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Occurrence of *Acidithiobacillus Ferrooxidans* Bacteria in Sulfide Mineral Deposits of Slovak Republic

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The formation and treatment of acid mine drainage represents one from the biggest environmental problems relating to mining and processing activities. When sulfide ores are exposed to air, water and microbes (autotrophic and heterotrophic archaea and bacteria) during the exploitation of coals and metal deposits, acid mine drainage is formed. The acid mine drainage genesis is mostly caused by bacteria of *Acidithiobacillus* genus. The sulfide ore deposits can form during the mining, but especially after the mines closing, the "natural biogeoreactors" producing these contaminated waters. In Slovak Republic there are many localities with existing acid mine drainage generation conditions. The continuance of acid mine drainage generation is not possible to stop and there is no chance of situation self-improvement. It is necessary to respect this situation, monitor the quality of these waters and develop the methods of their treatment. The main goal of the article was to monitor the occurrence of autochthonous chemolithotrophic Fe- and S-oxidizing bacteria *of Acidithiobacillus ferrooxidans* species in water out flowing from the selected sulfide mineral deposits and their waste deposits on the territory of Slovak Republic.

1. Introduction

Mining influenced water (MIW) is defined as any water which chemical composition has been affected by mining or mineral processing (Wolkersdorfer, 2008). There are several types of MIW: acid mine drainage, mineral processing waters, marginal waters and residual waters. Acid mine drainage (AMD) is a MIW with mineral acidity formed at the sites of active and abandoned mines by the dissolution of products resulting from the oxidation (chemically and microbiologically mediated) of the sulfide minerals, mainly pyrite or iron disulfide (Johnson and Hallberg, 2003). The specific issue clarifying the generation of AMD has an interdisciplinary character involving natural and technical sciences. It is primarily a function of the geology, hydrology and mining technology employed for the mine site. The explanation of AMD generation is based on the principle of the physical, chemical and biological processes running over in the contact zones of the geological and water environment. These processes are catalyzed by the mining activity resulting in the increase of contact surface and the access of oxygen to the system (Younger et al., 2002; Wolkersdorfer, 2008).
The most important chemical and biological-chemical reactions of the AMD generation are the best illustrated by examining the oxidation of pyrite (FeS_2), which is one of the most common sulfide minerals (Akcil and Koldas, 2006). There are four commonly accepted reactions that represent the chemistry of pyrite weathering to form AMD:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4H^+ + 4SO_4^{2-} \quad (1)$$

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$$4Fe^{2+} + 4H^{+} + O_2 \rightarrow 4Fe^{3+} + 2H_2O \quad (2)$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^{+} \quad (3)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^{+} \quad (4)$$

The equation (1) presents the chemical oxidation of the sulfide mineral by oxygen into dissolved iron, sulfate and hydrogen. The dissolved Fe^{2+}, SO_4^{2-} and H^+ record an increase in the total dissolved solids and acidity of the water and, unless neutralized, induce pH decrease. The second reaction involves the oxidation of the dissolved Fe^{2+} by oxygen, but also by *Acidithiobacillus ferrooxidans* bacteria (equation 2). This reaction is considered to be the rate-determining step in the overall AMD-generating sequence. Consequence, at the abiotic conditions, the reaction rate of the chemical oxidation (only by oxygen) is slowly. But at the presence of *Acidithiobacillus ferrooxidans* bacteria the reaction rate is multiple faster. The biological-chemical oxidation of the dissolved Fe^{2+} by bacteria depends on the oxygen concentration, pH and the bacterial activity (equation 2). At pH values between 2.3 and 3.5, ferric iron precipitates as $Fe(OH)_3$ and jarosite in the form of the orange-yellow precipitates (commonly referred to as "ochre" or yellow boy), leaving little Fe^{3+} in solution while simultaneously lowering pH (equation 3). Any Fe^{3+} from equation (2) that does not precipitate from solution through equation (3) may be used to oxidize additional pyrite, according to the equation (4).

