Lithium Bioleaching from Lepidolite Using the Yeast *Rhodotorula Rubra*

Renáta MARCINČÁKOVÁ[1], Jana KADUKOVÁ[2], Anna MRAŽÍKOVÁ[3], Oksana VELGOSOVÁ[4], Marek VOJTKO[5]

[1] RNDr.; Department of Material Science, Technical University in Kosice, Park Komenského 11, 042 00 Kosice, Slovak Republic; email: renata.marcincakova@tuke.sk

[1] Prof., RNDr., Ph.D.; Department of Material Science, Technical University in Kosice, Park Komenského 11, 042 00 Kosice, Slovak Republic; email: jana.kadukova@tuke.sk

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

[1] Doc. Ing., Ph.D.; Department of Material Science, Technical University in Kosice, Park Komenského 11, 042 00 Kosice, Slovak Republic; email: oksana.velgosova@tuke.sk

[1] Ing., Ph.D.; Department of Material Science, Technical University in Košice, Park Komenskeho 11, 042 00 Kosice, Slovak Republic; email: marek.vojtko@tuke.sk

Summary

In this present work lithium recovery from lepidolite (3.79% Li_2O) by bioleaching was investigated. Lithium due to its electrochemical reactivity and also other unique properties has attracted much attention for their application in many industrial fields such as batteries, ceramics and glass production, greases, pharmaceuticals and polymers and other uses. The tremendous growth in lithium demand for lithium batteries used in hybrid and electromobiles has raised great concern about the future availability of lithium.

In nature lithium is present in a variety of aluminosilicates and continental brines. One of the principal lithium minerals in the world is lepidolite. Its destruction and consequent lithium is a high capital and energy intensive process therefore it is necessary to seek an efficient, economic technique to handle this ore. Biohydrometallurgical approaches with low energy and cost requirement are coming into perspective. Some species of bacteria, fungi and yeasts contribute to weathering processes and mineralization of metal containing materials. The most active leaching fungi such as Penicillium simplicissimum and Aspergillus nige produce great amounts of organic acids which play an important role as leaching agents in metal dissolution. However, there is a lack of studies on metal bioleaching from solid substrates using the yeast Rhodotorula rubra*. In nature R. rubra may be found in silicates near lithium mining deposits. It is a slime producer and by means of macromolecules such as polysaccharides or polypeptides present in the capsule and wall can enhance silicate weathering processes.*

The main aim of this research work was to investigate lithium extraction from lepidolite using the yeast R. rubra and also the influence on nutrients on metabolic and leaching activity of the yeast. During the bioleaching of lepidolite using R. rubra Li extracted and accumulated in the biomass was 412.6 μg/g and 181.2 μg/g in nutrient and salt-limited medium, respectively. In leach liquor, lithium concentration was 25 μg/l and 89 μg/l in nutrient and salt-limited medium, respectively.

Keywords: Rhodotorula rubra, lepidolite, lithium, bioleaching

Introduction

Lithium is the lightest metal widely used in many commercial products. Lithium compounds have attracted great attention in many industrial areas, especially in ceramics, glass, pharmaceuticals, metallurgy (Ebensperger et al., 2005, Yan et al., 2012). Nowadays it is becoming more and more popular as the energy source for hybrid vehicles and electromobiles. The global consumption of lithium related to battery production has been increasing by more than 20% a year during the past several years (Wang et al., 2011). The tremendous growth of lithium in demand for lithium batteries used in power hybrid and fully electric automobiles has raised great concern about the future availability of lithium (Tahil, 2008, Yan et al. 2012).

The overall high energy and capital costs of lithium extraction from spodumene, lepidolite, petalite and pegmatites by conventionally methods leads to the search for new alternative technologies to be less energy intensive and competitively to pyro- or hydrometallurgical techniques. Metal bioleaching appears to be one of the promising and fast developing methods which use microorganisms and their metabolic activities for metal recovery from low grade ores and wastes (Luptakova et al., 2002, Kusnierova et al., 2011, Willner and Fornalczyk, 2013, Mrazikova et al., 2013).

In nature many bacteria, fungi and yeasts contribute to weathering processes and mineralization of metal containing materials. The most active leaching fungi are among the genera of *Penicillium* and *Aspergillus*. Within these two genera especially *P. simplicissimum* and *A. niger* can be considered the most important due to the ability to excrete great amounts of organic acids (Simonovicová et al., 2013). Microbial metabolites such as organic acids, amino acids, dissolve metals from minerals by displacement of metal ions, by changing redox condition or by the formation of soluble metal complexes and chelates (Burgstaler and Schinner, 1993, Hosseini, et al, 2007).

