Bacterial Reduction of Sulfates as Suitable Method for Removal of Sulfates from Acid Mine Drainage

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Abstract

The aim of this work was to investigate of the sulfates removal from acid mine drainage (AMD) by biological method using sulfate-reducing bacteria (SRB). A sample of AMD out-flowing from the Pech shaft of the abandoned and flooded Smolník deposit in Slovak Republic was studied. The untreated AMD (with contents metals) and treated AMD i.e. after of the metals removing by the synthetic sorbent Slovakite were used. The base of the biological sulfates elimination was the sulfates bacterial reduction under influence of SRB genera Desulfovibrio. These bacteria realize the sulfates reduction to hydrogen sulfide at the simultaneously oxidation of energetic substrate. Standard selective nutrient medium DSM-63 and modified selective nutrient medium DSM-63 (without sulfates contents) with standard amount of sodium lactate (as energetic substrate) were used in the experiments with untreated AMD. Standard selective nutrient medium DSM-63 and modified selective nutrient medium DSM-63 with standard, double and triple amount of sodium lactate were used in the experiments with treated AMD. In the case of untreated AMD has been reached low removing of sulfates - 20% efficiency (standard medium) and 26% (modified medium). The formation of heterogeneous precipitates containing metals pointed to the need for treatment of AMD by the initial removal of metals by sorption on Slovakite sorbent and subsequent removal of sulfates by bacterial reduction. The results of experiments with AMD treated in this way showed 49%, 70% and 88% efficiency of sulfate removal when using sodium lactate in standard, double and triple amounts. The results of this work suggest that ratio of substrate quantity and sulfate concentration is one of the key parameter of sulfate reducing condition. However, the price of the energy substrate is also an important factor. Therefore, subsequent experiments will be focused on the use of the more affordable substrates (e.g. whey) or mixed bacterial culture of SRB, which will also be able to use the products of decomposition of basic energetic substrates.

Keywords: laser scanning, Bacterial sulfate-reduction, Acid mine drainage, Sulfates, Metals sorption

Introduction

Sulfates (SO_4^{2-}) are one of the most commonly occurring anions in natural waters. Usually they are considered as a relatively harmless substance in the aquatic surroundings. But the sulfates concentration exceeds to 250 mg/L negatively affect the taste of water, concentration higher than 600 mg/L usually results in a laxative effect after usage that result to dehydration and high risk for infants. If level exceeding to 400 mg/ L, water is not used for baby usages (drinking, food, etc.) [1]. Next implications of high level of sulfate in waters present scaling in water pipes, which results in reduced diameter or blocked pipes. Besides elevated sulfate makes serious problem related of concrete pipelines bio corrosion [2, 3]. Contents of sulfates in waters increase especially by waste water discharge, which comes mainly from mining, metallurgical, chemical, paper and pulp industry, etc. In addition, sulfates are liberated to aquatic environment from natural sources through sulfur mineral dissolution, atmospheric deposition and sulfide oxidation from mineral, etc.

Mine waters origin during the exploitation, mainly after closing down the exploitation of mineral deposits running in the contact zones of water and geological environment [4]. The pH of mine water is determined by the quality and quantity of present minerals in the deposit and they may be in the range from 0 to 12. In the deposits with sulfides content occurs specific type of mine water, called acid mine water (AMD) with pH values <4.5. Their formation is related with the existence of autochthonous bacteria of the genus *Acidithiobacillus* [5]. The major components of AMD are sulfuric acid, metals in the form of sulfates and iron precipitates. The major focus on the treatment of AMD has been on acidity and dissolved metals, whereas minor regard has been paid to sulfates. But sulfates may be present in AMD at concentration ranging from few hundred to several thousand milligrams per liter. Nowadays concerns about sulfate discharge have increased and resulted in guidelines and regulations that limit the discharge into the aquatic ambient.

World-wide sulfates limit for surface water discharge range from 2,000 mg/L to 10 mg/L and generally vary between 250 and 1000 mg/L. Currently Government Regulation of the Slovak Republic No. 269/2010 [6] includes general requirements for water quality, where the recommended concentration of sulfates is 250 mg/L for surface waters, 150 mg/L (recommended value) or 250 mg/L (marginal value) for waters intended for drinking water abstraction and 250 mg/L for irrigation waters.

Chemical treatment, membrane technologies, ion exchange techniques and biological removal belong to suitable treatment processes for the removal of sulfate from mining effluents [1]. The biological removal of sulfates from AMD is possible to realize by way of anaerobic reduction to sulfide using sulfate-reducing bacteria (SRB).