AMD represent one of the major environmental problems concerning mainly the remains after termination of the mining activity (old mine loads) – exploitation of the polymetallic, precious metal and sulfide-containing deposits. AMD contain sulfuric acid and metals in soluble form (Fe, Cu, Zn, As, etc.). Their pH value is very low, about 1.5 – 2.0 (Lens and Pol, 2000). AMD cause the decomposition of other minerals, the devastation of the surrounding environment, the contamination of underground water and water streams by a wide range of elements, including the toxic ones, the penetration of metals into the food chain (Luptakova et al., 2002; Balintova et al., 2010). The fish kill belongs between the first observable negative impacts of the AMD inflow into the streams. Fish are exposed directly to metals and H^+ ions through their gills (the damage of the respiration) or indirectly through their ingestion of contaminated sediments and food items. The iron hydroxide formation (the orange-yellow precipitates) is a common weathering product of the sulfide oxidation found in thousands of miles of streams affected by AMD. Iron hydroxides and oxyhydroxides may physically coat the surface of stream sediments and streambeds destroying habitat, diminishing availability of clean gravels used for spawning, and reducing fish food items such as benthic macroinvertebrates. Acid mine drainage, characterized by acidic metalliferous conditions in water, is responsible for physical, chemical, and biological degradation of stream habitat (Jennings et al., 2008).

During exploitation, but mainly after the mines were closed, sulfide mineral deposits may become potential "natural biogeoreactors" producing AMD (Luptakova et al., 2002) and functioning on the principle of biogenous catalysis of chemical oxidation of both primary and secondary sulfide minerals by the above-mentioned bacteria species that live in symbiosis with other aerobic and anaerobic bacteria species. Due to this sulfide-weathering process metal in the water-insoluble sulfide is transformed into water-soluble sulfate (Johnson and Hallberg, 2003; Beolchini et al., 2011).

In Slovak Republic there are many localities with existing AMD generation conditions. Smolník (Figure1), Pezinok (Figure 2) and Šobov deposits are the typical examples. The fact that the AMD genesis is conditioned by the existence of autochthonous chemolithotrophic Fe- and S-oxidizing bacteria of *Acidithiobacillus ferrooxidans* species is taken into account only to a small extent. However, our previous research demonstrates the presence of the „in situ“ biological-chemical deposits oxidation in Smolník, Pezinok and Šobov (Luptakova et al., 2002). All three localities can be currently considered as natural biogeoreactors producing AMD with pH 2.0 – 3.5 and high content of heavy metals and sulfates.

There are currently about 400 million tons of ore material resources in Slovak Republic situated in 90 economically profitable deposits. Many of them were exploited short or long time ago or partly exploited within the geological survey.

Figure 1: The effluent of the acid mine drainage in Smolnik deposit (Slovak Republic).

Figure 2: The effluent of the acid mine drainage in Pezinok deposit (Slovak Republic).

At present only two deposits are exploited, namely Au-ore deposit in Hodruša and Fe-ore in Nižná Slaná. The other deposits are mostly flooded within the exploitation-attenuating process. The flooded mine area containing sulfide is a suitable environment for gradual generation and intensification of biological-chemical oxidation resulting in the increase of acidification and mineralization.

Except the three examples mentioned above, a little attention has been paid to study the occurrence of autochthonous Fe- and S-oxidizing microbial cultures with catalytic effect for the AMD formation. For the protection of the environment and in connection with new legislation being under way it is of great importance to obtain knowledge on the mentioned issues to prevent the unwanted effects of acid mine drainage (Plasari and Muhr, 2007). These were the reasons, why our research focused on the monitoring of the occurrence of autochthonous chemolithotrophic Fe- and S-oxidizing bacteria of *Acidithiobacillus ferrooxidans* species in the mine water form the selected sulfide mineral deposits on the Slovak Republic territory. Also the research was concentrated on the qualitative evaluation of AMD samples from the point of view of their acidity and heavy metals and sulfates content.

2. Materials and methods

Silverman´s and Lundgren´s selective nutrient medium 9K (Silverman and Lundgren, 1959) was used for the isolation and following cultivation of *Acidithiobacillus ferrooxidans* bacteria. The selective nutrient medium was inoculated by the mine water samples to obtain the enriched bacterial culture. This process was followed by the isolation of a clean bacterial culture using the plate dilution method (Karavajko et al., 1988). The bacteria identification by studying morphological, physiological and cultivation properties was carried out. The abiotic control was prepared without the selective nutrient medium inoculation by the mine water. The concentration of metals was determined by atomic absorption spectrometry (AAS) using a Varian 240FS/240Z spectrometer. The concentration of Fe^{3+} was determined by method based on absorption of ferric iron at 300 nm, using a Helios Gamma photometer. The nefelometric method was used to measure the concentrations of sulfate ion using a Spectromom195 instrument. The absorbance of the sample was measured at a wavelength of 490 nm. pH was measured using a pH-meter PHM210 MetLab. In process of the isolation the presence of bacteria was monitored by the microscopic observation (after the Gram stained of the microscopical preparations, by oil immersion, the magnification – 1000x) using the light microscope Nikon Eclypse 400.

