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Bacterial Elimination of Sulphates from Mine Waters

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Sulfates can be found in almost all types of water, in form of simple anion SO_4^{2-}. Together with hydrogen carbonates and chlorides, sulfates are the main anions in natural water ecosystems. Sulfate concentration in characteristic groundwater and surface water ranges from ten to hundreds mg/L. Nowadays, the importance of the control of sulfate concentration in waste water increases. Contents of sulfates in rivers increase especially by waste water discharge, which comes mainly from metallurgical, mining, chemical, textile industry. Sulfate concentration in these waters ranges from tens to thousands of grams per liter. There are many technologies for sulfate removal, used in the waste water treatment, including biological-chemical processes. One of these methods is the reduction of sulfates by sulfate - reducing bacteria. The object of our study was to verify experimentally the possibility of using sulfate-reducing bacteria to remove sulfates from mine water originating from coal mine Cígeľ (Slovakia). The main objectives were: sulfate elimination from mine water influenced by SRB, initial sulfate concentration effect on the bacterial sulfate reduction process and elemental sulfur preparation from hydrogen sulfide produced by bacteria.

1. Introduction

Sulfates occur in waters mainly as a simple anion SO_4^{2-}. In waters with high sulfate concentration also ion associate anions with some cation occurrence is possible. Together with bicarbonates and chlorides they form a major part of anions in natural waters. In common groundwaters and surface waters sulfate content ranges in tens to hundreds of milligrams/ liter. Particularly rich in sulfates are some mineral waters. Sulfate concentrations, occurring in surface and common ground waters are not of hygienic importance. However, high concentrations may affect the water taste and cause laxative effects (Jennings et al., 2008). Sulfate content should be monitored especially in industrial waste waters, because their higher contents are the cause of water aggressiveness to concrete in this case (Lens and Pol, 2000). This leads to the destruction of pipelines, concrete tanks, etc. Allowable sulfate concentrations depend on the quality of concrete and other factors. In the least favorable scenario, the allowable sulfate concentrations range around 100 mg/L. According to Slovak legislation – Government Regulation no. 269/2010, the limit concentration of sulfates in surface and drinking water is 250 mg/L.

The source of sulfates in industrial wastewaters can be: sulfuric acid, gypsum, by-products after coal desulphurization or metal sulfide biodegradation and other sulfate pollutants from chemical, textile, metallurgical, pharmaceutical, paper and mining industries − (Lens and Pol, 2000) treats the treatment technologies, (Wolkersdorfer, 2008) discusses application to mines. In these waters sulfate concentrations range is from tens to thousands g/L.

For sulfate removal from waters various physical-chemical and chemical methods are used the most - see (Younger et al., 2002) for a general background as well as (Balintova and Petrilakova, 2011) for a specific study of Ph as a factor. There are used ion exchangers or membrane filtration as an example of physical-chemical methods. Their advantage is a high cleaning effect, but the disadvantage is necessary water pre-treatment. The most used chemical methods are sulfate precipitation with calcium hydroxide. Again, this is an effective method, but the final product is a large volume of sewage sludge, which creates a problem with storage (Plasari and Muhr, 2007).

Biotechnology developments consider the possibility of using microorganisms to solve the problem of increased sulfate concentration in wastewater. A biotechnological process, which uses bacterial activity of

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sulfate reducing bacteria (SRB) and simulates the natural process occurring in the geochemical sulfur cycle, is a perspective solution (Odom and Rivers Singleton, 1993).
SRB may be one of the oldest forms of life on Earth. They can be found back billions of years in the geologic rock record to the Early Archean (3,900 to 2,900 million years ago) (Postgate, 1984). Ancient SRB left their first mark on their environment in pyrite minerals (FeS_2) as old as 3,400 million years. Today, these bacteria are widespread in marine and terrestrial aquatic environments. Their ability to adapt to extreme physical and chemical conditions enables them to play an important role in global geochemical cycles (Odom and Rivers Singleton, 1993) predominantly in the sulfur cycle that constitutes one of the best examples of the impact exerted by living organisms on geochemical cycles. SRB is a wide term that is applied to diverse collection of obligate anaerobic bacteria that use sulfates as an electron acceptor in the anaerobic oxidation of inorganic (equation 1) or organic substrates Eq(2) (Odom and Rivers Singleton, 1993):

$$4\,H_2 + SO_4^{2-} \xrightarrow{\textbf{SRB}} H_2S + 2\,H_2O + 2\,OH^- \qquad \text{Inorganic substrate} \qquad (1)$$

$$\text{Organic matter} + SO_4^{2-} \xrightarrow{\textbf{SRB}} HS^- + HCO^{3-} + H_2O \qquad \text{Organic substrates} \qquad (2)$$

