Bioleaching of Lithium from Lepidolite by the Mixture of *Rhodotorula Rubra* and *Acidithiobacillus Ferrooxidans*

Renata MARCINČÁKOVÁ[1], Jana KADUKOVÁ[2], Anna MRAŽÍKOVÁ[3]

[1] RNDr.; Technical University in Kosice, Faculty of Metallurgy, Park Komenskeho 11, 042 00 Kosice, Slovak Republic; email: renata.marcincakova@tuke.sk

[2] Prof. RNDr., Ph.D.; Technical University in Kosice, Faculty of Metallurgy, Park Komenskeho 11, 042 00 Kosice, Slovak Republic

[3] RNDr.; Technical University in Kosice, Faculty of Metallurgy, Park Komenskeho 11, 042 00 Kosice, Slovak Republic

Abstract

The objective of this study was to evaluate lithium bioleaching from lithium-rich ore lepidolite using the mixed culture of autotrophic bacteria Acidithiobacillus ferrooxidans *and heterotrophic yeast* Rhodotorula rubra. *Lithium, as an important part of cathode material and electrolyte, is one of the crucial elements in lithium – ion battery production. The conventional techniques concerning the treatment lithium ores involve pyrometallurgical and hydrometallurgical processes. The overall high energy and capital costs as well as serious second pollution of those processes urge a turn to alternative methods. Bioleaching appears to be one of the fast developing technologies, which uses different kinds of microorganisms (bacteria, fungi, yeast) and their metabolic products for the extraction of metals from low grade ores and wastes. The important microorganisms, which play an important role in metal recovery from sulphide minerals and waste, belong to autotrophic acidophilic group. Those microorganisms fix carbon dioxide and obtain energy from the oxidation of ferrous iron or reduced sulphur compounds. Contrary to bacterial leaching; the use of the yeast* Rhodotorula rubra *has also several advantages. These heterotrophic species are able to grow in acidic environment and due to their metabolites they can enhance metabolic activity of* A. ferrooxidans.

The bioleaching experiments were carried out in rich bioleaching media at the initial pH of 3.5. Results revealed that two main processes namely Li bioleaching (Li solubilisation) and Li bioaccumulation (Li uptake) were involved in Li bioleaching process. Li accumulation into the 1 g of microbial biomass was 47 μg. Lithium concentration in leach liquor was found to be 60 μg/l. During Li bioleaching using the mixture of R. rubra *and* A. ferrooxidans *almost 4905 mg biomass was generated in 1000 ml of the solution. The great biomass increase indicated the positive effect of synergistic interactions of heterotrophic yeast of* R. rubra *and autotrophic bacteria of* A. ferrooxidans *on metabolic activities of the microorganisms. However, no significant effect of the consortium on Li bioleaching efficiency was observed.*

Keywords: bioleaching, aluminosilicate, Rhodotorula rubra, Acidithiobacillus ferrooxidans*, Li recovery*

Introduction

The demand for lithium is ever increasing with advance in industrial world. 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 (Siame, 2011). Lithium extractions from aluminosilicates ores using conventional techniques such as pyrometalurgy or hydrometallurgy encompass several inherent constraints. The total high energy and capital inputs and serious second pollution as well, of these technologies urge a turn to alternative methods. Biohydrometallurgy, which utilizes microorganisms and their metabolic activities for metal extraction from primary and secondary sources, has become one of the most promising biotechnologies (Kusnierova et al., 2011, Willner and Fornalczyk, 2013). Industrial waste treatment mainly uses the ability of bacteria and fungi to produce acids, which can leach metals from various solid materials (Brandl and Faramarzi, 2006, Kadukova a Štofko, 2006, Hoque and Philip, 2011). The ability of heterotrophic microorganisms to attack aluminosilicate ores has been reported by many authors. Karavaiko et al. (1980) reported biodegradation of spodumene and subsequent Li extraction using the bacteria belonging to the genera of *Arthrobacter*, *Nocardia* and *Pseudomonas*. Ilgar et al. (1993) studied lithium bioleaching from spodumene using organic acids generated by *A. niger*. Rezza et al. (2001) utilized heterotrophic strains of *Aspergillus niger*, *Penicillium purpurogenum* and *Rhodotorula rubra* on lithium recovery from spodumene and Marcincakova et al. (2014) used *A. niger* on lithium bioleaching from lepidolite.