On the other hand, there the available information about the metal extraction from solid substrates using the yeast *Rhodotorula rubra* is limited. *R. rubra* was found in another silicate near the lithium mining deposits (Rezza et al., 1997). This microorganism is a slime producer and well-known by exopolymer production (Campbell, 1988, Rezza et al., 1997). *R. rubra* attacks the mineral and leaches by direct contact by means of its metabolic activity and also by means of macromolecules such as polysaccharides or polypeptides present in the capsule and the wall of the yeast (Rezza et al., 2001).

The aim of this study was to evaluate the influence of nutrient presence in the medium on metabolic and leaching activity of *R. rubra*.

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 Tab. 1.

Microorganisms

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 subcultured every month.

Bioleaching experiment

The bioleaching experiments were carried out in 250 ml Erlenmeyer flasks containing 2g lepidolite of different size fraction of +0.9–200 in 200 ml of a nutrient and salt-limited medium, respectively (Tab. 2). The media and mineral were sterilized by autoclaving for 20 min at 120°C. Prior to the bioleaching, *Rhodotorula rubra* was cultured on the slant Malt agar for 6 days and then used for the experiment. Each experiment was conducted in duplicate and non-inoculated blanks were performed in all cases. Erlenmeyer flasks were put on a rotary shaker at the operating speed of 160 rpm.

For elemental analysis the samples were collected by disposable sterilized pipettes and centrifugated 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 *R. rubra* is plotted in Fig. 1. As it can be seen there were differences in lithium dissolution using the yeast grown in two

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%

Tab. 2 Chemical composition of the bioleaching media

Tab. 2. Skład chemiczny obiektu ługującego

Chemicals	Nutrient medium	Salt-limited medium
glucose	20 g/l	5 g/l
$(NH_4)_2SO_4$	5 g/l	0.5 g/l
KH_2PO_4	5 g/l	-
$MgSO_4.7H_2O$	0.34 g/l	-
Yeast extract	7 g/l	-

different media. In the salt-limited medium metal leaching rate was the highest in the first 12 days, when 94 µg/l Li was released whereas in the nutrient medium only 25 µg/l Li on day 13 dissolved. Li concentration decreases observed on following days in both leaching systems may be explained lithium accumulation in the biomass. Since no Li was found out in the leach liquor in nutrient medium and pH started rapidly increasing the experiment was finished on day 28.

Given Li dissolution in the salt-limited medium, the changes of solution colour from pink to pale were observed on day 21, the yeast was added again to the solution and experiment went on. After that Li concentration in the solution slightly increased and on the last day of the experimental period 89 µg/l Li were determined.

The pH changes over the leaching time are plotted in Fig. 2. In both leaching systems the pH rapidly decreased in the first 12 days, from the initial pH of 5.1 up to 3.2 and 2.7 in the nutrient and salt-limited medium, respectively. Afterwards in the salt-limited medium it remained more or less constant until the end of the experimental period; however, in the case of Li bioleaching in the nutrient medium the pH rapidly increased up to 6.5 measured on day 20.

The results obtained, as depicted in Fig. 3 clearly showed that a much higher amount of biomass, almost 470 mg, was produced in the presence of all nutrients required for microbial growth, whereas in the absence of nutrients, only 182 mg of the biomass were generated. The results also revealed the higher biomass production the higher lithium accumulation. Li accumulated in the biomass during bioleaching in the nutrient medium was found to be 413 µg/g, while Li accumulated in the biomass produced in salt-limited medium was 181 µg/g (Tab. 3, Fig. 3).

The SEM photomicrograph of the lepidolite after bioleaching (Fig. 4) showed that *R. rubra* was attached to the mineral throughout the leach-

