

## THE EFFECT OF Fe(III) ON THE RATE OF MICROBIAL SULFATE-REDUCTION

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**ABSTRACT:** The effect of trivalent iron (Fe(OH)<sub>3</sub>) on microbial dissimilatory sulfate-reduction was studied in batch conditions with use of three different donors of electrons – Na-lactate, Na-acetate and solid organic matter (cow manure, spent mushroom compost and sawdust). The inoculum (mixed culture of sulfate-reducing and other metabolically related groups of microorganisms) was received from anaerobic cell for treatment of acid mine drainage. It was found maximal rate of the process sulfate reduction (147 mg SO<sub>4</sub><sup>2-</sup>/l.d) when lactate was used as sole source of carbon and energy. The Fe(III) reduction was prevalent process with acetate as donor of electrons (the rate of sulfate-reduction was only 13 mg SO<sub>4</sub><sup>2-</sup>/l.d). It was found that in the conditions, typical for anaerobic passive treatment, the rate of reduction of trivalent iron also is higher than the rate of sulfate-reduction. A part from reduced iron precipitated in the form of FeS. The concentrations of soluble Fe<sup>2+</sup> when using solid organic matter increased to rates above 1g/l after two months of the start of the experiment.

**KEY WORDS:** sulfate-reduction, Fe(III) reduction, mine waters

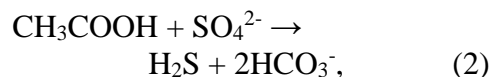
### 1. INTRODUCTION

Acidic sulfur-rich wastewaters causes environmental pollution and risk to the environment because they contain sulfur acid, high concentrations of metals (Fe, Mn, Cu, Zn, Al) and metalloids (mainly As). The major producer of acid mine drainage is the mining industry [1]. Conventionally, hydroxide precipitation is the most commonly applied method for the treatment of metal containing waters. In recent years, the process dissimilatory sulfate-reduction has been proposed as an alternative to hydroxide precipitation [2]. Sulfate-reducing bacteria oxidize simple organic compounds (lactate, acetate, butirate, and other products of fermentations) under anaerobic conditions with sulfate as acceptor of electrons. The SRB can be subdivided into two groups depending on their oxidative capability:

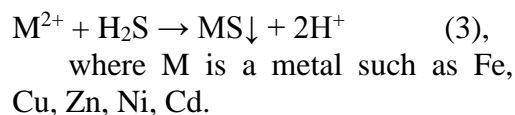
- The first group includes SRB that oxidize the organic compound incompletely usually with acetate as an end product:



- The second group covers the genera that completely oxidize the organic substrate to CO<sub>2</sub>:



The hydrogen sulfide reacts with the metals in AMD to form insoluble metal sulfides:



The  $\text{HCO}_3^-$  ions increase pH and alkalinity of the water. Due to these advantages, the process finds different applications for mine water treatment by active systems and passive systems. At first case the microbial sulfate reduction is carried out in anaerobic bioreactors (upflow anaerobic sludge bed reactor, the fluidized bed reactor and the anaerobic filter) with use of different types of soluble organic electron donor (ethanol, lactate and their mixtures).

At second case the process is realized in passive systems. Different types of passive systems with bacterial sulfate-reduction are used for mine water treatment: anaerobic, compost wetlands with significant surface flow; mixed compost/limestone systems, with predominantly subsurface flow (so-called Reducing and Alkalinity Producing Systems (RAPS)) and subsurface reactive barriers to treat acidic, metalliferous ground waters. But bacterial sulfate reduction is limited to certain environmental conditions. The bacteria require the presence of sulfate, suitable concentrations of low-molecular weight carbon compounds and absence of oxidizing agents such as  $\text{O}_2$ , Fe(III) and Mn(VI) [3].

