# The Possibilities of Sulphate-Reducing Bacteria Use in Mine Drainage Waters Remediation

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*Summary*

*The objectives of the present work are to give some view on some biological-chemical methods used for the mine drainage water treatment. These waters are often characterized by typical high concentrations of sulphates and metals as a consequence of the mining industry of the sulphide minerals. The principles of the biological-chemical methods of the metals and sulphates elimination from mine drainage waters include sulphate reduction, bioprecipitation by the application of sulphate-reducing bacteria (SRB) and sorption by the biogenic sulphides.*

*The culture of sulphate-reducing bacteria was isolated from the mineral spring Gajdovka (Košice, Slovakia) in Postgate medium C. Mine drainage water samples were collected in Slovak localities Banská Štiavnica and Smolník.*

*Keywords: mine drainage water, remediation, sulphate-reducing bacteria*

## Introduction

Current and past mining activities, as well as various industries discharge large quantities of wastewater. Many processes, especially in the mining and metallurgical processing industry, produce acidic effluents containing significant amounts of dissolved metals. When these metal-contaminated wastewaters are exposed to the environment they can cause serious and sometimes permanent ecological damage (Hallberg, 2010). Acid mine drainages (AMD) usually contain high levels of sulphates and metals, such as copper, iron, manganese, zinc, cadmium and lead, which are not biodegradable and thus tend to accumulate in living organisms, causing various diseases and disorders. The amount and toxicity of the generated mine drainage waters depends on several factors such as mineralogy of the rock material, surface area, crystallography, temperature, oxygen concentration, etc. (Pinto et al., 2011).

Numerous techniques and methods have been studied and developed for mine drainage waters treatment. The use of biological-chemical treatment processes, based on the activity of sulphate-reducing bacteria (SRB), for remediation of metals and sulphates contaminated mine drainage waters from 2 Slovak localities – Smolník and Banská Štiavnica had been investigated in this work.

Under anaerobic conditions, sulphate can be used as a terminal electron acceptor by sulphate-reducing bacteria that couple the oxidation of the substrate (organic or inorganic compounds) to the reduction of sulphate and use the energy produced for growth and maintenance. The anaerobic reduction of sulphate has been used as a means for treating a variety of sulphate-containing industrial effluents (Moosa et al., 2002). Sulphates as the terminal electron acceptor, a carbon source and an electron donor are the primary nutrient requirements for SRB. Possible electron donors include: hydrogen, organic acids (acetate, propionate), various alcohols (methanol, ethanol) as well as fumarate and aromatic compounds (Cao et al., 2012). In most cases the organic carbon source can also act as the electron donor (Postgate, 1984).

An important component of acid mine drainage remediation systems based on bacterial sulphate reduction is metal sulphide precipitation. In these treatment processes, sulphate reducing bacteria generate sulphide and bicarbonate ions that are used to neutralize acidic effluents while the sulphide ions are used to effect precipitation of the dissolved metal ions as metal sulphides (Mokone et al., 2012). Sulphide precipitation is the desired mechanism of contaminant removal because metal sulphides are highly insoluble and less bio-available compared with other metal species. This process is particularly effective for removing heavy metals such as cadmium, copper, lead, mercury, zinc and iron to low concentration (Kuyucak, 2002). By other studies was observed that Fe, Mn, Al, and Zn seemed to be removed following precipitation or co-precipitation as hydroxides (Mačingová et al., 2011, Zaluski et al., 2003). Metals can also be removed by co-precipitation with (or adsorption onto) Fe and Mn oxides and bacterially produced metal sulphides (Jong and Parry, 2003). It was found that iron sulphide material, produced by sulphate-reducing bacteria, is an excellent adsorbent for a wide range of heavy metals and had a very high specific uptake from solution for metal ions (Jong and Parry, 2004).

## Materials and methods

### *Sulphate-reducing bacteria*

A mixed culture of sulphate-reducing bacteria was isolated using medium Postgate C from mineral water collected at Gajdovka spring (Košice, Slovakia). It is water with pH 7.5, $H_2S$ odour and with natural content of SRB. The predominant genus in mixed cultures of SRB is usually *Desulfovibrio*. Bacteria were grown for 10 days at 30°C in glass reaction flasks in anaerobic conditions that had been generated by introducing an inert gas ($N_2$) and chemically with sodium thioglycollate.

