THE METAL AND SULPHATE REMOVAL FROM MINE DRAINAGE WATERS BY BIOLOGICAL-CHEMICAL WAYS

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Abstract: Mine drainage waters are often characterized by high concentrations of sulphates and metals as a consequence of the mining industry of sulphide minerals. The aims of this work are to prove some biological-chemical processes utilization for the mine drainage water treatment. The studied principles of contamination elimination from these waters include sulphate reduction and metal bioprecipitation by the application of sulphate-reducing bacteria (SRB). Other studied process was metal sorption by prepared biogenic sorbent. Mine drainage waters from Slovak localities Banská Štiavnica and Smolník were used to the pollution removal examination. In Banská Štiavnica water, sulphates decreased below the legislative limit. The elimination of zinc by sorption experiments achieved 84 % and 65 %, respectively.

Key words: metals, mine drainage water, sulphate-reducing bacteria

1. Introduction

Industrial wastewater streams containing heavy metals such as copper, zinc, lead, chromium, nickel, cadmium, manganese, aluminium, cobalt, arsenic, silver, etc. are produced from different industries and from a variety of applications.

Mining influenced water (MIW) is generated from chemical and biological processes in which sulphidic minerals, pyrite being the most common, are oxidized to sulphates and metallic hydroxides. The amount and toxicity of the generated water depends on several factors such as mineralogy of the rock material, surface area, crystallography, temperature, oxygen concentration, and the amount of water contacting the material (PINTO *et al.,* 2011). MIW which is also defined as any water whose chemical composition has been affected by mining or mineral processing (WILDEMAN and SCHMIERMUND, 2004) is often characterized by low pH (acid mine drainage) and high dissolved metal concentrations discharging to surface waters (MILLER *et al.*, 2011). Acidic drainage from abandoned metal mines is a widespread and persistent form of aquatic pollution (MAYES *et al.,* 2011).

The conventional techniques for removing heavy metals from water include many processes such as chemical precipitation, flotation, ion exchange, electrochemical deposition, and adsorption. The adsorbents may be of mineral, organic or biological origin, zeolites, industrial byproducts, agricultural wastes, biomass, and polymeric materials (BARAKAT, 2011; HERKOVA *et al.,* 2013; SCHÜTZ *et al.,* 2013).

A possible alternative to the chemical treatment of these effluents is bioremediation using anaerobic sulphate-reducing bacteria (SRB), taking advantage of the fact that these microorganisms grow in mining environments (DUARTE *et al.,* 2008).

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Sulphate-reducing bacteria are important members of microbial communities with economic, environmental and biotechnological interest. They can exist in a variety of environments such as soils, sediments and domestic, industrial and mining wastewaters. SRB are included in a group of chemoorganotrophic and strictly anaerobic bacteria, which contains representatives of the genera *Desulfovibrio*, *Desulfomicrobium*, *Desulfobacter* and *Desulfotomaculum*, among others (LUPTÁKOVÁ and KUŠNIEROVÁ, 2005; MARTINS *et al.*, 2009).

SRB are useful to AMD remediation due to two reasons. At first, because of their ability to reduce sulphates to hydrogen sulphide that additionally reacts with certain metals dissolved in the contaminated waters. On the other hand, the system acidity is reduced by their own action of sulphate reduction and by the carbon metabolism of the bacteria (GARCIA *et al.*, 2001).

Anaerobic processes based on the use of sulphate reducing bacteria are able to use sulphate as an electron acceptor and to form hydrogen sulphide that leads to an increase in the pH of the water and the precipitation of heavy metals, forming insoluble sulphides. In addition, SRB also produce bicarbonate as a by-product of the sulphate reduction, which also contributes to an increase in the pH (JIMENEZ-RODRIGUEZ *et al.*, 2009). SRB require a substrate composed of simple organic compounds using sulphate as a terminal electron acceptor. SO_4^{2-} converts into H_2S and HCO_3^{-}, according to the following equation.

$$SO_4^{2-} + \text{nutrients} + H_2O \rightarrow H_2S + HCO_3^{-} \tag{1}$$

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).

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). Iron sulphide solids may be beneficial for a variety of biogeochemical applications (ZHOU *et al.*, 2014). It was found that iron sulphides 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).

The aim of this work was to examine a suitability of sulphate-reducing bacteria use to treat the mine drainage waters polluted with high concentration of sulphates and heavy metals from 2 Slovak places (Banská Štiavnica, Smolník). The next step was to evaluate the success rate of studied processes – sulphates elimination, metals precipitation and sorption.