Equations above (1) and (2) indicate the possibility of using SRB to remove sulfates from mine water, because sulfates contained may be utilized by SRB and form hydrogen sulfide, which can be used then for:

- preparation of metal sulfides by selective heavy metal precipitation – see e.g. (Luptakova et al., 2012) for a general review and (Kaksonen and Puhakka, 2007) for sulphate reduction; from industrial waste water, because hydrogen sulfide in aqueous solution, depending on pH, reacts easily with heavy metal cations to form low soluble metal sulfides, which describes equation (3):

$$Me^{2+} + H_2S \longrightarrow MeS + 2\,H^+ \qquad (Me^{2+} \text{ - metal cation}) \qquad (3)$$

- bacterial sulfate-reduction result is a simultaneous sulfates and heavy metal elimination, which is considered to be this method's advantage in comparison with physical-chemical and chemical methods. The method is suitable for treatment of acid mine drainage, which constitute one of the main environmental problems related to mining (Cruz Viggi et al., 2011) ;
- elemental sulfur preparation because hydrogen sulfide produced by bacteria can be chemically or bacterially oxidized to sulfur. This is represented by equations (4) and (5):

$$2\,Fe3^+ + H_2S \longrightarrow 2\,Fe^{2+} + S^0 + 2\,H^+ \qquad \text{(chemical oxidation of hydrogen sulfide)} \qquad (4)$$

$$2\,H_2S + CO^{2-} \xrightarrow[\text{light}]{\textbf{\textit{Chromatium vinosum}}} 2\,S^0 + C + 2\,H_2O \qquad \text{(biological oxidation of hydrogen sulfide)} \qquad (5)$$

2. Materials and methods

2.1 Sulfate-reducing bacteria

A bacterial culture of SRB (Desulfovibrio sp. and Desulfotomaculum sp.) isolated from potable mineral water (Gajdovka spring, Kosice-north, Slovakia) was used for the experiments (Luptakova and Kusnierova, 2005). It was selected from the mixed cultures by Postgate′s method (Postgate, 1984) and modified dilution method (Karavaiko et al. 1988).

2.2 Nutrient medium for sulfate-reducing bacteria

The SRB isolation and subsequent cultivation for the production of active SRB cultures were carried out using Postgate′s nutrient medium C (Postgate, 1984). For the experiments of sulphate elimination was used complete same nutrient medium i.e. with sulfates and incomplete (without sulfates). The chemical composition of the complete medium was as follows (in g/L): 0.5 K_2HPO_4, 1.0 NH_4Cl, 1.0 Na_2SO_4, 0.1 $CaCl_2{\cdot}2H_2O$, 2.0 $MgSO_4{\cdot}7H_2O$, 3.5 sodium lactate, 1.0 yeast extract, 0.5 $FeSO_4{\cdot}7H_2O$, 0.1 ascorbic acid and 0.1 thioglycolic acid. The incomplete Postgate′s nutrient medium C was non-involved Na_2SO_4, $MgSO_4{\cdot}7H_2O$ and $FeSO_4{\cdot}7H_2O$. The pHs of the media were adjusted to 7.5 using 5M NaOH.

2.3 Mine water

The experiments were carried out at the laboratory scale using the mine water coming from the coal mine Cígeľ (Slovak republic). The annual average pH values, sulfates and metal concentration of the studied water described in Table 1.

Table 1: pH and chemical composition of mine water Cígeľ

pH	Concentration of sulfates and chosen metals (mg/L)							
	SO_4^{2-}	Fe^{2+}	Mn	Sb	Al	Ba	Sr	Zn
7.3	460	0.2	1,6	0.02	0.3	0,09	0.24	0,3

2.4 Solution for hydrogen sulfide oxidation

For chemical oxidation of bacterial produced hydrogen sulfide was used a solution containing Fe^{3+} cations prepared from $Fe_2 (SO_4)_3.9 H_2O$ with Fe^{3+} concentration of 8 g / L.

2.5 Elimination of sulfates from mine water

The principle of sulfate elimination from waste water was SRB cultivation in anaerobic conditions, lasted 8 days in hermetically sealed reactor, using Postgate′s nutrient medium C (with and without sulfates), with an initial pH 7,5 , temperature 30 ºC, 15 % SRB inoculum, stationary discontinuous, anaerobic conditions (inert gas – nitrogen). Total liquid phase volume, consisting of mine water, nutrient medium and SRB inoculum, was 200 ml. The ratio of mine water volume and culture medium volume with inoculum was in case of Postgate′s nutrient medium C with sulfates, as without sulfates 3:1, 1:1, 1:3. During the experiments, sulfate concentration, pH, presence of hydrogen sulfate and SRB was monitored. Abiotic experiments were performed under the same conditions but without the presence of SRB.

