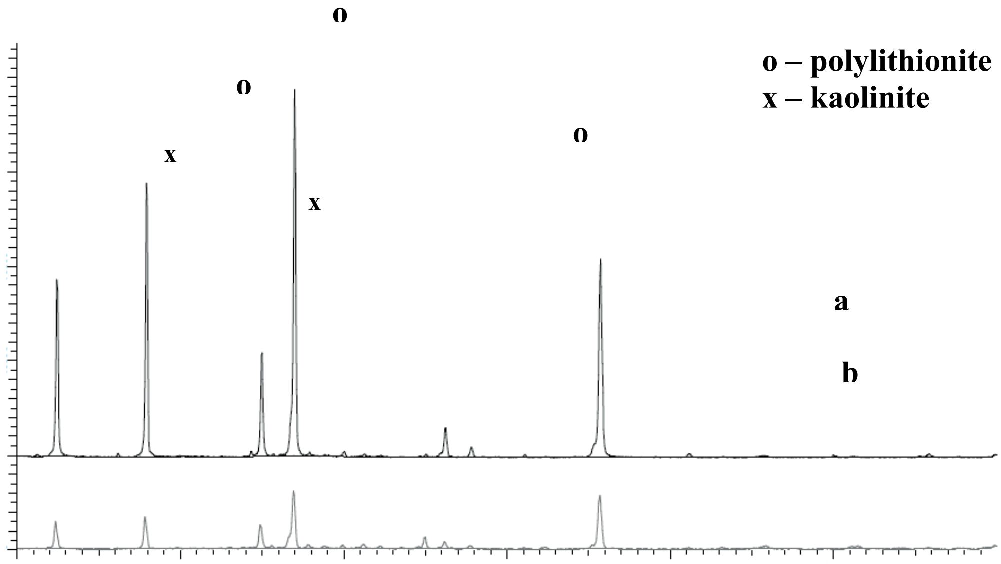

Fig. 4 X-ray analysis of the mineral before a) and after b) bioleaching with *A. niger*

Rys. Prześwietlenie rentgenowskie minerału przed (a) i po (b) biołlugowaniu z użyciem *A. niger*

mulate various metals it was assumed that lithium was accumulate into the biomass. Therefore, at the end of the experiments lithium concentration was also determined in the biomass obtained from the leach liquor. Li accumulated in the biomass produced by 4-day and 12-day old spores was found to be 121µg/l and 545µg/l, respectively. In the medium with short-term cultured spores of A.niger much less biomass was produced compared to long-term cultured spores. The revealed that the highest amount of Li (645µg/l) was dissolved using long-term cultured spores. By the usage of short-term cultured spores only 189µg/l Li dissolved.

The results of a scanning electron micrograph (SEM) examination of the mineral before and after the bioleaching process (Fig. 3) point out the structural changes of the mineral surface after the attack by A. niger. In the Fig. 3b arrows point out the spores of the fungus.

The changes in crystalline structure of the mineral before and after the bioleaching process were also confirmed by X-ray analysis (Fig. 4).

Conclusion

In summary it may be concluded that organic acids generated by A. niger were involved in the leaching process. The age of spores or conidia of the heterotrophic fungus can influence lithium dissolution from the mineral. Despite a rather low Li leaching efficiency reached it may be concluded that Aspergillus niger could to certain extent disrupt crystalline structure of the mineral.

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Wpływ wieku zarodników Aspergillus niger *na roztwory litowe z lepidolitu*

Lit i jego związki mają kilka zastosowań komercyjnych, np. w rafinacji metali, syntezach organicznych i polimeryzacji, produkcji leków, szkła, ceramiki czy baterii. Obecnie lit staje się coraz bardziej interesujący i atrakcyjny jako składnik baterii do pojazdów elektrycznych i hybrydowych. W naturze lit jest najczęściej występującym metalem, jednakże w niskim stężeniu. Konwencjonalnie przetwarzanie pegmatytów (glinokrzemiany) zawierających lit są czaso-, energo- i kosztochłonne. Biohydrometalurgia uważana jest za technologię o niskich kosztach i niskich wymagań energetycznych. Niektóre gatunki heterotroficznych organizmów, takich jak Aspergillus *i* Penicillium *wykazują wielki potencjał w bioługowaniu metali z rud I różnych typów odpadów, np. popiołów lotnych, zużytych katalizatorów czy odpadów elektrycznych. Mikroorganizmy heterotroficzne z rodzaju* Aspergillus *posiadają potencjał w produkcji kwasów organicznych, głównie szczawiowego, cytrynowego i glukonowego, działających na metale wydobywane z niskiej jakości rud i odpadów. Artykuł prezentuje badania nad wpływem wieku zarodka* Aspergillus niger *na wydobycie litu z glinokrzemianów. Zarodki i związki* conidia *użyte w doświadczeniu były hodowane 4 i 12 dni. Doświadczenia bioługowania metali zostały przeprowadzone w pożywce o niskiej temperaturze otoczenia. Po raz pierwszy lit pojawia się w roztworze 26 dnia w obu przypadkach w ilości 60 µg/l i 26 µg/l przy użyciu odpowiednio 4 i 12 dniowych zarodków. Jako, że* A. Niger *cechuje się znaczną możliwością kumulacji różnych metali, lit znaleziono także w biomasie. Badania pokazują, że dłużej hodowane zarodniki wytwarzają więcej biomasy niż krócej hodowane. Stężenie litu w biomasie wyprodukowanej przez 4 i 12 dniowe zarodniki wynosiło odpowiednio 121µg/l i 545µg/l. Pomimo niskiej wartości pH (pH = 3) wyższa wydajność bioługowania została otrzymana z dłużej hodowanych zarodników. Wyniki badań na elektronowym mikroskopie skaningowym (SEM) przed i po bioługowaniu wykazały zmiany strukturalne powierzchni minerału po zaaplikowaniu* A. Niger. *Analiza rentgenowska potwierdziła zmiany w sieci krystalicznej minerału przed i po procesie bioługowania.*

Słowa kluczowe: bioługowanie, glinokrzemian, Aspergillus niger, *odzysk litu*