Introduction

The sulphate rich waste waters are generated by a large spectrum of industrial processes including the paper and pulp production, sea food processing, potato starch production, tanneries, fermentation, fertilizers, ore processing and mining. Declining coal and metal mining industries have caused the problem of acid mine drainage (AMD) – the highly acidic, dissolved metals and sulphates containing waste waters. In the treatment of AMD a little attention has focused on the mitigation of dissolved sulphates. This may be attributed to its lower environmental risks – sulphates emissions are not a direct threat for the environment as sulphates are chemically inert, non volatile, non toxic and non bioaccumulative compounds. In general, organisms have a relatively high tolerance for sulphates. The consumption of the drinking water containing sulphates concentration in excess of 600 mg l\(^{-1}\) commonly results in laxative effects. The taste threshold for the most prevalent sulphates salts ranges from 250 to 500 mg l\(^{-1}\) (Davies et al., 2003; INAP, 2003). In term of the protection of aquatic life, sulphates toxicity is dependent on chloride and hardness concentrations of the water and it is different case by case (Davies et al., 2003; IDNR, 2009). However, the discharge of the sulphate-rich industrial waste water into the surface waters contributes to the increase of the corrosion potential of receiving waters and augments the total dissolved solid contents causing a problem with the cooling in the industries. Moreover, high sulphates concentration induces an unbalance in the natural sulphur cycle (Sarti et al., 2009).

Sulphates may be present in the waste waters at concentrations ranging from a few hundred to several thousand milligrams per liter and they should not be discharged into water bodies without sulphates removal.

The current Government Regulation No. 296/2005 of the Slovak body of laws includes general requirements for the surface water quality in the Slovak Republic, where the recommended sulphates concentration is 250 mg l\(^{-1}\).

Various treatment techniques of the mine waters have been developed during the recent time. The selection criteria for each of the processes are based on a number of technical, economical, environmental and legislative factors. The treatment processes removing sulphates include these categories: chemical treatment with the mineral precipitation, membrane technologies, ion exchange technologies and biological technologies (INAP, 2003).

The biological removal of sulphates from the waste waters can be realized by the way of anaerobical reduction to sulphide using sulphate-reducing bacteria (SRB). SRB are a heterogeneous group of microbes, which use sulphate as terminal electron acceptor. They use simple inorganic and organic compounds like hydrogen, lactate and acetate as electron donors (growth substrates) and reduce the sulphate to hydrogen sulphide as the end product by dissimilatory sulphate reduction pathway (Postgate, 1984).

Materials and analytical methods

Acid mine drainage

The investigation has been carried out at a laboratory scale, utilizing AMD discharged from the shaft Pech located at Smolník (abandoned and flooded Slovak sulphidic deposit), containing 2 150 mg l sulphates. Before the use, many metals (Fe, Al, Mn, Zn and Cu) have been eliminated from AMD by selective sequential precipitation. These metals were removed by the precipitation in the
form of hydroxides using sodium hydroxide as reagent and sulphides using bacterially produced hydrogen sulphide as reagent at the various values of pH (Mačingová et al., 2009).

Microorganisms

In the experiment the culture of the sulphate-reducing bacteria (genera Desulfovibrio) has been used, isolated from a mixed culture of SRB obtained from the mineral water Gajdovka (Košice, Slovakia). For their isolation and cultivation the selective nutrient Postgate´s medium C has been used (Postgate, 1984) at 30 °C at anaerobic conditions.

Analytical procedures

The nephelometric method was used to determine the concentration of sulphates at 490 nm using the Spectromom 195 spectrophotometer. The pH of the cultivation media was determined by the potentiometric method using the PHM 210 MeterLab pH meter.

Experimental procedure

Batch studies on sulphates removal from the pre-treated AMD using SRB were carried out using four reactors containing 250 ml modified nutrient Postgate’s medium C. The modification in each case was removing the sulphate containing salts from the prescribed media and adding 100 ml of pre-treated AMD containing the soluble sulphate. Here was also used the different growth substrates: sodium lactate according to prescribed media (sample marked La-Na I.), double volume of sodium lactate (sample marked La-Na II.), calcium lactate according to prescribed media (sample marked La-Ca I.) and double volume of calcium lactate (sample marked La-Ca II.). The pH value of nutrient medium was adjusted at 7.5 using 5 M sodium hydroxide. Each of the reactors was inoculated with 10 % of a 3-day SRB culture. The abiotic controls with substrates sodium lactate and calcium lactate respectively have been prepared (samples marked La-Na AC and La-Ca AC). The rest of the conditions for the growth were kept the same as that of the prescribed media.

The regular samples were taken every day over a week to monitor levels of sulphates.

Results and discussion

The aim of our work was the experimental verification of the possibilities of the sulphate rich waste waters utilization as the source of sulphate for the cultivation of SRB. During our study we obtained the evidence for the bacterial sulphate reduction to hydrogen sulphide:

(i) by formation of a black precipitate of FeS when sulphide is formed (for diagnostic purposes media contain a ferrous salt),

(ii) by decreasing of sulphates concentration in the nutrient mediums.

Figs. 1 and 2 show sulphate reduction kinetic when using substrates sodium lactate and calcium lactate respectively.

Results of our experiment demonstrate the high efficiency of the AMD treatment. Depend on the quality and quantity of used substrates there was reached 100 % (La-Na II.), 97 % (La-Ca II.), 61.39 % (La-Na I.) and 57.55 % (La-Ca I.) removing of sulphates by the dissimilatory sulphate reduction. In the abiotic controls the concentration of sulphates did not decrease. Fig. 3 shows the efficiency of sulphate elimination.

Technologies include SRB are promising for their ability to reduce soluble sulphates and produce high amount of hydrogen sulphide which has a great affinity to react with divalent metals to form of insoluble metal sulphides thus removing toxic metals from waste waters (Kaksonen et al., 2007). Sulphide precipitation of metals has been demonstrated to have several benefits over the usually used hydroxide precipitation, such as lower effluent metal concentrations, better thickening characteristics of the metal sludge and the possibility to recover valuable

![Fig. 1. Sulphate reduction kinetics using natrium lactate as substrate for cultivation of SRB.](image)
metals (Huisman et al., 2006). Obtained hydrogen sulphide may be also oxidized to elementary sulphur by way of chemical oxidation using ferric ions (Luptáková et al., 2002) or by Thiopaq gas purifying technology. This is a biotechnological process for removing $\text{H}_2\text{S}$ from gaseous streams by absorption into a mild alkaline solution followed by the oxidation of the absorbed sulphide to elementary sulphur by naturally occurring microorganisms – Thiobacillus bacteria (THIOPAQ, 2004).

**Conclusion**

The possibility of utilization of the sulphate reducing bacteria to remove the sulphates from the waste water was the aim of our research. The sulphate concentration in six reactors contained modified nutrient Postgate’s medium with the pre-treated AMD as a source of sulphates were monitored during one week. Four reactors were inoculated with SRB cultures and two reactors without inoculation served as an abiotic control. A quantitative elimination of the sulphate (100 and 97 %) has been achieved in our experiment. Results have demonstrated the technical feasibility of the use of sulphate-reducing bacteria to biological sulphate removal from pre-treated AMD.

**Acknowledgements.** This work was supported by the Slovak Research and Development Agency under the contract APVV-51-027705, the Slovak Grant Agency VEGA under the Project 2/0075/08 and by the Italian National Council Researches under CNR Project No. 6-1-132.48.2.

**References**