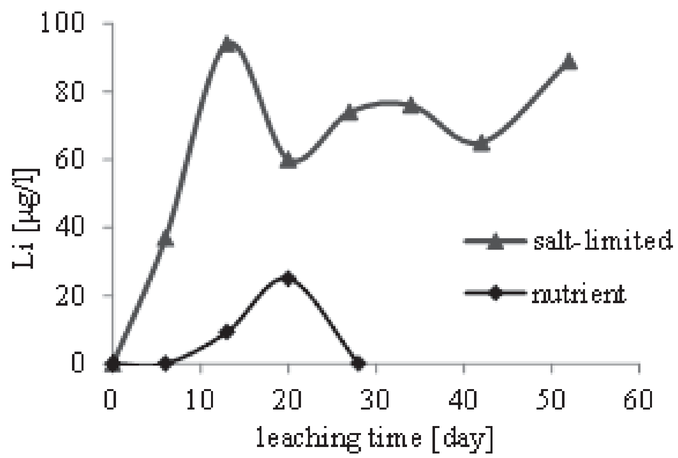

Fig. 1.um concentrations in the leach liquor during the bioleaching in the nutrient and salt-limited medium using *R. rubra*

Rys. 1. Stężenie uM w płynie ługującym podczas bioługacji w nutriencie i medium solnym przy użyciu *R. rubra*

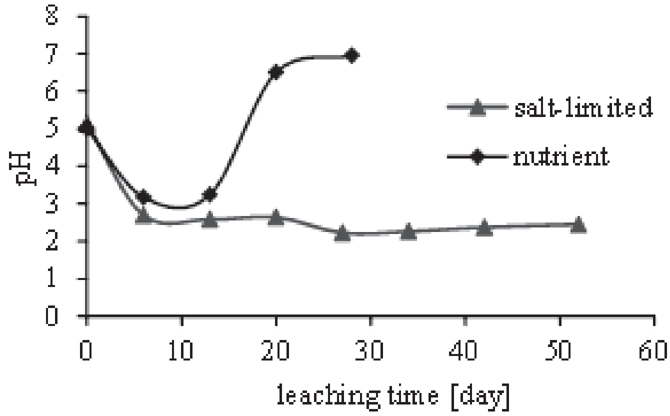

Fig. 2. pH changes over the leaching time during the bioleaching in the nutrient and salt-limited medium using *R. rubra*

Rys. 2. Zmiany ph w relacji do czasu ługowania podczas bioługowania w nutriencie i medium solnym przy użyciu *R. rubra* ing process. It can be seen a higher concentration of the yeast *R. rubra* covered by slime and capsular exopolymers in the nutrient medium compared to those in salt-limted medium.

After these results it may be concluded that highly limited medium is not appropriate for Li extraction from lepidolite using the yeast *R. rubra* since greater Li extraction was obtained when all nutrients required for microbial growth were present. There is necessary to stress that the results are in contradiction with those described by Rezza et al. (1997) who supposed that microbial adaptation to a low nutrient medium can enhance bioleaching process. According to the literature (Rezza et al., 1997, Salinas et al., 200) it is also obvious that capsular exopolymers played an important role in lithium recovery from minerals. The cell wall of the red yeast contains fucogalactomannan type polymer containing fucose and galactose and has highly branched structure which may contrib-

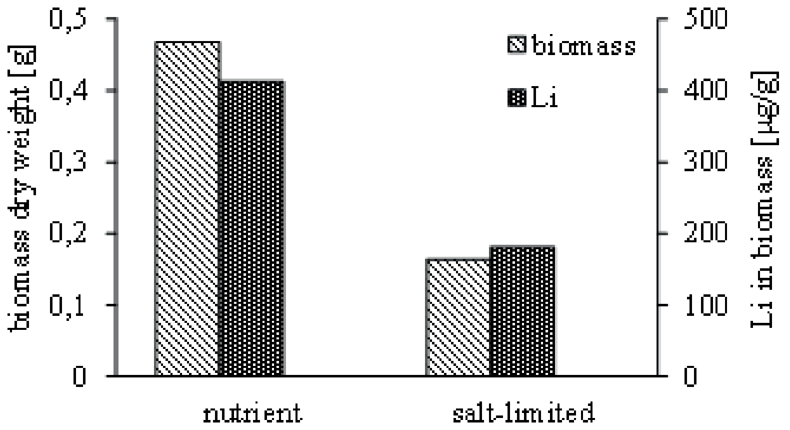

Fig. 3. Biomass dry weight [g] and Li concentration accumulated in the during the bioleaching in the nutrient and salt-limited medium using *R. rubra*

Rys. 3. Waga suchej biomasy [g] oraz nagromadzenie stężenia Litu podczas bioługowania w nutriencie i medium solnym przy użyciu *R. rubra*