It has been found that mixture of autotrophic and heterotrophic microorganisms is much more effective in sulphur oxidation of pyrite than the pure microbial culture (Borovec, 1990). Fournier et al. (1998) and Zhou et al. (2013) also reported that heterotrophic microorganisms such as *Rhodotorula*

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%

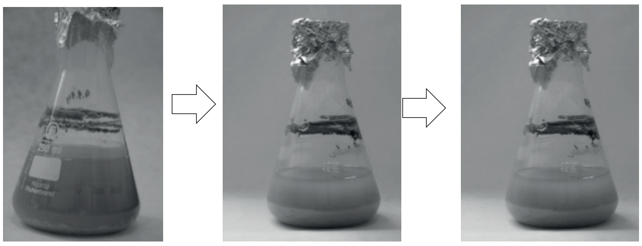

Fig. 2. Colour changes of the leach liquor on day 6, 29 and 69 during Li bioleaching by mixture of *R. rubra* and *A. ferrooxidans*

Rys. 2. Zmiana koloru roztworu w czasie bioługowania Li w 6,29 I 69 dniu ługowania za pomocą mieszaniny *R. rubra* i *A. ferrooxidans*

rubra and *Galactomyces sp.* can promote metabolic and leaching activity of *A. ferrooxidans* and *A. thiooxidans*, respectively. In the case of acidophilic bacteria it is considered that the heterotrophs contribute to the stability of the mixed mineral-oxidizing population. The heterotrophic microorganisms use extracellular metabolites and cell lysates from autotrophs as carbon source resulting in the removal of inhibitory excess of carbon and therefore stimulating growth of chemolithoautotrophs (Fournier et al., 1998, Ilyas et al., 2007).

The present study was undertaken to evaluate the potential of mixed culture of autotrophic bacteria of *A. ferrooxidans* and heterotrophic yeast *R. rubra* to solubilize lithium from lepidolite.

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

The acidophilic bacteria of *Acidithiobacillus ferrooxidans* were obtained from the Institute of Geotechnics of Slovak Academy of Science in Kosice, Slovakia and cultured in the 9K medium (Silverman and Lundgren, 1968). The yeast of *Rhodotorula rubra* was bought from the Culture Collection of Yeasts, Institute of Chemistry of Slovak Academy of Sciences and maintained at 4°C on a solid Malt Agar. Stock cultures were sub-cultured every month. Prior to the bioleaching experiments, *R. rubra* was cultured on the slant Malt agar for 6 days.

Bioleaching experiment

The experiments were carried out in 250 ml Erlenmeyer flasks containing 2 g mineral in 200 ml of liquid bioleaching media composed of 9K medium, glucose – 1 g/l yeast extract – 0.1 g/l and 1000 ml distilled water. The initial pH was adjusted to 3.5 by 10M H_2SO_4. Each experiment was conducted in duplicate.

For elemental analysis the samples were collected by disposable sterilized pipettes and centrifuged at 4500 rpm for 5 min. At the end of the experiments the solid residue were filtered, air-dried for 24 h and then burnt at 500°C for 4 hours to remove the biomass.

Lithium was determined in leach liquor and biomass. The biomass was digested by the hydrochloric acid method to determine Li accumulated in the biomass. Lithium present in the solution was determined by Atomic Absorption Spectrophotometer (Perkin Elmer 3100) at 670 nm.

Results and discussion

Lithium concentration determined in leach liquor during Li bioleaching using the mixture of *R. rubra* and *A. ferrooxidans* is plotted in Fig. 1.

As it can be seen the highest Li bioleaching rate was in the first 13 days, when 84 µg/l Li dissolved. Afterwards Li concentration in the leach liquor sharply decreased and on day 28 no Li was found. Since *R. rubra* is characterized by a high ability to accumulate various metals into their cells the decrease of Li concentration may be explained by lithium accumulation into the biomass (Fournier et al., 1998, Salinas et al., 2000). This fact was proven later by a consequent biomass analysis. In the first 28 days of the Li bioleaching the solution colour changed from greenish-brown to grey (Fig. 2).

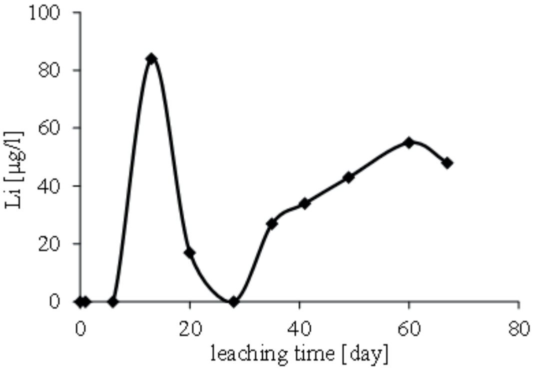

Fig. 1. Lithium bioleaching from lepidolite by mixture of *R. rubra* and *A. ferrooxidans*