2. Material and methods

2.1 Sulphate-reducing bacteria

A mixed culture of sulphate-reducing bacteria (with predominant genus *Desulfovibrio*) was isolated from mineral water collected at Gajdovka spring (Košice, Slovakia) using medium Postgate C (POSTGATE, 1984). It is water with pH 7.5, H_2S odour and with natural content of SRB. 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.

2.2 Mine drainage water

Samples of the mine drainage water were collected in Slovak localities Banská Štiavnica and Smolník. First sample from Banská Štiavnica (marked BŠ) is the outflow from dump of "New Shaft" (where was the Pb-Zn ore deposit). Second sample (marked Sm) is from the shaft Pech (Smolník), which is acid mine drainage from the enclosed and flooded sulphide deposit (mainly FeS_2). Therefore, waters outflows from dumps can contain increased concentrations of metals (such as Fe, Cu, Zn, Pb). They were analyzed by atomic absorption spectrometry – AAS (Spectrometer Varian).

2.3 Sulphates and metals removal

Process of sulphate reduction was monitored in both mine drainage water samples. 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. After inoculation, 1 mL of liquid phase from each sample was taken out. Solutions were transferred to plastic tubes, centrifuged at 10 000 rpm for 10 minutes to small solid particles separation and then were extracts diluted and prepared for analysis. Sampling was realized every second day during 2 weeks. "Abiotic controls" were prepared by filling the flasks with modified Postgate medium C and mine drainage water, without SRB inoculation. Sampling was the same. Bottles were all the time stored in thermostat at 30 °C and enclosed to avoid an oxygen entry. The concentration of the sulphates in the solutions was determined by the DIONEX ICS-5000 Ion Chromatograph.

Moreover, at the beginning and at the end of experiments mentioned above, 5 mL of each sample were taken out, filtrated, stabilized by HNO_3, diluted and prepared for the measurement of selected metal ions concentration by AAS. There was metal bioprecipitation by the SRB application and concentration decrease expected at the end.

2.4 Biogenic sorbent preparation and utilization

The creation of biogenic sorbent in the form of iron sulphides was realized in a modified growth medium for SRB cultivation - Postgate C. The modification in this case consist of an addition of Fe ions in form of sulphates ($FeSO_4.7H_2O$, $Fe_2(SO_4)_3.9H_2O$) and double dose of sodium lactate as carbon and energy source. The production was performed in glass bottles containing growth medium with pH around 7.5 % and 10 % of bacteria inoculum, at 30°C, under anaerobic conditions. The samples composition was evaluated by using X-ray powder diffraction measurement on Bruker-AXS D8 Advance device.

Sorption tests were conducted by mixing 0.1 g of sorbent samples with 100 mL of mine drainage water samples at room temperature in plastic Erlenmeyer flasks for 24 hours. The mixture was during experiment continuously stirred using mechanical laboratory shaker at 250 oscillations per minute. 2 mL of liquid phase were taken out in predetermined intervals, filtered and prepared for analysis. The concentrations of the metal ions in the filtrates were determined by AAS.

3. Results and discussion

The results of mine drainage waters analysis from Banská Štiavnica and Smolník are summarized in Table 1.

Table 1. The analysis of mine drainage water (selected parameters).

Sample	pH	Sulphates	Fe	Zn	Cu
		mg/L			
Banská Štiavnica (BŠ)	6.1	950	<0.05	5.6	0.2
Smolník (Sm)	3.8	1660	205.1	6.9	1.3

In the past ore minerals such a sphalerite, pyrite, chalcopyrite, marcasite were mined in Banská Štiavnica. In Smolník it was mainly pyrite and chalcopyrite. Therefore, higher concentrations of Fe, Zn and Cu were found in sampled waters. Water from Banská Štiavnica contained higher amount of zinc. Very high concentration of iron besides increased zinc presence was confirmed in water from Smolník.

Next research was focused on metals (iron, zinc) and sulphates elimination from mine drainage waters by biological-chemical processes caused by SRB performance and H_2S presence. The pH of nutrient medium before inoculation and mine drainage water addition was adjusted to 7.5, the value suitable for SRB growth. All three components were then mixed in the bottles and sealed with butyl rubber stoppers. The pH conditions very probably caused immediate precipitation of some amount of metals dissolved in mine drainage water samples. Remaining metals were possibly eliminated by following SRB activity - hydrogen sulphide production and bioprecipitation or adsorption onto bacterially produced sulphides. The specific responsible mechanism was not examined.