### *Mine drainage water*

Samples of the mine drainage water were collected in Slovak localities Banská Štiavnica and Smolník. In the past in Banská Štiavnica were mined ores minerals such sphalerite, pyrite, chalcopyrite, marcasite, galenite. The predominant in Smolník was pyrite and chalcopyrite. Therefore waters outflowing from dumps contain increased concentrations of metals (Fe, Cu, Zn, Pb). In our work sample from Banská Štiavnica is the outflow from dump of "New Shaft" (where the Pb-Zn ore deposit was). Second sample is from the shaft Pech (Smolník), which is acid mine drainage from the enclosed and flooded sulphide deposit (mainly $FeS_2$).

The most expected heavy metals in the mine drainage – Fe, Zn and Cu were analyzed by atomic absorption spectrometry – AAS (Spectrometer Varian). Sulphate determinations were made by the DIONEX ICS-500 Ion Chromatograph. Data for pH, metals and sulphates concentrations in water samples are shown in Table 1.

### *Sulphates elimination*

The mine drainage water samples analyses confirmed high sulphates concentrations and therefore were prepared 6 experiments with mixtures of mine drainage waters with bacteria and medium that is specific for the nutritional needs of the genus *Desulfovibrio* in order to study an activity of SRB (reduction of sulphates) under created conditions and to follow the possibility of removing sulphates by SRB from these waters to minimum levels. Four 250 ml bottles were filled with modified Postgate medium C (without sulphates usually present in standard medium), with mine drainage water samples (BŠ, Sm) and inoculated with a mixture of SRB (inoculum 10%) taken from cultivation flasks. Experiment 2 was provided without SRB, such as "abiotic control". Two 100 ml bottles were filled only with mine drainage water samples and inoculated with 15% of SRB mixture. Experiments conditions are resumed in Table 2. After inoculation 1 ml of liquid phase from each sample was taken out. Next sampling was realized during 2 weeks. Solutions were transferred to plastic tubes, centrifuged at 10000 rpm for 10 minutes to small solid particles separation and then were extracts diluted and analyzed. The concentration of the sulphates in the solutions was determined by ion chromatography. During experiments were also measurements of pH values carried out. Bottles were all the time stored in thermostat at 30°C and enclosed to avoid an oxygen entry.

Table 1. The analysis of mine drainage water samples

Tabela 1. Analiza próbek ścieków

| Sample | pH | Sulphates (mg/l) | Fe (mg/l) | Zn (mg/l) | Cu (mg/l) |
|---|---|---|---|---|---|
| Banská Štiavnica (BŠ) | 6.15 | 980 | <0.05 | 5.7 | 0.1 |
| Smolník (Sm) | 3.80 | 1690 | 206.9 | 6.3 | 1.0 |

Table 2. Experimental conditions for sulphates elimination

Tabela 2. Warunki eksperymentalne dla usuwania siarczanów

| Experiment | Nutrient medium (ml) | Mine drainage water (ml) | SRB inoculum (vol. %) | Type of mine drainage water sample |
|---|---|---|---|---|
| 1 | 225 | – | 10 | – |
| 2 | 250 | – | – | – |
| 3 | 125 | 100 | 10 | BŠ |
| 4 | 125 | 100 | 10 | Sm |
| 5 | – | 90 | 15 | BŠ |
| 6 | – | 90 | 15 | Sm |

***Metal removal***

Following the theoretical findings about metal removal by the application of SRB, were concentrations of copper, zinc and iron ions in extracts from 6 above mentioned experiments on the beginning and at the end of each of them analyzed by AAS. Under created conditions (nutrients, pH, temperature) was metabolic activity of SRB - hydrogen sulphide production, metals bioprecipitation or adsorption onto bacterially produced sulphides expected. The specific mechanism responsible to metal removal was not examined.