Rys. 1.Biołgowanie Li za pomocą mieszaniny *R. rubra* i *A. ferrooxidans*

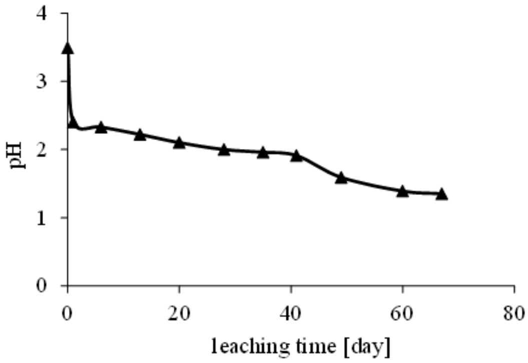

Fig. 3. The pH changes over the time during Li bioleaching by mixture of *R. rubra* and *A.ferrooxidans*

Rys. 3. Zmiana pH w czasie biołgowania Li za pomocą mieszaniny *R. rubra* i *A. ferrooxidans*

It was assumed that inhibition of metabolic activity of the yeast occurred therefore on day 29 *R. rubra* was added to the solution again. On following days Li solubilisation continued and on day 60 reached concentration of 50 µg/l Li.

Within several hours a pH decreased sharply and on the first day reached value of 2.3 (Fig. 3). Throughout the experimental period pH values slightly decreased up to value of 1.35 measured on day 69. Low pH values indicated a good metabolic activity of *A. ferrooxidans* (Brandl, 2008, Valdés et al., 2008).

Results showed that two main processes namely Li bioleaching (Li solubilisation) and Li bioaccumulation (Li uptake) were involved in Li bioleaching process. It may be also assumed that Li bioaccumulation process occurred during the whole experimental period. The amount of Li accumulated into the 1 g of fungal biomass was found to be 47 µg. During Li bioleaching using the mixture of *R. rubra* and *A. ferrooxidans* almost 4905 mg biomass was generated in 1000 ml of the solution. The great biomass increase indicated the positive effect of synergistic interactions of heterotrophic yeast of *R. rubra* and autotrophic bacteria of *A. ferrooxidans* on metabolic activities of the microorganisms. However, no significant effect of the consortium on Li bioleaching activity was observed since only 11 mg Li were extracted from 1 kg of lepidolite.

Conclusion

The present work showed that two processes are involved in Li extraction from lepidolite using the mixture of *R. rubra* and *A. ferrooxidans*. Bioaccumulation appears to be one of the dominant processes. The present study also showed that synergistic interactions of both microorganisms were very effective in metabolic activity of the microorganisms (high biomass production). On the other hand, no significant effect of the consortium on Li bioleaching efficiency was observed.

Acknowledgements

The authors would like to thank Dr. N. Rowson for the supply of the lepidolite used in this work.

The work was fully supported by the grant from the Slovak National Grant Agency under the VEGA Project 1/0235/12 and 1/0197/15.

Literatura – References

1. BRANDL, H. FARAMARZI, M. 2006. "Microbe-metal interactions for the biotechnological treatment of metal-containing solid waste." *China Particuology* 2: 93–97.
2. BRANDL, H. 2008. *Microbial leaching of metals, In Biotechnology Set*. 2nd edition (Eds H.-J. Rehm and G. Reed), Weinheim, Germany: Wiley-VCH Verlag GmbH.
3. HOQUE, MD. E., PHILIP, O.J. 2011. "Biotechnological recovery of heavy metals from secondary sources." *Materials Science of Engineering* C, 31: 57–66.