Lovley (1991) reported that sulfate reduction is generally inhibited in sediments in which organic matter oxidation is being coupled to Fe(III) reduction [4]. The available evidence suggests that the inhibition of sulfate reduction can be attributed primarily to Fe(III)-reducing microorganisms maintaining the concentration of electron donors at level too low for sulfate reducers to metabolize them. The reduction of the trivalent iron is also as a result of enzymatic reduction of ferric-reduced bacteria and the chemical reduction of organic compounds and microbial produced hydrogen sulfide (Rioux and Fortin, 2004).

García-Balboa et al., 2010 were studied the effect of sulfate reduction on

Fe(III) reduction [5]. The results obtained show that both processes took place in a successive way and none synergetic effect was detected. The authors suggest that ferric reduction is a preferential metabolic process over sulfate reduction when both electron acceptors coexist.

In this work the effect of Fe(III) on sulfate reduction was studied with use of two different soluble donors of electrons (lactate and acetate) and the main goal was to clarify the influence of the ferric ions on two different groups of sulfate-reducing bacteria. Also both microbial processes were realized in a model of anaerobic cell, filled with solid organic matter.

## 2. MATERIALS AND METHODS

Batch experiments were carried out in 300 ml glasses bottles containing organic substrates and nutrient solution. Simple organic compounds (Na-lactate and Na-acetate) or solid organic matter (cow manure, spent mushroom compost and sawdust) were used as organic substrates. The nutrient solution used in these tests contained (in g/l):  $\text{Na}_2\text{SO}_4$  - 3.0;  $\text{KH}_2\text{PO}_4$  - 0.2;  $(\text{NH}_4)_2\text{SO}_4$  - 0.5;  $\text{KCl}$  - 0.5;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  - 0.15;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 1.0; yeast extract - 0.1. Trivalent iron is imported in the form of suspension  $\text{Fe}(\text{OH})_3$ . The concentrations of sulfates and iron are respectively 2.5 and 3.0 g/l. In two variants Na-lactate and Na-acetate – 8 g/l each were used separately as source of carbon and energy for bacteria. In the third bottle solid organic matter (100 g dry weigh) was added to nutrient solution. The bottles were inoculated with mixed enrichment cultures of ferric, sulphate-reducing bacteria and other metabolically related groups of microorganisms. The inoculum was received from laboratory anaerobic cell for treatment of acid water, contained Fe – 200 mg/l, Zn – 50 mg/l and Cu – 20 mg/l. The bacteria were incubated at 30 °C.

### Analytical techniques

The pH was measured using pH electrode (VWR) and pH meter HANNA HI 9021. The Eh was measured using Electrode Sen Tix ORP (WTW). The sulphate concentration was determined using spectrophotometric method by BaCl<sub>2</sub> as reagent at a wavelength of light of 420 nm. The concentration of Fe(II) was measured by sulfosalicylic acid method.

The identification and enumeration of the microorganisms inhabiting the bottles were carried out by standard microbiological methods.

### 3. RESULTS AND DISCUSSION

It was found that the Fe(III) has the least impact on microbial sulfate-reduction when lactate was used as a sole source of

carbon and energy (Figure 1). The rate of sulfate-reduction in these conditions was 147 mg SO<sub>4</sub><sup>2-</sup>/l.d. This result as well as microbiological analyses (Table 1) showed that independently of competition between Fe(III)-reducing and SRB for electron donors, the sulfate-reducing bacteria, oxidizing incompletely organic compounds are able to outcompete bacteria which reduce trivalent iron. In this variant Fe(II) was established on the 20-th day after beginning of the experiment. In the previous days the ferrous iron wasn't detected, because iron precipitated in the form of FeS by microbial hydrogen sulfide. The concentration of Fe(II) increases in time after full reduction of sulfate, and reaches to 0.56 g/l in the end of the experiment.