In generally SRB are prokaryotic microorganisms, both bacteria and archaea, which can use sulfate as the terminal electron acceptor in their energy metabolism. The principle of the SRB metabolisms is the sulfates reduction to hydrogen sulfide while oxidizing the inorganic or organic substrates [7]. Based on this feature of the SRB, there is a possibility the possibility of using SRB to remove sulfates from mine water. Bacterial sulfate-reduction gives out in the simultaneous sulfates and heavy metal elimination. As well bacterially produced hydrogen sulfide can be used for the preparation of metal sulfides from mine waters or other industrial waste water by the selective sequential precipitation [8, 9] as well as the elementary sulfur preparation by chemically or bacterially oxidation to sulfur [10].

This paper presents the results of the sulfates biological removal from real AMD at laboratory condition. AMD without of the treatment i.e. with naturally contents metals and sulfates and the treated AMD by the metals sorption using the synthetic sorbent Slovakite were used. For the SRB growth the sodium lactate as organic substrates were used.

Materials And Methods

Acid mine drainage

The AMD was sampled in the shaft Pech, which receives waters draining the abandoned Smolnik sulfide deposit (Slovak Republic). In the experiments the untreated AMD (marking of samples AMD-UT) and the treated AMD (marking of samples AMD-T) were used. The base of the AMD treatment were three steps: 1 - the iron removing by oxidation with 31% H_2O_2; 2 - subsequent precipitation with 0.1 M NaOH; 3 - the metals removing by sorption using inorganic composite sorbent Slovakite [11]. The majority dissolved metals concentration, content of sulfates and pH values of studied AMD samples are presented in the Table 1.

Tab. 1. Parameters of studied AMD

	pH	Fe_{total}	Al	Mn	Zn	SO_4^{2-}
		mg/L				
AMD-UT	3.98	260	50	23	7.9	2348
AMD-T	6,74	0,02	1,2	9,4	0,2	1420

Bacterial cultures

In the experiments culture of SRB genera *Desulfovibrio* has been used, isolated from the mineral water Gajdovka (Kosice – North, Slovak Republic). For isolation and cultivation, the selective culture media DSM-63 was used at anaerobic conditions and temperature 30° C. The sodium lactate was used as the energetic substrate for the growth of bacteria. The composition of culture media creates optimal conditions for the growth of SRB genera *Desulfovibrio* [12].

Analytical Methods

Concentration of sulfates during studied process were analyzed by ion chromatography Dionex ICS 5000 (Sunnyvale, CA, USA), equipped with an IonPac AS11-HC anion column and suppressed conductivity detector. pH values were determined by pH meter inoLab ph 730 (WTW, Germany).

Removal of Sulfates

Experiments were realized in the reagent bottles under anaerobic conditions at 30 °C for 24 days. The samples of liquid phase were collected to monitor the concentration of sulfates and pH values. Standard selective nutrient DSM-63 and modified selective nutrient DSM-63 media - without sulfates contents and standard, double and triple amount of sodium lactate (as energetic substrate) were used. The untreated (AMD-UT) and treated acid mine drainage (AMD-T) served as a source of sulfates for the sulfate bacterially reduction. For control the abiotic samples (without the application of SRB) were prepared at identical conditions. Composition of biotic and abiotic samples in the process of the biological sulfates removal describers Table 2.

Tab. 2. Composition of S/AMD and P/AMD studied samples

Sample	Amount of Sodium Lactate	AMD	DSM-63 (with sulfates)	DSM-63 (without sulfates)	Inoculum of SRB
		ml			
AMD-UT-S-1	standard	100	80	-	20
AMD-UT-S-1-C		100	100	-	-
AMD-UT-1		100	-	80	20
AMD-UT-1-C		100	-	100	-
AMD-T-S-1	standard	100	80	-	20
AMD-T-S-1-C		100	100	-	-
AMD-T-1		100	-	80	20
AMD-T-1-C		100	-	100	-
AMD-T-2	double	100	-	80	20
AMD-T-2-C		100	-	100	0
AMD-T-3	triple	100	-	80	20
AMD-T-3-C		100	-	100	0

UT- untreated, T – treated, S – with sulfates, C- abiotic control

Results and Discussion

Successful process of the bacterial sulfates reduction it was possible to confirm on the basis of monitored parameters:

- the formation of the black precipitates FeS (Fig. 1) according of equation (1); Fe^{2+} is a basic constituent of the modified medium DSM-63 [7]:

$$Fe^{2+} + H_2S \rightarrow FeS + 2H^+ \quad (1)$$

- the formation of hydrogen sulfide according the orientation test (equation (2) when the hydrogen sulfide reacts with Cu^{2+} under acidic conditions (Fig. 2). The intensive brown coloring is proportional to the hydrogen sulfide volume [13]. Cu^{2+} is a basic constituent of the test chemical reagent:

$$Cu^{2+} + H_2S \rightarrow CuS + 2H^+ \quad (2)$$

- the decrease of sulfate concentrations (Figures 3 – 6). None or the minimal decreasing of concentration of sulfates in abiotic controls was observed.