SONG *et al.* (2001) state once sulphate-reducing conditions are established, sulphide precipitation becomes the predominant mechanism of metal removal from acid mine drainage. WATSON and ELLWOOD (1994) performing industrial effluents treatment found out that many of the removed metals do not form insoluble sulphides, but were removed when iron sulphide was produced. They came to the conclusion that the bacterially created iron sulphide was acting as an adsorbent for a wide range of heavy metals including many not normally precipitated as sulphides. Next study revealed that metal sulphide solid phases produced by sulphate-reducing bacteria can remove Co, Cr, Cu, Hg, Pb, Zn, Mn, Fe, As and Al. The metal ion concentration was reduced from 10 mg/L to a few µg/L (WATSON *et al.*, 1995). The batch adsorption experiments realized by MARIUS *et al.* (2005) shown very successful removal of cadmium.

The changes in metal concentrations in solutions with SRB are summarized in Table 2. Iron and zinc elimination in sample with water from Banská Štiavnica was not very significant. But iron concentration decrease in Smolník water was evident, from 64 to final value 0.7 mg/L. In blank (abiotic) samples were no or very little concentration changes and therefore are not listed. Acid mine drainage treatment by SRB by COSTA *et al.* (2008) showed almost complete precipitation of the main metals (Fe, Cu, Zn). Metals removal was attributed to the combined result of precipitation as metal sulphides, (oxy)hydroxides, coprecipitation and sorption onto the matrix materials surface. No enough sulphate reduction efficiency was obtained, which corresponds to less than 50 % of removal. MACHEMER and WILDEMAN (1992) pointed out to similar finding, where most or all of the Fe, Cu and Zn were removed. Manganese elimination was significantly lesser, about 30-40 %. The efficiency of sulphate decrease did not reach 25 %.

Table 2. Iron and zinc concentrations in solutions.

Sample	Fe		Zn	
	mg/L			
	Initial concentration (after mixing)	**Final concentration**	**Initial concentration** (after mixing)	**Final concentration**
Medium+SRB+BŠ	2.1	1.4	0.4	0.3
Medium+ SRB+Sm	64.3	0.7	0.3	<0.03

The elimination of sulphates from mine drainage water samples (BŠ, Sm) by sulphate-reducing bacteria is shown in Fig. 1. Metabolic processes of bacterial culture isolated from Gajdovka spring were in both experiments as expected and the required sulphate-reduction was running in created conditions. Almost all sulphates in sample with Banská Štiavnica mine water were removed within the experiment duration. The concentration in water from Smolník did not decrease below 250 mg/L, which is the limit value for sulphates in water in legislative. There is the need of supplementary nutrients for bacterial activity to lower the sulphates under the limit.

It is also evident that in “abiotic controls” (AC-BŠ, AC-Sm) were no changes in sulphates concentration noticed.

The other step of investigation was aimed at biogenic sorbent composition study. As it has already been mentioned, hydrogen sulphide generated as a consequence of SRB metabolic activities reacts in medium with soluble metal (iron) ions. Final products are strong dependent on solution chemistry, temperature and last but not least, on length of creation. Previous examinations on biogenic iron sulphide formation in natural conditions have identified poorly crystalline mackinawite and greigite as major solid phases (SCHOONEN, 2004). In addition, many intermediate iron sulphides may exist between disordered mackinawite and well crystallized pyrite in anaerobic environments, including greigite, marcasite, smythite and pyrrhotite (MOKONE *et al.*, 2010).

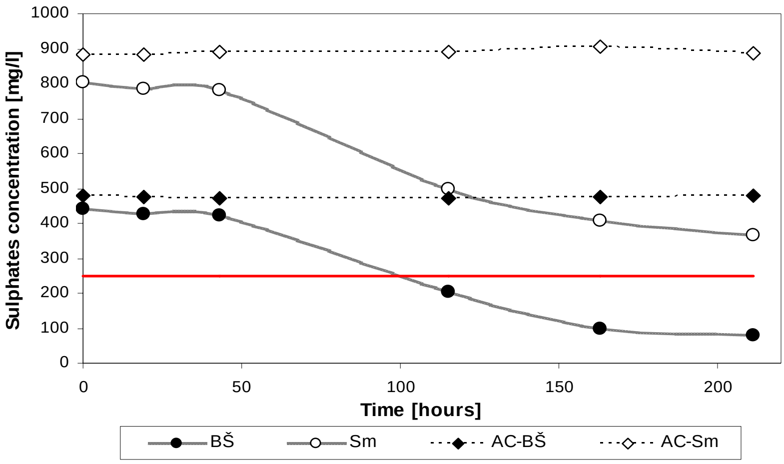

Fig. 1. Decreasing of sulphates concentration during bacterial sulphate-reduction in mine drainage waters.