Tab. 3 Biomass dry weight and Li concentration in the biomass and leach liquor

Tab. 3. Waga suchej biomasy i stężenie Litu w biomasie i płynie ługującym

Medium	Biomass dry weight [mg/l]	Li in the biomass [µg/g]	Li in the solution [µg/l]
nutrient	468	412.6	25
salt-limited	182	181.2	89

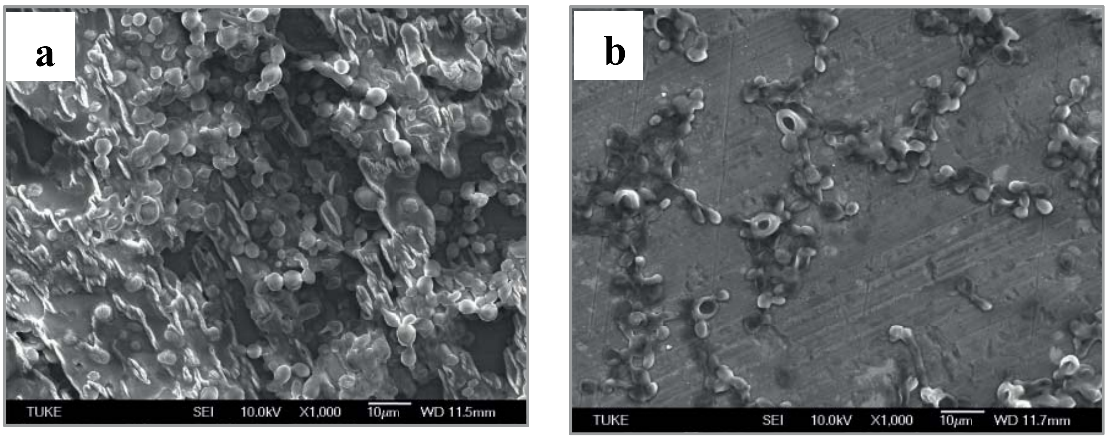

Fig. 4 SEM microphotograph of *R. rubra* attachewd to the during the bioleaching in nutrient a) and salt-limited media, b). Magnification 1000 x.

Rys. 4. Mikrofotografia SEM drożdży *R. rubra* obecnych podczas bioługowania w nutriencie a) i solnym medium b) w powiększeniu 1000x ute to the higher adsorption capacity of *R. rubra*. WThe mechanisms involved in bioleaching of aluminosilicates by the yeast have not been fully understood; however, according to the literature (Salinas et al., 2000, Pankiewicz et al., 2006) it may be supposed that lithium accumulation involves two processes: (1) a passive adsorption of the metal on the external cell surface; and (2) a transport of the metal through the cell membrane into the cytoplasm that involves metabolic process.

Conclusions

The results from this present fundamental study demonstrate that 0.17% and 0.045% Li can be recovered from lepidolite by microbial leaching using the heterotrophic microorganism of *Rhodotorula rubra* in rich nutrient salt-limited medium, respectively. It may be also emphasized that low nutrient conditions did not enhance leaching process as Rezza et al (1997) assumed since the highest amount of biomass and Li dissolution was reached in the environment in which all nutrients needed for microbial growth were present.

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 a grant from the Slovak National Grant Agency under the VEGA Project 1/0235/12.

Received January 7, 2015; reviewed; accepted February 28, 2015.

Literatura - References

1. *Campbell, Y. Culture, storage, isolation and identification of yeast. In: Yeast a Practical Approach ed. Campbell, Y. and Duffus, J.H., Washington, D.C. IRL Press, 1988, p. 1–8.*
2. *Danch, A., Chmielowski, J. Selenium bioaccumulation in Saccharomyces cerevisiae, Acta Biologica Siles, 18, 1985, p. 57–64.*
3. *Ebensperger, A. et al. The lithium industry: its recent evolution and future prospects, Resource Policy, 30 (3), 2005, p. 218–231.*
4. *Hosseini, M.R., et al. Bioleaching of iron from highly contaminated Kaolin clay by Aspergillus niger, Applied Clay Science, 37, 2007, p. 251–257.*
5. *Kusnierová, M. et al. Energetic wastes as an equivalent for primary non-metallic materials, Inżynieria Mineralna, 1, 27, 2011, p. 73–78.*
6. *Luptáková, A. et al. Minerálne biotechnológie II., Sulfuretum v prírode a v priemysle, VŠB Technická univerzita v Ostrave, 2002. ISBN 80-248-0114-0.*
7. *Mražíková, A. et al. Influence of used bacterial culture to copper bioleaching from printed circuit boards, Inzynieria Mineralna, 14, 2, 2013, p. 59–62.*
8. *Pankiewicz, U. et al. Optimization of selenium accumulation in Rhodotorula rubra cells by treatment of culturing medium with pulse electric field, International Agrophysics, 20, 2006, p. 147–152.*
9. *Rezza, I. et al. Extraction of lithium from spodumene by bioleaching, The Society for Applied Bacteriology, Letters in Applied Microbiology, 25, 1997, p. 172–176.*
10. *Rezza, I. et al. Mechanisms involved in bioleaching of an aluminosilicate by heterotrophic microorganisms, Process Biochemistry, 36, 2001, p. 495–500.*
11. *Salinas, E. et al. Removal of c.admium and lead from dilute a aqueous solution by Rhodotorula rubra, Bioresource Technology, 72, 2000, p. 107–112.*
12. *Šimonovičová, A. et al. Influence of the environment on the morphological and biochemical characteristics of different Aspergillus niger Wild Type Strain, Indian Journal of Microbiology, 53 (2), 2013, p. 187–193.*