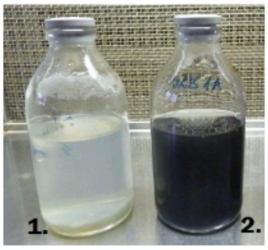

Fig. 1. Formation of black precipitates FeS (1 – abioic control, 2 – at presence of SRB)

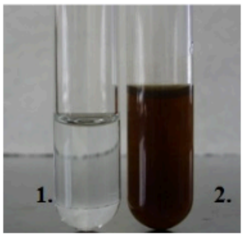

Fig. 2. Orientation test of presence of hydrogen sulfide (1 – abioic control, 2 – at presence of SRB)

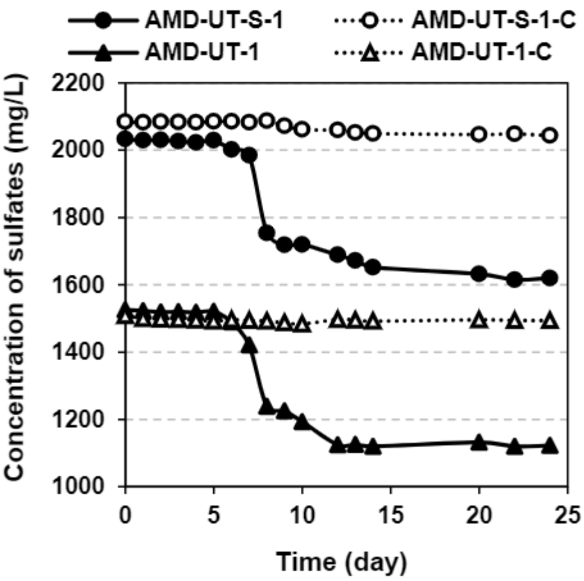

Fig. 3. Decrease of sulfate concentrations using untreated AMD and DSM-63 medium with or without SO_4^{2-}

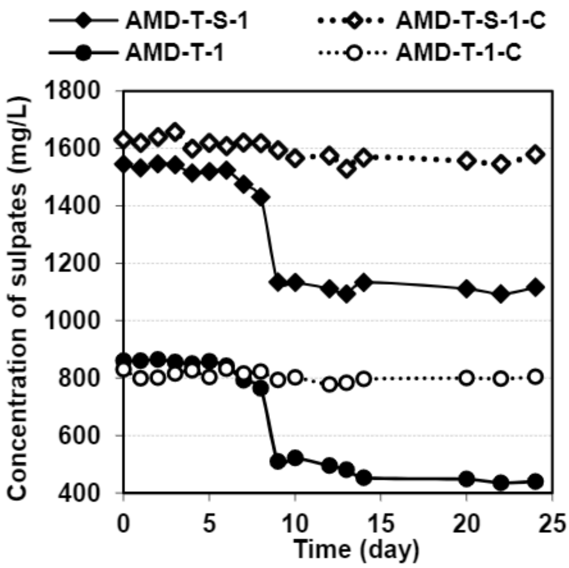

Fig. 4. Decrease of sulfate concentrations using treated AMD and DSM-63 medium with or without SO_4^{2-}

At untreated AMD has been reached low removing of sulfates - 20% efficiency (standard medium) and 26% (modified medium) – Fig. 3 and 6. The formation of heterogeneous precipitates containing metals (namely iron ions) pointed to the need for treatment of AMD by the initial removal of metals by sorption on Slovakite sorbent and subsequent removal of sulfates by bacterial reduction. For the reason before the sorption experiments AMD was pretreated due to high concentration of iron cations. Concentration of these cations is several times higher than the others (Table 1.) and could distort the sorption. From this reason, iron cations were removed by oxidation using 31% H_2O_2 and subsequent precipitation with 0.1 M NaOH. It was determined that Fe^{3+} ions form iron (III) hydroxyl-sulfates in presence of sulfuric acid in water environment according to equation (3):

$$Fe^{3+} + OH^- + SO_4^{2-} \rightarrow Fe(OH)SO_4 \quad (3)$$

In this stage about 99.9% of iron was eliminated from AMD samples. But iron cations form Fe(OH)SO4 and from this reason it was possible to remove also part of sulfates equivalent to the amount of iron cations. The removal efficiency for sulfates was above 40 %. After filtration of iron precipitates, the filtrate for removal of other metals by sorption on Slovakite sorbent was used. Filtrate after sorption experiments represented treated AMD (AMD-T), which for the bacterial sulfate reduction was used.