2.6 Sulfur production

Sulfur production from bacterially produced hydrogen sulfide that was created in sulfate elimination process from mine water, realized by chemical oxidation of Fe^{3+} ions in two interconnected tanks. It was followed by “starting” of bacterial sulfate reduction (3-4 days from SRB culture beginning) by continuous supplying of the gas phase with inert gas (nitrogen) from the culture tank to a second container filled with a solution containing Fe^{3+}. The sulfur precipitation was performed in the second reactor.

2.7 Analytical methods

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 (APHA, 1989). Values of pH were measured using a pH-meter PHM210 MetLab. At the beginning and end of the process 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. Formation and presence of hydrogen sulfide was determined by an indicative test, the principle of which is the reaction of Cu^{2+} cations with hydrogen sulfide in an acidic medium while formation a brown color-formation of CuS, whose intensity is proportional to the amount of hydrogen sulfide. The qualitative analysis of precipitates obtained by the sulfur production process was done using energy dispersive spectrometry (EDS) analysis, using instruments, which included a scanning electron microscope BS 300 (Tesla, Czechoslovakia) and an X-ray microanalyser EDAX 9100/60 (Philips, Holland). The precipitates were dried and coated with gold before the EDS analysis.

3. Results and discussion

3.1 Elimination of sulfates from mine water

After 2 – 4 days from the beginning and until finishing sulfate elimination from mine water Cígeľ were, in comparison with abiotic control, observed following changes:

- black precipitates formation, i.e. FeS formation according to equation (6), because Fe^{2+} cations are a standard component of Postgate′s nutrient medium C and are present in studied mine water

$$Fe^{2+} + H_2S \longrightarrow FeS + 2H^+ \quad (6)$$

- positive result of hydrogen sulfide presence screening test (Figure 1);
- SRB presence (Figure 2).

These changes suggested the growth of SRB and thus to bacterial sulfate reduction and hydrogen sulfide production.

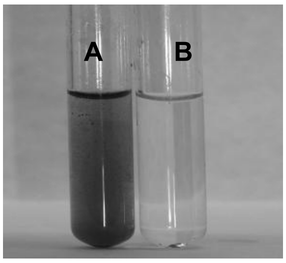

Figure 1: Testing of H_2S presence (A - positive result; B – negative result)

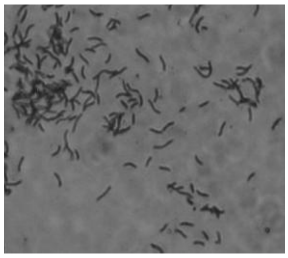

Figure 2: The microphotograph of SRB Desulfovibrio sp. (light microscope, oil immersion, magnification – 1000x)

Figures 3 − 5 show the changes in sulfate concentration in the liquid phase, influenced by SRB, depending on its composition. These results, compared to abiotic controls, point to:

- 40% efficiency of sulfate elimination (or reduction) by SRB in case of using complete Postgate′s nutrient medium C (Figures 3 to 5, curve COM-1, COM-2 and COM-3);
- 100% efficiency of bacterial sulfate elimination when applied Postgate′s nutrient medium C without sulfates (Figures 3 - 5, curve INCOM-1, INCOM-2 and INCOM-3);
- the fact that the ratio of mine water volume and nutrient medium with SRB inoculum due to sulfate elimination efficiency, using incomplete Postgate′s nutrient medium C is 1:1 (Figure 4, curve INCOM-2).

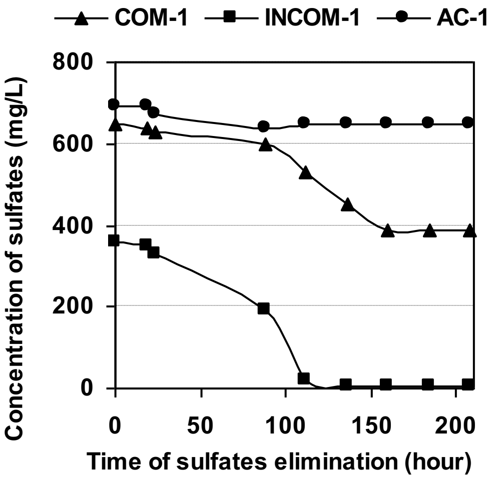

Figure 3: Elimination of sulfates from mine water Cígeľ by cultivation of the sulfate-reducing bacteria (ratio of mine water volume and nutrient medium with inoculum – 3:1). COM – complete Postgate′s nutrient medium C (with sulfates); INCOM – incomplete Postgate′s nutrient medium C (without sulfates); AC – abiotic control experiment