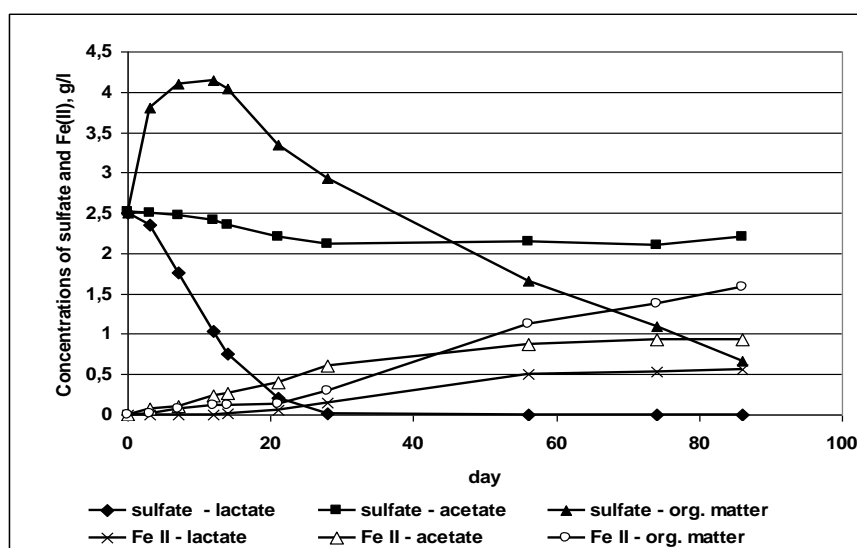


Figure 1. Dynamic of the concentrations of sulfate and Fe(II) at concomitant carried out of sulfate and ferric reduction.

When acetate was used as sole donor of electrons, the rate of sulfate reduction was only 13 mg SO<sub>4</sub><sup>2-</sup>/l.d. Divalent iron was found at the beginning of the experiment, and the concentration of ferrous ions after three months was 0.93 g/l. The data shows that in these conditions Fe(III) reducing bacteria compete with populations of SRB, oxidizing completely the organic substrate to CO<sub>2</sub>. Some authors

reported that the acetate is one of preferring donors of electrons for ferric-reducing bacteria [4]. Rioux and Fortin, 2004 studied the mechanism for Fe(III) reduction in mine tailings [6]. According to the authors SRB growth did not appear to be a function of the type of electron donors in the systems, but the presence of both sulfate reducing bacteria and Fe(III)-reducing bacteria limited the growth of

iron-reducing bacteria in the acetate systems. The same authors suggested that Fe(III)-reducing bacteria cannot compete with SRB when acetate is the only electron donor present. Also Li et al. (2006) suggested that sulfate-reducing bacteria play important roles in direct iron reduction in sulfidic sediments, such as the marine sediments and sulfate-type lakes [7]. In our study the results showed that Fe(III) bacteria are able to outcompete SRB, oxidizing completely the organic substrate to CO<sub>2</sub>.

Barlett et al. (2012) were studied the interaction of growth of ferric-reducing bacteria *Geobacter* and SRB when acetate is added to subsurface sediments to promote in situ uranium reduction [8]. It is apparent from both the experimental and modeling approaches that the acetate-oxidizing *Geobacter* and SRB have little impact on each other as long as acetate is maintained in excess. Probably under different environmental conditions and different composition of microbial consortium, at the same donors of electrons dominant microbial process can be either a sulfate-reduction, or ferric-reduction.