To verify the adsorption properties of biogenic sulphides, separately from previous experiments, were sorption tests realized. They were conducted by mixing 0.1 g of sorbent samples with 100 ml of mine drainage water samples. Biogenic sorbent was prepared by SBR cultivation in modified medium Postgate C (Jenčárová and Luptáková, 2012). The mixture was stirred for 4 hours in plastic Erlenmeyer flasks using mechanical laboratory shaker. 2 ml of liquid phase were taken out in predetermined intervals and filtered. The concentrations of the metal ions (Cu and Zn) in the filtrates were determined by AAS.

## Results and discussion

The presence of SRB in cultivation flasks was verified by the microscopic examination. In addition the smell provoked by the hydrogen sulphide was obvious. This mixture of SRB isolated from Gajdovka spring was used for experiments of sulphates removal. Results of this research work are shown on Figure 1.

From the results is possible to state that for sulphates elimination from mine drainage waters by SRB are necessary both compounds, i.e. nutrient medium with carbon source and bacteria inoculum. It is obvious that in Experiments 5 and 6, where only mine drainage water and inoculum were used, no visible changes in sulphates decreasing are present. Bacteria without nutrients were not able to grow and realize sulphate reduction. In Experiments 3 and 4, where nutrient medium took part, is decrease evident, especially in water from Banská Štiavnica, where almost all sulphates were removed. In Experiment 1, where only nutrient medium with little initial sulphate concentration was used, were all sulphates eliminated by SRB within 5 days. In “abiotic control” (Experiment 2) were no changes noticed.

The pH value of nutrient medium before SRB inoculation and mine drainage water addition was in all experiments adjusted to 7.5 that is the suitable pH for SRB growth (optimum 6.5–7.5). During experiments duration were changes of pH measured and are resumed in Table 3. It is evident that after mine drainage waters addition, especially AMD from Smolník (pH 3.8), the pH of solutions fell down. In Experiment 6 it reached the value that limits SRB activity. This induces that not only nutrient presence but also pH influences sulphates elimination from mine drainage water samples (Fig. 1). pH values of other 5 samples are in an appropriate range for SRB existence and activity. Also 6.7–6.8 (Experiment 4) is satisfactory enough.

Metals removal from solutions in 6 experimental bottles (composition explained in Table 2) was next investigated activity. Concentrations of copper, zinc and iron ions in extracts on the beginning and at the end (“final concentration”) of individual experiments were analyzed by AAS. The determined concentrations are in Table 4. The total amount of metals in solution after SRB inoculation and mine drainage water addition into the bottles with (or without) nutrient medium was marked as “initial concentration”. In the case of copper were values very low on the beginning already. Concentrations of zinc decreased in Experiments 1–5, probably because of bioprecipitation or adsorption. Only in Experiment 6 was the increase recorded, caused most probably by dissolving of solid particles from nutrient medium and SRB inoculum consist of

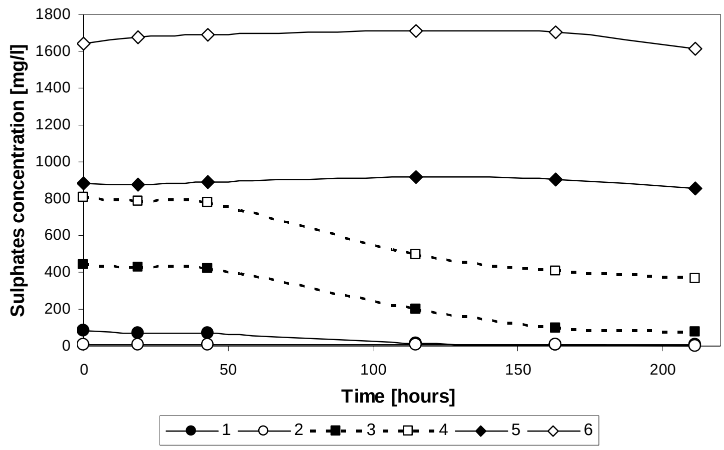


Fig. 1. Changes of sulphates concentration during bacterial sulphate-reduction

Rys. 1. Zmiany stężenia siarczanów podczas zastosowania bakterii redukujących siarczany

