BACTERIAL REDUCTION OF BARIUM SULPHATE BY SULPHATE-REDUCING BACTERIA

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Abstract: Acid mine drainage (AMD) is a worldwide problem leading to contamination of water sources. AMD are characterized by low pH and high content of heavy metals and sulphates. The barium salts application presents one of the methods for the sulphates removing from AMD. Barium chloride, barium hydroxide and barium sulphide are used for the sulphates precipitation in the form of barium sulphate. Because of high investment costs of barium salts, barium sulphide is recycled from barium sulphate precipitates. It can be recycled by thermic or bacterial reduction of barium sulphate. The aim of our study was to verify experimentally the possibility of the bacterial transformation of $BaSO_4$ to BaS by sulphate-reducing bacteria. Applied $BaSO_4$ came from experiments of sulphates removal from Smolnik AMD using $BaCl_2$.

Key words: acid mine drainage, sulphate-reducing bacteria, barite, bacterial reduction.

1. Introduction

Acid mine drainage (AMD) is a big problem in mining industry and major environmental concern. It causes surface water pollution, which may impact aquatic life as well as whole ecosystem (JOHNSON and HALLBERG, 2003). Acid mine waters are characterized by low pH and high content of heavy metals and sulphates. In order to minimize negative impacts of AMD appropriate treatment process has to be chosen (JENČÁROVÁ, 2008; MAČINGOVÁ, 2008). These processes are focused on neutralizing, stabilizing and removing pollutants. From this reason efficient and environmental friendly methods are needed to be developed in order to reduce heavy metals as well as sulphates (SINGOVSZKÁ, 2013; HOLUB, 2014).

According to Slovak legislation – Government Regulation no. 269/2010*,* the limit concentration of sulphates in surface and drinking water is 250 mg/L. Increased content of sulphates concentrations can be remediate with various existing sulphate treatment processes (SINGH and CHUGH, 2010): precipitation of sulphates with lime and limestone, precipitation using barium salts, sulphate precipitation in the presence of aluminium and calcium ions, sulphate removal using aluminous cement, membrane processes, ion exchange process, sorption and biological sulphate removal methods using sulphate reducing bacteria (SRB). Biological reduction, compared to chemical treatment, has more advantages: it is effective in sulphate elimination, it does not produce

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amounts of waste, it is cost effective and has added advantage of removing trace metals from mine water.

Principle of the chemical precipitation methods using barium salts is sulphate precipitation in form of barium sulphate (insoluble compound). Barium chloride, barium hydroxide and barium sulphide are used for precipitation:

$$BaCl_2 + H_2SO_4 \rightarrow BaSO_4 + 2HCl \quad (1)$$

$$Ba(OH)_2 + H_2SO_4 \rightarrow BaSO_4 + 2H_2O \quad (2)$$

$$BaS + H_2SO_4 \rightarrow BaSO_4 + H_2S \quad (3)$$

Because of high investment costs of barium salts, barium sulphate precipitates are transformed to the form of barium sulphide. BaS can be transformed (i.e. recycled) chemically by thermic reduction of barium sulphate with carbon in the form of coal at a temperature of 1200 °C to barium sulphide (HAMMACK and HEDIN, 1995):

$$BaSO_4 + 2C \rightarrow BaS + 2CO_2 \quad (4)$$

Like that the recycled BaS can be used in the process of the sulphates removal from waste waters again. The key factor affecting the process economy during the chemical reduction is high energy consumption. Bacterial reduction of barium sulphate, to obtain barium sulphide by SRB, could be used as an alternative and more economical recycling method.

SRB represent the bacteria that use sulphates as a terminal electron acceptor for their metabolism. These bacteria realize the conversion of sulphate to hydrogen sulphide under anaerobic conditions (ODOM and SINGLETON, 1993) and utilize organic compounds which range from simple organic acids (acetate and lactate) and ethanol to long-chained fatty acids and certain aromatic compounds.

The aim of our study was to verify experimentally the possibility of the BaS recycling by the bacterial reduction of $BaSO_4$ using SRB. Applied $BaSO_4$ came from experiments of sulphates removal from Smolnik AMD using $BaCl_2$.