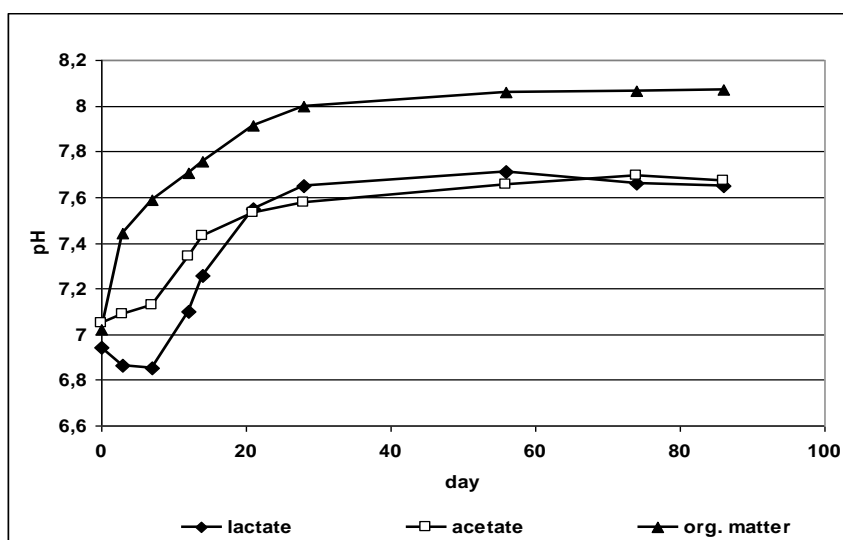


Figure 2. Dynamic of pH at concomitant carried out of sulfate and ferric reduction.

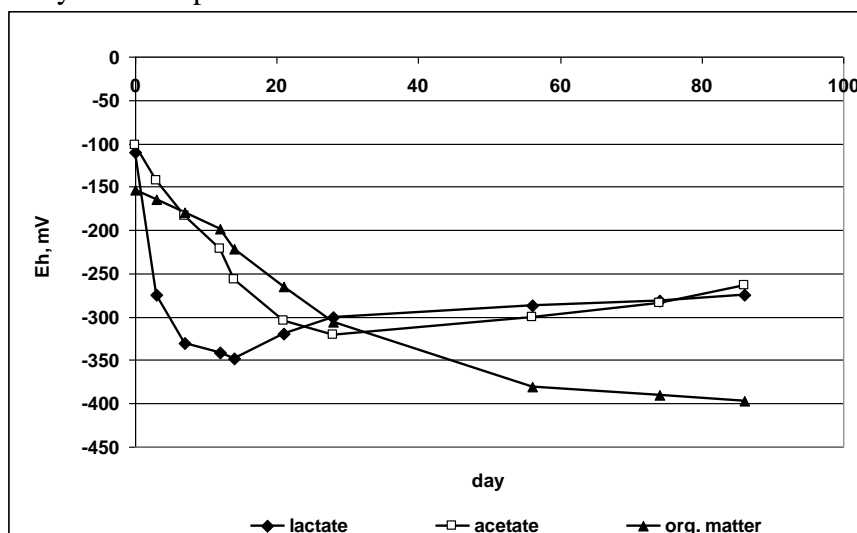


Figure 3. Dynamic of Eh, mV at concomitant carried out of sulfate and ferric reduction.

In the variant with the solid mixed organic concentration of sulfate increases to 4.15 g/l due to dissolution of the gypsum in spent mushroom compost. The concentration of sulfate was decreased after the second week, and the maximal rate of the process 99 mg SO<sub>4</sub><sup>2-</sup>/l.d was established between 14 – 21 days. But dynamic of concentration of ferrous ion shows, that the rate of ferric-reduction is higher than sulfate-reduction. Even in the period with maximal rate of reduction of sulfates, the concentration of Fe(II) was in the interval 20-140 mg/l due to the insufficient amount of microbial hydrogen sulfide. The concentration of ferrous ions in this variant was 1.58 g/l in the end of experiment. The higher concentrations of iron in this variant are due to enough amount of available sources of carbon and energy, received by the hydrolysis of the

biopolymers in solid organic matter. Even in the end of experiment, concentration of organic carbon was over 2500 mg/l, while in the variants with lactate and acetate, the organic carbon were respectively 45 and 54 mg/l. All the microorganisms were limited to source of donors of electrons and the rates of the both processes greatly decreased.

The pH in all variants increased during concomitant running of both processes (Figure 2). The most marked increase in pH (pH above 8.0) was detected when solid organic matter was used as source of carbon and energy. In the variants with lactate and acetate, pH increased to level around 7.6. The slight decrease of pH in the variant with lactate at the first days is due to fermentations of lactic acid.