3. Results and discussion

During the years 2006 – 2010 in the spring (March – April) and the autumn (September – October) the samples collection was carried out from 60 sampling points from 24 sulfide mineral deposits. The 120 samples of the mine water per year were taken from 24 sulfide mineral deposits in eastern, central and western Slovak Republic. On the basis of the chemical analysis of metals, sulfates and measurement of pH it can be concluded that the current state of mine waters is satisfactory in most deposits, except for Smolník, Pezinok and Šobov with an intensive generation of acid mine drainage (Table 1).

*Table 1: Values of pH, concentrations of metals and sulfates in some AMD sample (the collection of samples – April 2010). *Limit values according of Regulation of the Government of the Slovak Republic no. 269/2010*

	pH	Concentration of metals and sulfates [mg/L]						
		Cu	As	Zn	Mn	Fe	Al	SO_4^{2-}
Smolník, Pech shaft	3.8	8.4	0.05	12.0	35.5	405.3	108	2940
Pezinok, Pernek tunnel	5.5	<0.02	<0.005	0.16	28.2	24.5	-	400
Šobov, under heap	2.3	4.71	0.365	5.03	41.3	2454	647	1170 0
Rožňava, Maria mine	2.8	0.44	<0.005	0.09	49.0	66.3	1.0	940
Slovinky, Alžbeta shaft	5.2	<0.02	<0.005	0.23	24.8	38.2	-	850
Rudňany, New tunnel	4.6	<0.02	<0.005	1.2	56.2	72.1	1.5	1280
Limit values*	6-8.5	0.02	0.03	0.1	0.3	2	0.2	250

Yearly the isolation of acidophilous Fe- and S-oxidizing bacteria of *Acidithiobacillus ferrooxidans* species was realized from all mine water samples (i.e. from the spring and the autumn collection of the mine water). The bacteria isolation was realized until 24 hours after the samples collection. The orange precipitates occurrence is the typical attribute of the *Acidithiobacillus ferrooxidans* bacteria positive growth (according to the equations 2 - 3). During the follow-up period this effect was detected at 32 sampling points in 12 sulfide deposits (Figure 3). Predominantly occurrence of the studied bacteria was detected in Smolník and Šobov deposits. The presence of the *Acidithiobacillus ferrooxidans* bacteria was confirmed also in the case of the mine water with pH > 5.0 (for example the Pernek tunnel – Pezinok deposit or the Slovinky deposit). This fact suggested that the presence of the *Acidithiobacillus ferrooxidans* bacteria was not connected solely with a high acidity of water, but corresponded to the increased concentration of iron and sulfates. The primary isolation of *Acidithiobacillus ferrooxidans* bacteria was carried out in the following sampling points of the sulfide mineral deposits: Rožňava – Maria mine (Figure 4), Nižná Slaná – Jozef shaft, Slovinky – Alžbeta shaft (Figure 5), Rudňany – New tunnel, Kremnička – Dedičná tunnel, Voznica – Voznická dedičná tunnel, Voznica – New drain tunnel and Fichtenhűbel – Raky tunnel. The obtained results demonstrate a real possibility of the acid mine drainage generation in Slovinky, Rožňava and Rudňany deposits.

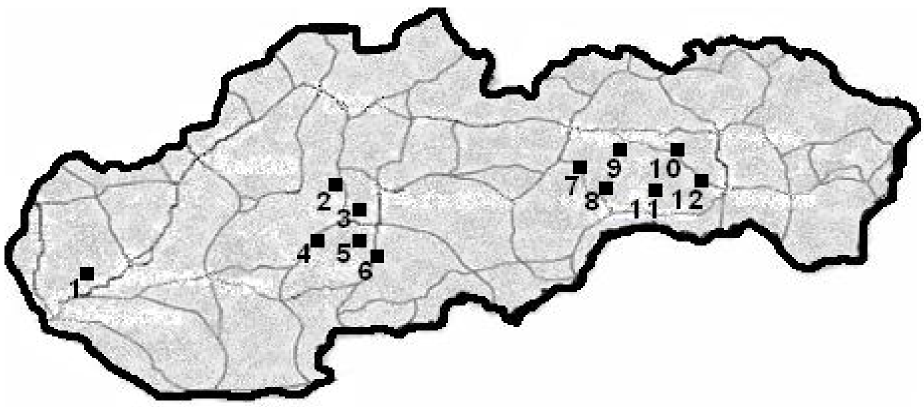

Figure 3: The occurrence of Acidithiobacillus ferrooxidans bacteria in Slovak Republic. 1 – Pezinok, 2 – Kremnička, 3 – Horná Ves, 4 – Voznica, 5 – Šobov, 6 – Odkalisko sedem žien, 7 – Nižná Slaná, 8 – Rožňava, 9 – Rudňany, 10 – Slovinky, 11 – Smolník, 12 – Fichtenhűbel.