2. Materials and methods

In the experiment a culture of SRB (genera *Desulfovibrio*) was used. Bacteria were isolated from natural mineral water Gajdovka (source in the area of Kosice - North). For the isolation and cultivation of SRB the selective medium DSM-63 also known as J. Postgate was used. For the experiment of the sulphates reduction the modified medium DSM – 63 was used. This medium contents sodium lactate as carbon source and barium sulphate as sulphate source. Applied $BaSO_4$ came from experiments of sulphates removal from Smolnik AMD, using $BaCl_2$ (DEMČÁK, 2014). The amount of $BaSO_4$ was calculated on the base of sulphates content in the selective medium DSM-63 (LUPTÁKOVÁ *et al*., 2010). Experiments were realized in the reagent bottles at 30 °C for 40 days under static and anaerobic conditions. The inoculum of SRB was 10% (v/v). The initial pH value of the modified medium was 7.2. The abiotic control was carried out without the SRB application at the same conditions. Composition of samples was as follows: Sample SRB – 180 ml modified medium DSM-63 + 20 ml SRB inoculum + 0.66 g $BaSO_4$; Sample K (abiotic control) – 200 ml modified medium DSM-63 + 0.66 g $BaSO_4$.

Sulphates content was determined by ion chromatography method. Soluble sulphide was analyzed using a methylene blue method based on a colorimetric determination of dissolved H_2S and HS^- by *N*,*N*-dimethyl-*p*-phenylenediamine. The composition of precipitates was analyzed by X-ray diffraction technique.

3. Results and discussion

We assumed the bacterial reduction of sulphates by SRB in the presence of sodium lactate as an electron donor and $BaSO_4$ as an electron acceptor under anaerobic conditions according to equations (5) and (6) (BALDI *et al*., 1996):

$$\text{Na-lactate} + BaSO_4 \xrightarrow{\text{SRB}} \text{biomass} + BaCO_3 + BaS + CO_2 + H_2S \quad (5)$$

$$BaSO_4 + 2H_2S \longrightarrow BaS + SO_2 + 2H_2O + S \quad (6)$$

The changes of sulphates and hydrogen sulphide concentrations are plotted in Fig. 1 and Fig. 2. In the case of biotic conditions the initial sulphates concentration (20.00 mg/L) was caused by the sulphates concentration from the SRB inoculum. After inoculation during the first and the second days the sulphates concentration was rapidly increased. Consequently the decreasing of sulphates concentration and the increasing of hydrogen sulphide concentration were recorded (Eq.5).

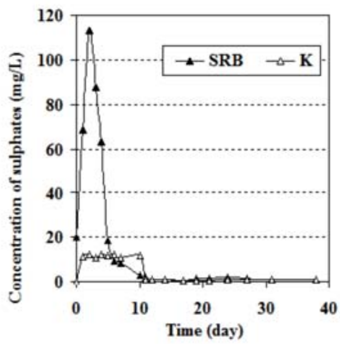

Fig. 1. Changes of sulphates concentration.

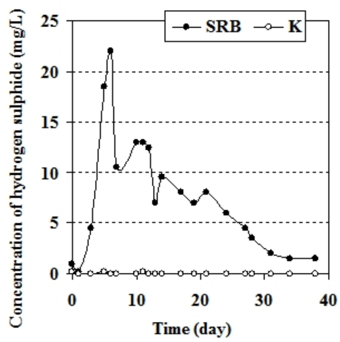

Fig. 2. Changes of hydrogen sulphide concentration.

The presence of sulphates was estimated in solution at abiotic conditions too. Probably the culture medium was caused the dissolution of $BaSO_4$ by the influence of the calcium chloride which is a basic constituent of the applied culture medium DSM-63 (HUGDAHL *et al*., 2007). However, the listed culture medium influence was not observed after the tenth days of experiments.

On the fifth day the hydrogen sulphide concentration maximum (18.5 mg/L) was observed. At the same time the acetate i.e. the metabolic product of SRB was detected as the next carbon source for SRB (Fig. 3). Consequently the decreasing of lactate concentration and the increasing of acetate concentration were recorded. In the experiment a culture of SRB was substituted by the genera *Desulfovibrio*. This genus cannot utilize of acetate (ODOM and SINGLETON, 1993) and the process of the bacterial sulphate reduction was gradually stopped. In the course of experiments the release of barium to the liquid phase was observed (Fig. 4). Ten times the quantity of barium was dissolved by bacteria (2.96 mg/L) than in the un-inoculated medium (0.35 mg/L).

In the case of abiotic conditions, the presence of hydrogen sulphide and the acetate was not proved (Fig. 2 – sample K and Fig. 3 – sample K-acetate); in the course of 10 days the culture medium was caused the dissolution of $BaSO_4$ probably by the influence of the calcium chloride and the final concentration of Ba was 0.35 mg/L (Fig. 4).