**Table 1.** Dynamic of the number of different groups of microorganisms

Donor of electrons	day					
	1	7	20	38	55	70
<b>lactate</b>	<b>cells/ml</b>					
Aerobic heterotrophic bacteria	10 <sup>3</sup>	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>
Anaerobic heterotrophic bacteria	10 <sup>3</sup>	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>
Bacteria fermenting sugars with gas production	10 <sup>3</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>4</sup>
Sulphate-reducing bacteria, using lactate	10 <sup>2</sup>	10 <sup>5</sup>	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>6</sup>
Sulphate-reducing bacteria, using acetate	10 <sup>2</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>
Fe <sup>3+</sup> -reducing bacteria	10 <sup>1</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>5</sup>
Cellulose-degrading microorganisms	10 <sup>1</sup>	10 <sup>1</sup>	10 <sup>1</sup>	<10 <sup>1</sup>	<10 <sup>1</sup>	<10 <sup>1</sup>
<b>acetate</b>						
Aerobic heterotrophic bacteria	10 <sup>3</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>4</sup>
Anaerobic heterotrophic bacteria	10 <sup>3</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>
Bacteria fermenting sugars with gas production	10 <sup>3</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>3</sup>
Sulphate-reducing bacteria, using lactate	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>3</sup>	10 <sup>2</sup>
Sulphate-reducing bacteria, using acetate	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>3</sup>
Fe <sup>3+</sup> -reducing bacteria	10 <sup>1</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>4</sup>
Cellulose-degrading microorganisms	10 <sup>1</sup>	10 <sup>1</sup>	10 <sup>1</sup>	<10 <sup>1</sup>	<10 <sup>1</sup>	<10 <sup>1</sup>
<b>Solid organic matter</b>						
Aerobic heterotrophic bacteria	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>5</sup>
Anaerobic heterotrophic bacteria	10 <sup>5</sup>	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>5</sup>
Bacteria fermenting sugars with gas production	10 <sup>3</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>3</sup>
Sulphate-reducing bacteria, using lactate	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>
Sulphate-reducing bacteria, using acetate	10 <sup>2</sup>	10 <sup>4</sup>	10 <sup>4</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>4</sup>
Fe <sup>3+</sup> -reducing bacteria	10 <sup>5</sup>	10 <sup>7</sup>	10 <sup>7</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>4</sup>
Cellulose-degrading microorganisms	10 <sup>3</sup>	10 <sup>5</sup>	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>	10 <sup>3</sup>

Data about Eh (Table 3) showed that most reducing conditions at first days were created in the variant with lactate. In the end of experiment lowest values of Eh (-390 mV) are established when solid organic matter was used as a source of donor of electrons. Due to the depletion of lactate and acetate around 20<sup>th</sup> day, the redox potential begins to increase, keeping negative values in the range - 250 to - 300 mV. Data about number of different microorganisms shows presence of a rich microbial community. The number of SRB and Fe<sup>3+</sup>- reducing bacteria cultivated with lactate was highest between 7 and 38 days (10<sup>6</sup> - 10<sup>7</sup> cells/ml). It was found that in presence of acetate the number of Fe<sup>3+</sup>-reducing bacteria was an order of magnitude higher than number of SRB.

This correlates with established lowest rate of microbial sulphate reduction in the use of acetate as a sole source of carbon and energy. The microbiological analysis shows the largest amount of population of all the bacteria in variant with mixed organic matter. Due to high concentration of dissolved organic substances the number of Fe<sup>3+</sup>-reducing bacteria was in a range 10<sup>6</sup> – 10<sup>7</sup> cells/ml.

#### 4. CONCLUSIONS

The type of the source of carbon and energy has a great influence on the rate of the sulphate and ferric reduction, in the conditions of concomitant presence of both acceptors of electrons. The microbial composition of inoculum has an important role to determine which process will dominate with the use of the same electron donor. The mixed culture, isolated from anaerobic cell for mine water treatment consisted of SRB, which fully outcompete the Fe<sup>3+</sup>-reducing bacteria when lactate was used as source of carbon and energy. At the conditions, typical for passive treatment of acid mine water, the increase of the rate of sulfate reduction will be possible if the growth of SRB, oxidizing

incompletely organic compounds are stimulated by adding of wastewater, containing suitable for them donors of electrons.

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