13. *Tahil, W. The trouble with lithium? under the microscope. Meridian International Research, Martainville, France, 2008, available at: http://www.mer-idian-intres.com/Projects/LithiumMicroscope.pdf*

14. *Wang, H. Electrochemical property of $NH_4V_3O_8$.$0.2H_2O$ flakes prepared by surfactant assisted hydrothermal method, Journal of Power Sources, 196, 2, 2011, p. 788–792.*

15. *Willner, J., Fornalczyk, A. Extraction of metals from electronic waste by bacterial leaching, Environmet Protection Engineering, 2013, p. 197–208.*

16. *Yan, Q. et al. Extraction of lithium from lepidolite by sulfation roasting and water leaching, International Journal of Mineral Processing, 110–111, 2012, p. 1–5.*

Bioługowanie litu z lepidolitu przy pomocy drożdży Rhodotorula Rubra

W niniejszej pracy badany jest odzysk litu z lepidolitu (3.79% Li_2O poprzez bioługowanie). Lit, ze względu na elektrochemiczną reaktywność, jak również inne unikalne cechy znalazł zastosowanie w wielu branżach przemysłowych m.in. przy produkcji baterii, ceramiki i szkła, stosuje się go również do smarów, farmaceutyków, polimerów itp. Ogromny wzrost popytu na lit i litowe baterie, używane w hybrydowych i elektrycznych samochodach, wzbudził obawy względem zasobów litu w przyszłości.

W naturze lit występuje we wszelkim rodzaju glinokrzemianu i solnisk. Jednym z głównych minerałów na świecie, zawartym w licie jest lepidolit. Jego destrukcja z uzyskaniem litu to wysoce kosztowny i energochłonny proces, dlatego bardzo ważne jest znalezienie skutecznej i wydajnej techniki wydobycia tego kruszcu. Pojawiają się nowe możliwości w związku z metodami biohydrometalurgicznymi, które nie wymagają dużego nakładu energii i kosztów. Niektóre gatunki bakterii, grzybów i drożdży przyczyniają się do procesów wietrzenia oraz mineralizacji metali zawierających pierwiastki. Najaktywniejsze z grzybów ługujących, takie jak Penicilliumsimplicissimum *oraz* Aspergillusnige*, produkują znaczne ilości kwasów organicznych, które grają ważną rolę jako czynniki ługujące w procesie rozpadu metalu. Niemniej jednak, brak jest badań nad bioługowaniem metalu ze stałego substratu z użyciem drożdży* Rhodotorula rubra*. W naturze* R. rubra *występuje w krzemianach przy złożach kopalnianych litu.*

Głównym założeniem niniejszych badań jest sprawdzenie wydobycia litu z lepidolitu przy użyciu drożdży R. rubra *jak również zbadanie wpływu na odżywcze wartości aktywności metabolicznej i ługującej drożdży. Podczas bioługacji lepidolitu przy pomocy* R. rubra*, wydobycie i gromadzenie litu w biomasie wyniosło odpowiednio 412,6 µg/g oraz 181.2 µg/g dla wartości odżywczych i medium solnego. W płynie ługowym, stężenie litu wyniosło odpowiednio 25 µg/l i 89 µg/l dla wartości odżywczych i medium solnego.*

Słowa kluczowe: Rhodotorula rubra, lepidolit, lit, bioługowanie