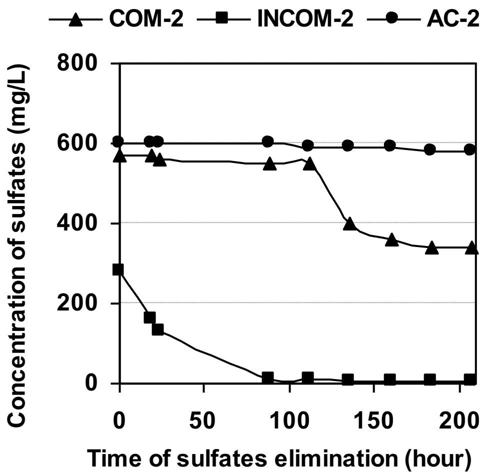

Figure 4: Elimination of sulfates from mine water Cígeľ by cultivation of the sulfate-reducing bacteria (ratio of mine water volume and nutrient medium with inoculum – 1:1). COM – complete Postgate′s nutrient medium C (with sulfates); INCOM – incomplete Postgate′s nutrient medium C (without sulfates); AC – abiotic control experiment

Figures 3-5 illustrates, that in experiments with using complete Postgate′s nutrient medium C, the sulfate concentration initially started to fall, but then the process of bacterial reduction was stopped. Probably it had been depletion of organic substrate (sodium lactate). Quite different situation was observed using incomplete Postgate′s nutrient medium C. Then SRB reduced sulfates only from the mine water and the organic substrate amount was sufficient.

3.2 Sulfur production

Production of elemental sulfur from bacterially produced hydrogen sulfide with Fe^{3+} cations assistance indicates following reaction:

$$2\ Fe3^{+} + H_2S \longrightarrow 2\ Fe^{2+} + S^0 + 2\ H^{+} \qquad \text{(chemical oxidation of hydrogen sulfide)} \qquad (4)$$

Figure 6 shows EDS qualitative analysis of emerging precipitates, which demonstrate sulfur formation.

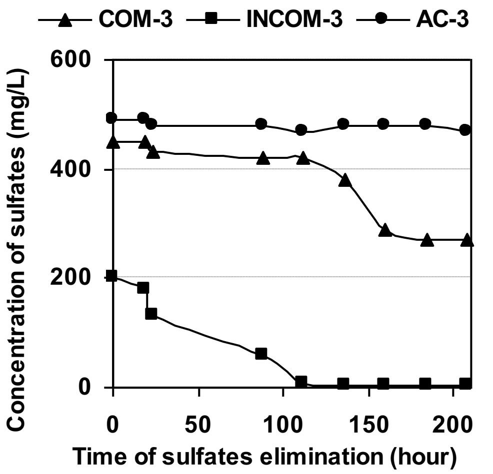

Figure 5: Elimination of sulfates from mine water Cígeľ by cultivation of the sulfate-reducing bacteria (ratio of mine water volume and nutrient medium with inoculum – 1:3). COM – complete Postgate's nutrient medium C (with sulfates); INCOM – incomplete Postgate's nutrient medium C (without sulfates); AC – abiotic control experiment

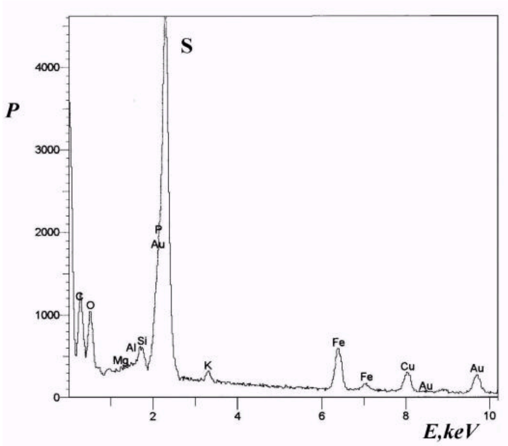

Figure 6: EDS qualitative analysis of produced precipitates from biologically produced hydrogen sulfide by chemical oxidation with Fe $^{3+}$, P – impulse count, E - energy

4. Conclusions

Using cultivation of sulfate-reducing bacteria and using complete Postgate's nutrient medium C was achieved about 40% efficiency of sulfate elimination (or reduction), in the case of medium without sulfates 100% efficiency. These results referred to the need to ensure sufficient concentration of organic substrate for the growth of sulfate-reducing bacteria with respect to initial sulfate concentration. The best ratio of mine water volume, nutrient medium with SRB inoculum due to sulfate elimination efficiency using incomplete Postgate's nutrient medium C was 1:1. Presented theoretical knowledge as well as experimental results from sulfate elimination from Cígeľ mine water using SRB cultivation allows to note that their natural metabolic activity can be used in environmental technology for cleaning industrial waste water with excessive content of sulfate and elemental sulfur products.

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