The results of experiments with AMD-T in this way showed 49%, 70% and 88% efficiency of sulfate removal when using sodium lactate in standard, double and triple amounts (Fig. 5). The kinetic of bacterial sulfate reduction depends on the several factors. The ratio of substrate quantity and sulfate concentration in the feed is an important factor related to electron flow in anaerobic metabolism [14]. Experimental data showed that the higher substrate quantities led to higher efficiency of the sulfates removal. Fig. 5 documented the satisfactory reduction of sulfates meeting the limit for surface waters established by Slovak legislation of 250 mg/L only in the case of sample AMD-T-3. Total elimination of sulfates was not achieved. **Probably** bacterial sulfate reduction was stopped at the influence of suitable organic substrate absence.

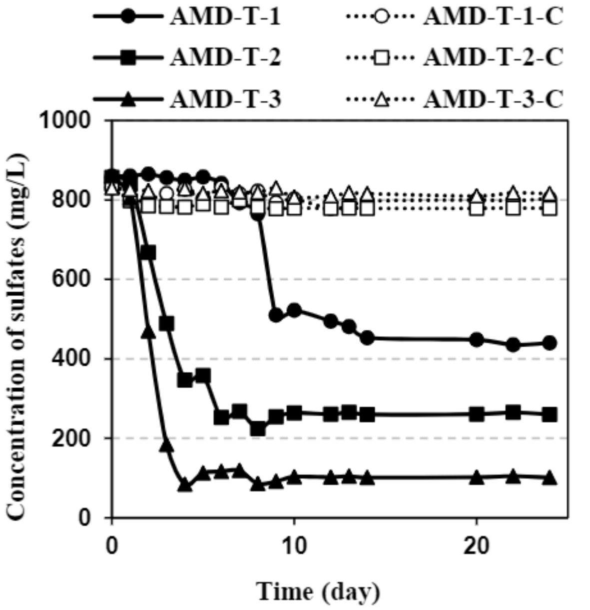

Fig. 5. Decrease of sulfate concentrations using treated AMD and DSM-63 medium without SO_4^{2-} contents and standard (1), double (2) and triple (3) amount of sodium lactate (as energetic substrate)

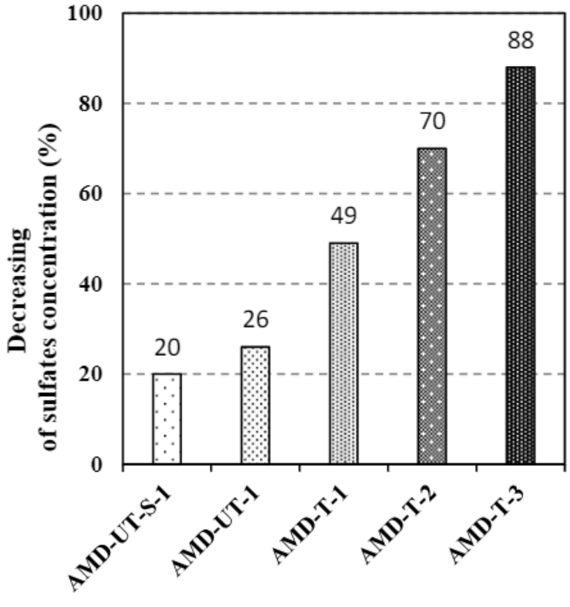

Fig. 6. Efficiency of bacterial sulfates reduction by SRB using untreated and treated AMD at different amount of substrates.

Conclusions

Achieved results demonstrated the possibility of a biological treatment of acid mine drainage with high concentration of sulfates. At untreated AMD i.e. with naturally contents metals and sulfates has been reached low removing of sulfates, only 20% efficiency using standard medium DSM-63 and 26% using modified medium i.e. without sulfates. A higher sulfate removal efficiency was achieved by pretreatment of the studied AMD. This pretreatment was carried out in three steps: 1- oxidation of Fe^{2+} as the majority metal to Fe^{3+} using H_2O_2; 2 - precipitation of Fe^{3+} with 0.1 M NaOH; 3- the other metals removing by sorption using inorganic composite sorbent Slovakite. Experiments with AMD treated in this way pointed to removing of sulfates by dissimilatory sulfate reduction has been reached with 49%, 70% and 88% efficiency in depending on amount of used organic substrate – sodium lactate at using modified DSM-63 medium i.e. without sulfates. Only in the case of the using of sodium lactate in triple amounts was achieved sulfate concentration below the limit value in view of Government Regulation no. 269/2010. The results of this work suggest that ratio of substrate quantity and sulfate concentration is one of the key parameter of sulfate reducing condition. However, the price of the energy substrate is also an important factor. Therefore, subsequent experiments will be focused on the use of the more affordable substrates (e.g. whey) or mixed bacterial culture of SRB, which will also be able to use the products of decomposition of basic energetic substrates.

Acknowledgments

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