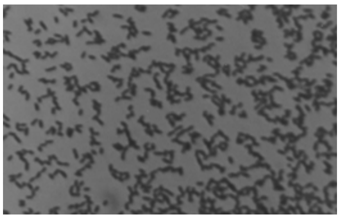

Figure 4: Acidithiobacillus ferrooxidans bacteria isolated from the acid mine drainage of the Maria mine (Rožňava deposit, Slovak Republic).

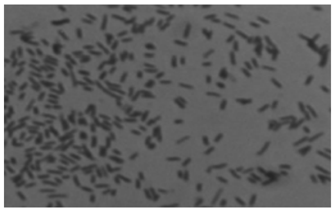

Figure 5: Acidithiobacillus ferrooxidans bacteria isolated from the acid mine drainage of the Alžbeta shaft (Slovinky deposit, Slovak Republic).

There was an increased content of sulfate and Fe observed in all samples taken from these localities, as well as the occurrence of *Acidithiobacillus ferrooxidans* bacteria, indicating the biological-chemical oxidation of Fe- and S-containing minerals and the possibility of certain changes in the water mineralisation. These facts support the need to monitor the quality of water after the completion of deposit flooding, mainly as regards the content of iron and sulfates and other toxic metals contained in paragenesis (Plasari and Muhr, 2007).

On the basis of the obtained results it was possible to come to a conclusion that 12 samples from the researched set of localities did not meet the criteria concerning the quality of special waters according to a regulation of the Slovak Government (2010), which include waste waters originating from the exploitation and processing of sulfide ores, as well as seepage waters from the relevant landfills. Smolník, Šobov and Pezinok deposits were identified as localities at risk due to significant activity of autochthonous acidophilous chemolithotrophic Fe- and S-oxidizing bacteria *of Acidithiobacillus ferrooxidans* species that basically operate as natural biogeoreactors producing AMD. At these deposits was observed the mine water effluences with pH ranging from 2.0 to 5.1 and with increased or even extremely high levels of heavy metals and sulfates.

The very negative influence of AMD on the surrounding environment was documented in the abandoned and flooded Smolník deposit. It is historically one of the best-known and richest Cu – Fe ore deposits in Slovak Republic, geomorphologically located in the Slovenské Rudohorie Mountains (West Carpathians). The main minerals of the deposit are pyrite and chalcopyrite. In 1990 mining was stopped. The mine was flooded until 1994. At the same year an ecological collapse occurred. Consequently the fish kill and the decline of the Smolník stream quality were observed. At the present time AMD drains from the flooded mine through the Pech shaft into the Smolník stream and is negatively affecting the stream biotope. In addition, the gradual neutralization of AMD as it comes into contact with the surface water causes the precipitation of Fe and Al oxy-hydroxides that are carried by water into the Hnilec river and further to the Ružín water-basin (Balintova et al., 2010). The generation of AMD at the Smolník deposit is not possible to stop. It is necessary to develop methods for its treatment (Luptakova and Kusnierova, 2005; Luptakova et al., 2010).

4. Conclusion

The occurrence of acidophilus autochthonous S- and Fe-oxidizing *Acidithiobacillus ferrooxidans* bacteria as one of basic factors of bio-catalysis supporting the generation of strongly mineralized acid mine drainage was confirmed at 32 sampling points situated in 12 old mine loads in Slovak Republic. The results obtained proved a real possibility of acid mine drainage generation in Slovinky, Rožňava and Rudňany deposits. Some strongly mineralized acid mine drainage can be considered as atypical source of a wide range of elements (Luptakova and Kusnierova, 2005; Kumar et al., 2007; Balintova and Petrilakova, 2011). There is a prospective possibility of using sulfate-reducing bacteria of *Desulfovibrio and Desulfotomaculum* genera to eliminate negative effects of the acid mine drainage. The recent research is focused on the development of the chemical and biological-chemical methods for the metals selective recovery from the acid mine drainage. These methods constitute the possibility of the recovery metals in a suitable form for commercial or industrial utilization. The combination of the metal precipitation using the sodium hydroxide (chemical methods) with the metal precipitation using the bacterially produced hydrogen sulfide (biological-chemical method) presents the base of the selective sequential precipitation (SSP) (Tabak et al., 2003; Luptakova et al., 2010).

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