Table 3. pH values during experiments

Tabela 3. Wartości pH podczas eksperymentów

| Experiment | pH | | | |
|---|---|---|---|---|
| | Day 3 | Day 6 | Day 10 | Day 13 |
| 1 | 7.2 | 7.0 | 7.1 | 7.2 |
| 2 | 7.2 | 7.1 | 7.1 | 7.2 |
| 3 | 7.3 | 7.4 | 7.3 | 7.4 |
| 4 | 6.2 | 6.7 | 6.7 | 6.8 |
| 5 | 7.3 | 7.5 | 7.7 | 7.6 |
| 6 | 4.9 | 4.8 | 4.6 | 4.5 |

Table 4. Metal concentrations in solutions

Tabela 4. Stężenia metali w roztworach

| Experiment | Fe (mg/l) | | Zn (mg/l) | | Cu (mg/l) | |
|---|---|---|---|---|---|---|
| | Initial concentration | Final concentration | Initial concentration | Final concentration | Initial concentration | Final concentration |
| 1 | 0.45 | 0.5 | 0.25 | 0.2 | <0.03 | <0.03 |
| 2 | <0.05 | <0.05 | 0.85 | 0.35 | 0.9 | <0.03 |
| 3 | 1.9 | 1.3 | 0.4 | 0.35 | <0.03 | <0.03 |
| 4 | 62.1 | 0.5 | 0.3 | <0.03 | <0.03 | <0.03 |
| 5 | 1.35 | 0.45 | 0.7 | <0.03 | <0.03 | <0.03 |
| 6 | 172.85 | 192.5 | 0.7 | 4.55 | <0.03 | <0.03 |

zinc precipitates because of pH under 5. The most notable removal of iron from solution appeared in Experiment 4, where final concentration was 0.5 mg/l. It can be explained by very suitable conditions for SRB activity – hydrogen sulphide production as a consequence of their metabolic processes and precipitation of iron in form of insoluble iron sulphides. The increasing of iron concentration occurred only in Experiment 6, where probably dissolution of iron solid phase from SRB inoculum decreased the Fe amount.

Sorption experiments carried out by mixing the biogenic sorbent samples (iron sulphides) with mine drainage water samples (BŠ and Sm) to confirm adsorption properties of sorbent created by SRB were investigated for 4 hours. The efficiency of copper and zinc sorption from waters are illustrated on Figure 2.

In both cases the initial concentration of copper was not very high and therefore almost 90% of Cu ions were removed very quickly (within 15 minutes). The overall removal was 99.7% and 98.4%, respectively. The zinc ions sorption was slower, after 240 minutes were from solutions 87% (sample BŠ) and 74% (sample Sm) of Zn ions removed.

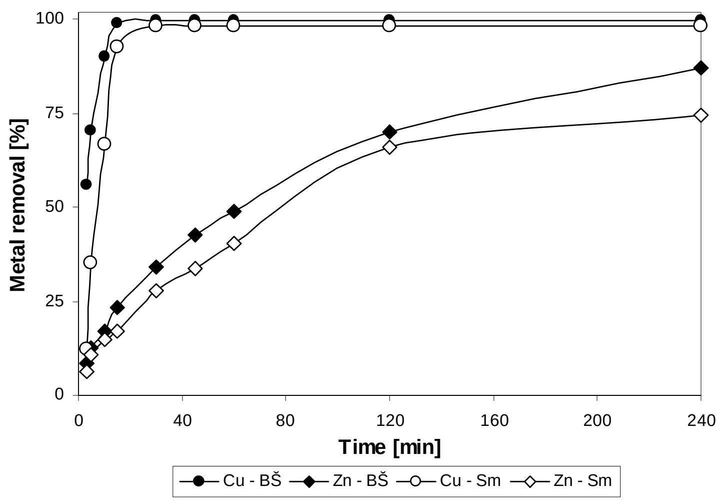


Fig. 2. Metal removal by sorption experiments from mine drainage waters

Rys. 2. Usuwanie metali przez sorpcję

## Conclusion

The purpose of this work was to investigate some biological-chemical methods, specifically sulphate-reducing bacteria utilization, for the mine drainage water remediation. The results of realized experiments reveal that the major mine drainage water contaminants (sulphates and heavy metals) can be eliminated by SRB activities under appropriate conditions. The biggest amount of sulphates was removed in Experiment 3 with mine drainage water sample from Banská Štiavnica. The highest elimination of iron from solution was attained in Experiment 4, where AMD from Smolník was used. Sorption experiments confirmed a suitability of biogenic sorbent created by SRB cultivation to treat the mine drainage waters polluted with copper and zinc.