4. FOURNIER, D., LEMIEUX, R., COUILLARD, D. 1998. "Essential interactions between *Thiobacillus ferrooxidans* and heterotrophic microorganisms during a wastewater sludge bioleaching process." *Environmental Pollution* 101: 303–309.
5. ILYAS, S. et al. 2007. "Bioleaching of metals from electronic scrap by moderately thermophilic acidophilic bacteria." *Hydrometallurgy* 88(1–4): 180–188.
6. KADUKOVÁ, J., ŠTOFKO, M. 2006. *Environmentálne inžinierstvo pre hutníkov*, Košice: Equilibria
7. KARAVAIKO, G.I. et al. 1988. *Biotechnology of metals*, Manual, Moskva: Centre for International Project GKNT.
8. KUSNIEROVA, M. et al. 2011. "Energetic wastes as an equivalent for primary non-metallic materials" *Inżynieria Mineralna* 1(27): 73–78.
9. LUPTÁKOVÁ, A., KUŠNIEROVÁ, M., FEČKO, P. 2002. *Minerálne biotechnológie II. Sulfuretum v prírode a priemysle*, Ostrava.
10. MARCINCAKOVA, R. et al. 2014. "The influence of spore age of Aspergillus niger on lithium dissolution from lepidolite." *Inżynieria Mineralna*, 15: 211–216.
11. REZZA, I. et al. 2001. "Mechanisms involved in bioleaching of an aluminosilicate by heterotrophic microorganisms." *Process Biochemistry* 36: 495–500.
12. SALINAS, E. et al. 2000. "Removal of cadmium and lead from dilute a aqueous solution by *Rhodotorula rubra*." *Bioresource Technology* 72: 107–112.
13. SIAME, E. 2011. *Recovery of lithium from China clay waste using a combination of froth flotation, magnetic separation, roasting and leaching.* University Exeter as a thesis for the degree of Doctor of Philosophy in Earth Resources, February.
14. SILVER, M., LUNDGREN, D.G. 1968. "Sulfur-oxidizing enzyme of Ferrobacillus ferrooxidans.", *Canadian Journal of Biochemistry* 46: 1215–1220.
15. VALDÉS, J. et al. 2008. *Acidithiobacillus ferrooxidans metabolism: from genome sequence to industrial applications*, In BMC Genomics.
16. WILLNER, J., FORNALCZYK, A. 2013. "Extraction of metals from electronic waste by bacterial leaching." *Environmet Protection Engineering:* 197–208.
17. ZHOU, J. et al. 2013. "Degradation of inhibitory substances in sludge by Galactomyces sp. Z3 and the role of its extracellular polymeric substances in improving bioleaching." *Bioresource Technology* 132: 217–223.

Biołgowanie litu z Lepidolitu przy użyciu mieszanki drożdży Rhodotorula Rubra i bakterii Acidithiobacillus Ferrooxidans

Celem niniejszych badań była ocena biołgowania litu z rudy lepidolitu bogatej w lit przy użyciu mieszanej kultury bakterii autotroficznych Acidithiobacillus ferrooxidans oraz heterotroficznego drożdży Rhodotorula rubra. Lit, jako ważny składnik materiału katody oraz elektrolitu, jest jednym z najważniejszych elementów w produkcji akumulatorów litowo-jonowych. Konwencjonalne techniki przetwarzania rud litu uwzględniają procesy pirometalurgiczne i hydrometalurgiczne. Całkowity koszt energetyczny i finansowy, jak również poważne zanieczyszczenie wtórne tych procesów, zmusiły do poszukiwania metod alternatywnych separacji. Biołgowanie jest obecnie jedną z najszybciej rozwijających się technologii, która wykorzystuje różnego rodzaju mikroorganizmy (bakterie, grzyby, drożdże) i ich produkty metaboliczne do wydzielania metali z niskiej jakości rud i odpadów. Mikroorganizmy, które odgrywają ważną rolę w procesie odzysku metalu z minerałów siarczkowych i odpadów, należą do grupy autotroficznej, acidofilowej. Te mikroorganizmy przyłączają się do dwutlenku węgla i pobierają energię z oksydacji żelazowego żelaza lub zredukowanych związków siarki. W przeciwieństwie do ługowania bakteryjnego, użycie drożdży Rhodotorularubra ma również kilka zalet. Te heterotroficzne gatunki są w stanie dojrzewać w kwaśnym środowisku i dzięki swoim metabolitom mogą wspomagać aktywność metaboliczną bakterii A. ferrooxidans.

Testy biołgowania zostały przeprowadzone w bogatych nośnikach biołgujących o początkowej wartości pH wynoszącej 3,5. Wyniki pokazały, że dwa najważniejsze procesy, tj. biołgowanie Li (rozpuszczanie Li) oraz bioakumulacja Li (pobór Li), były włączone do procesu biołgowania Li. Akumulacja Li w 1g. biomasy mikrobiologicznej wyniosła 47 µg. Stężenie litu w płynie ługującym wyniosło 60 µg/l. Podczas biołgowania Li przy użyciu mieszanki R. rubra oraz A. ferrooxidans wygenerowano blisko 4905 mg biomasy w 1000 ml roztworu. Duży wzrost biomasy wskazuje na pozytywne działanie interakcji synergistycznych heterotroficznego drożdżaka R. rubra i autotroficznej bakterii A. ferrooxidans na aktywność procesów metabolicznych mikroorganizmów. Niemniej jednak, nie zaobserwowano znacznego wpływu konsorcjum na skuteczność biołgowania Li.

Słowa kluczowe: biołgowanie, glinokrzemiany, Rhodotorula rubra, Acidithiobacillus ferrooxidans, odzysk Li