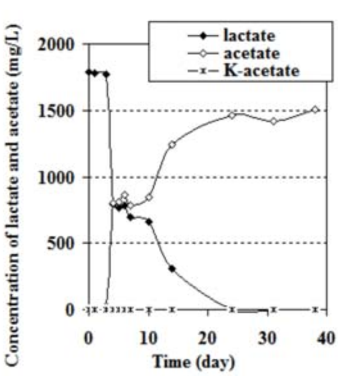

Fig. 3. Utilization of lactate by SRB and creation of acetate.

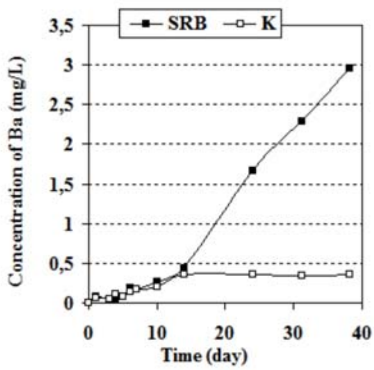

Fig. 4. Release of barium to liquid phase.

After 40 days accrued precipitates from the samples SRB and K were separated from liquid phase, saved at anaerobic conditions and analysed by X-ray diffraction technique. The presence of BaS was not confirmed in the precipitates composition (Fig. 5).

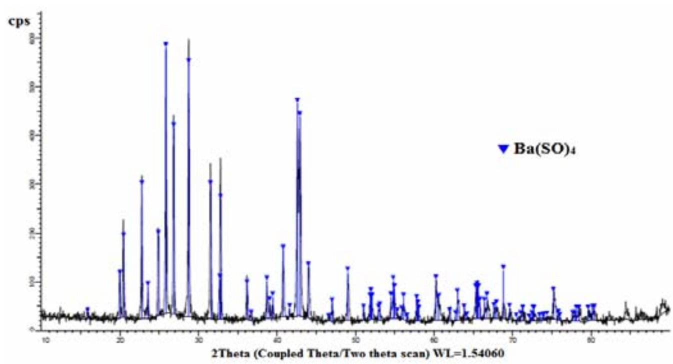

Fig. 5. X-ray diffraction spectrum of precipitates (sample SRB).

For all the informative experiments of the sulphates elimination from AMD by the recycled BaS were realized. The obtained results were assigned the sulphates decreasing only in the case of BaS prepared at the SRB influence (Fig. 6). The bacterially prepared BaS as the sulphates precipitating agent was effected 24.4 % decreasing of sulphates from AMD. The precipitates take from abiotic conditions were induced the very minimal decreasing of sulphates from AMD – 0.11%.

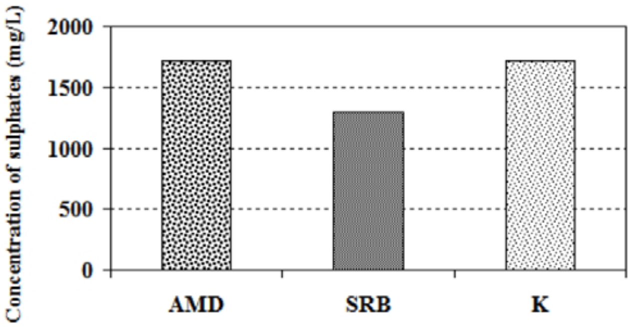

Fig. 6 Sulphates elimination from AMD. AMD – amount of sulphates in AMD before elimination (1720 mg/L); SRB – amount of sulphates in AMD after application of bacterially prepared BaS (1400 mg/L); K - amount of sulphates in AMD after application of precipitates from abiotic control (1718 mg/L).

BALDI *et al.* (1996) observed that barium sulfide may form in the process of bacterial barite reduction and dissolution, it could not be positively identified in the solid phase. Despite its instability in aqueous solution, probably BaS is a reactive transient species stabilized on a colloidal or solid phase. Polysaccharide complexes which frequently form organic coatings on suspended matter may account for barium sulfide stabilization. These organic coatings i.e. biofilms could be associated with the biomass production. The polysaccharides of bacterially cells represent the suitable specific microenvironment for the BaS stabilization.

4. Conclusions

The main purpose of this work was to investigate the possibility of the BaS recycling by the bacterial reduction of $BaSO_4$ using the sulphate-reducing bacteria. The obtained results demonstrate the influence of SRB (genera *Desulfovibrio*) on the studied $BaSO_4$ came from experiments of sulphate removal from Smolnik AMD. In spite of the presence of BaS was not confirmed by the X-ray diffraction methods in the precipitates composition. For all the informative experiments of the sulphates elimination from AMD by the bacterially recycled BaS were assigned the sulphates decreasing. Specific explanation could be associated with the research of BALDI *et al.* (1996). Probably accrued BaS was stabilized on a colloidal or solid phase by the bacterially cells polysaccharides.

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