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## Literatura – References


1. *Cao J. et al.: Influence of electron donors on the growth and activity of sulfate-reducing bacteria. International Journal of Mineral Processing, 106–109, 2012, p. 58–64.*
2. *Hallberg K.B.: New perspectives in acid mine drainage microbiology. Hydrometallurgy, 104, 2010, p. 448–453.*
3. *Jenčárová J., Luptáková A.: The elimination of heavy metal ions from waters by biogenic iron sulphides. Chemical Engineering Transactions, 28, 2012, p. 205-210.*
4. *Jong T., Parry D.L.: Removal of sulphate and heavy metals by sulphate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. Water Research, 37, 2003, p. 3379–3389.*
5. *Jong T., Parry D.L.: Adsorption of Pb(II), Cu(II), Cd(II), Zn(II), Ni(II), Fe(II), and As(V) on bacterially produced metal sulfides. Journal of Colloid and Interface Science, 2004, 275, p. 61-71.*
6. *Kuyucak N.: Role of microorganisms in mining: generation of acid rock drainage and its mitigation and treatment. The European Journal of Mineral Processing and Environmental Protection, 2 (3), 2002, p. 179-196.*
7. *Mačingová E. et al.: Recovery of metals from acid mine drainage. In Biotechnology & Metals 2011: 2nd International Conference, Slovakia. Ed. Alena Luptáková, Jana Kaduková, Hedviga Horváthová, Mária Praščáková. - Technical University of Košice, Faculty of Metallurgy; Slovak Academy of Sciences, Institute of Geotechnics : Equilibria, Košice, 2011, p. 75-78. ISBN 978-80-553-0723-7.*
8. *Mokone T.P. et al.: Metal sulphides from wastewater: Assessing the impact of supersaturation control strategies. Water Research, 46, 2012, p. 2088-2100.*
9. *Moosa S. et al.: A kinetic study on anaerobic reduction of sulphate, Part I: Effect of sulphate concentration. Chemical Engineering Science, 57, 2002, p. 2773 – 2780.*
10. *Pinto P.X. et al.: Biosorption of heavy metals from mining influenced water onto chitin products. Chemical Engineering Journal, 166 (3), 2011, p. 1002-1009.*
11. *Postgate J.R.: The sulphate-reducing bacteria. 2nd edition. Cambridge: Cambridge University Press, 1984, p. 208.*
12. *Zaluski M.H. et al.: A modular field-bioreactor for acid rock drainage treatment. In: Barnhisel R.I. (Ed.), Paper Presented at the 7th International Conference on Acid Rock Drainage (ICARD), March 26–30, 2006, St. Louis MO, American Society of Mining and Reclamation (ASMR), p. 2575–2584.*


## *Możliwość redukcji siarczanów za pomocą bakterii z kwaśnych odcieków kopalnianych*


*Celem niniejszej pracy jest zasugerowanie pewnego punktu widzenia na charakterystykę metod biologiczno-chemicznych zastosowanych w drenażowych oczyszczalniach ścieków. Ścieki te charakteryzują się często wysokim stężeniem siarczanów i metali będących efektem przemysłu górniczego minerałów siarczkowych. Do zasad biologiczno-chemicznych metod usuwania metali i siarczanów ze ścieków można zaliczyć redukcję siarczanów, biologiczne wytrącanie przez zastosowanie bakterii redukującej siarczany (SRB) oraz sorpcję przez siarczki biogeniczne.*
*Kultury bakterii redukującej siarczany zostały wydzielone ze źródła mineralnego Gajdovka (Koszyce, Słowacja). Ścieki pobrano ze słowackich okolic Banská Štiavnica i Smolník.*



*Słowa kluczowe: odciek wód kopalnianych, oczyszczanie, bakterie redukujące siarczany*