The result of X-ray analysis of sorbent sample prepared by SRB cultivation is shown in Fig. 2. It revealed three compounds, identified as mackinawite, greigite, and sulphur alpha. This corresponds to GRAMP *et al.* (2010), where major peaks for biologically produced precipitates have been identified as iron sulphide minerals - mackinawite and greigite. The presence of sulphur alpha may be explained by incomplete sulphate reduction or sulphides oxidation.

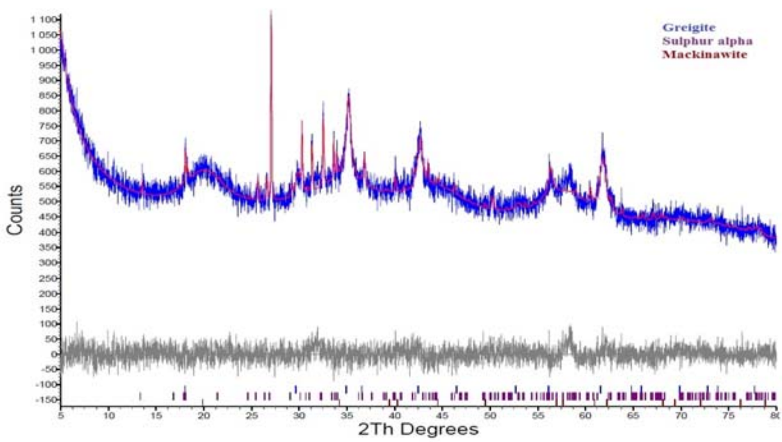

Fig. 2. XRD patterns of biogenic sorbent.

Figure 3 illustrates the sorption experiments results carried out by mixing the biogenic sorbent with mine drainage water samples (BŠ and Sm) to verify sorption properties of created sorbent. Because of low initial copper concentration in waters, only zinc removal was spotted.

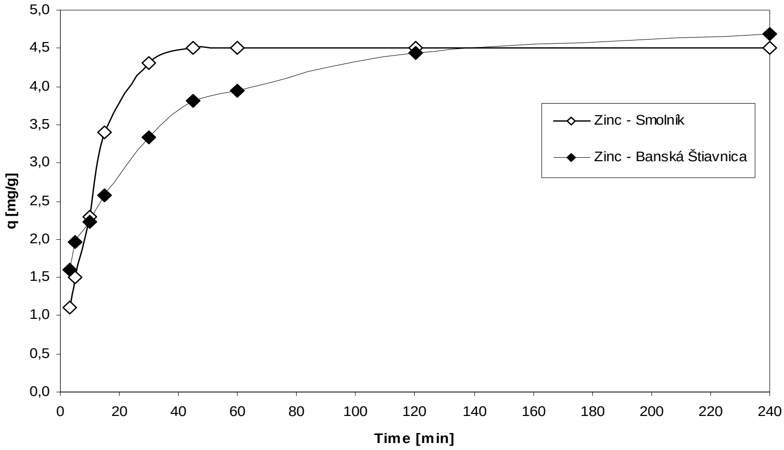

Fig. 3. Zinc sorption from mine drainage water samples by biogenic sorbent.

It is visible, that sorption from both samples became more stabilized after 120 minutes. The total zinc elimination after 4 hours in sample Sm achieved 65 %, in sample BŠ it was about 84 %. The removal percentage value for zinc obtained by JONG and PARRY (2004), which studied zinc sorption by bacterially produced metal sulphides from synthetic solution (pH = 7), was 93.3 %.

The progress of each experiment and total removal could be influenced by different factors, such as an initial zinc concentration, pH of water, presence and amount of other metal ions in solution, etc. Any other mechanism such as sorption was not in this case assumed and examined.

4. Conclusion

This work was oriented on the sulphate-reducing bacteria application for heavy metals and sulphates elimination from mine drainage waters. The results confirmed that SRB from Gajdovka spring were utilizable and under appropriate conditions (nutrient, pH, temperature), able to reduce high concentrations of sulphates in water samples to minimum and precipitate dissolved metals to insoluble form. Sorption experiments showed a suitability of biogenic sorbent created by SRB cultivation to treat the mine drainage waters polluted with metal ions.

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