



Removal of Contaminants from Water by Bacterial Activity

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Abstract

High concentration of sulphates and metals in waters is often as a consequence of anthropogenic activity and industry. The principles of the biological-chemical methods for pollution removal include various processes. The most widely metabolic pathway of sulphate-reducing bacteria - overall dissimilatory reduction - is the complete reduction of sulphate to hydrogen sulphide. Two major metabolic groups are known, depending on whether or not they can oxidize acetate. One group utilizes lactate, fumarate, propionate, butyrate, pyruvate, and aromatic compounds, which they typically oxidize to acetate, while the other group oxidizes acetate to CO₂ and H₂O. Sulphate is reduced to H₂S through a series of intermediate reactions. The end product of this reaction, hydrogen sulphide, can react with metal ions to form insoluble metal sulphides or reduce soluble toxic metals, often to less toxic or less soluble forms. This way, sulphate-reducing bacteria are utilizable in bio-elimination of sulphate and metal from water.

Keywords: pollution, sulphates, bacteria, reduction

Introduction

Wastewaters coming from various industries are often characterized by numerous pollutants. High levels of metals, sulphates and other salt constituents and low pH are common characteristics of wastewater produced in mining and metal processing (Lens and Pol, 2000). The concentration of sulphates often exceeds legislative limits. 250 mg/L is a value allowed to be discharged according to SR Government Regulation 269/2010. Therefore, there is a need to treat these waters before being released to the environment.

Many techniques exist to remove sulphates from water, but in many cases they are generally expensive and produce high sludge volumes. There is an increasing interest in the potential biotechnological applications of bacterial sulphate reduction as an alternative method for sulphate removal from environmental contamination.

Sulphate-reducing bacteria (SRB) are those prokaryotic microorganisms that can use sulphate as the terminal electron acceptor in their energy metabolism, i.e. that are capable of dissimilatory sulphate reduction. Most of the SRB belong to one of the four following phylogenetic lineages (with some examples of genera): (i) the mesophilic δ -proteobacteria with the genera *Desulfovibrio*, *Desulfobacterium*, *Desulfobacter*; (ii) the thermophilic Gram-negative bacteria with the genus *Thermodesulfovibrio*; (iii) the Gram-positive bacteria with the genus *Desulfotomaculum*; and (iv) the Euryarchaeota with the genus *Archaeoglobus* (Castro et al., 2000). They are considered to be chemoorganotrophic and strictly anaerobic bacteria with the ability to perform dissimilatory sulphate reduction with the simultaneous oxidation of the organic substrates (Postgate, 1984). Two major metabolic groups are known, depending on whether or not they can oxidize acetate (Widdel et al., 1993). Group of complete oxidizers (acetate oxidizers) has the ability to oxidize the organic compound to carbon dioxide, incomplete oxidizers (non-acetate oxidizers) carry out

the incomplete oxidation of the organic compound to acetate. Some species of the genera *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, *Desulfoarculus*, *Desulfomonile*, as well as *Desulfotomaculum acetoxidans* and *Desulfovibrio baarsii* belong to the group of complete oxidizers (Rabus et al., 2006). The incomplete oxidizers include *Desulfovibrio*, *Desulfomicrobium*, *Desulfobotulus*, *Desulfotomaculum*, *Desulfobacula*, *Archaeoglobus*, *Desulfobulbus* and *Thermodesulfovibrio* (Colleran et al., 1995). The growth kinetics for incomplete oxidizers is generally faster than the complete oxidizers. While the group of "complete oxidizers" oxidizes acetate to CO₂, the incomplete oxidizers utilize lactate, fumarate, propionate, butyrate, pyruvate, and aromatic compounds, which they typically oxidize to acetate (Cao et al., 2012; Teclu et al., 2009; Liamleam and Annachatre, 2007). Sulphate is reduced to H₂S through a series of intermediate reactions that include trithionate, thiosulphate and some organic sulphur compounds (Oluwaseun et al., 2009; Peck, 1993). The energy produced serves for growth and maintenance.

The aim of this work is to study the removal of sulphates from waters as a consequence of metabolic activity of bacteria and follow the associated processes and by-products.

In some cases, treatment by biological sulphate reduction is not pleasant because of odorous sulphide production. Nevertheless, it has been recognized as an efficient method for removing sulphate from wastewater and as a mean for treating a variety of sulphate-containing industrial effluents (Moosa et al., 2002). The main advantages of using SRB are minimal sludge production and removal of metals (Van den Brand et al., 2015). Biogenically produced hydrogen sulphide can react with dissolved metals to form metal sulphide precipitates since the solubility of most toxic metal sulphides is generally very low (Jong and Parry, 2006). Moreover, valuable metals from biologically precipitated metal sulphide can be recovered and recycled.

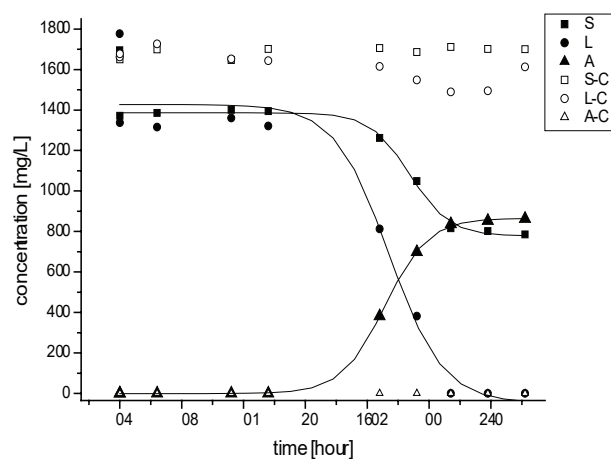


Fig. 1. Changes in nutrient medium Postgate's C influenced by bacterial sulphate reduction (S - sulphates, L - sodium lactate, A - acetate, S-C - sulphates in control sample, L-C - sodium lactate in control sample, A-C - acetate in control sample)

Rys. 1. Zmiany w pożywce C pod wpływem bakteryjnej redukcji siarczynu (S - siarczany, L - mleczan sodu, A - octan, S-C - siarczany w próbce kontrolnej, L-C - mleczan sodu w próbce kontrolnej, A-C - octan w próbce kontrolnej)

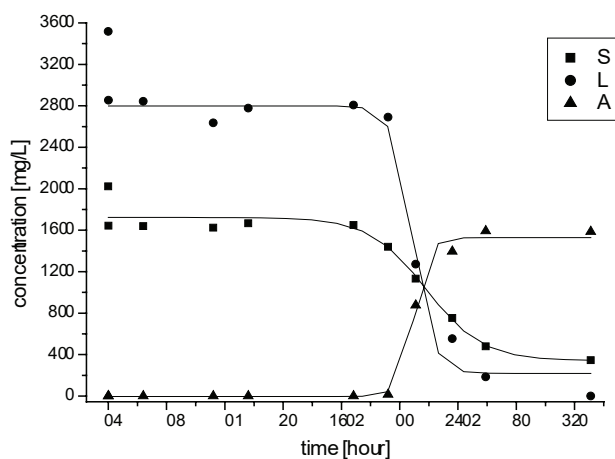


Fig. 2. Changes in modified nutrient medium Postgate's C influenced by bacterial sulphate reduction (S - sulphates, L - sodium lactate, A - acetate)

Rys. 2. Zmiany w zmodyfikowanej pożywce C pod wpływem bakteryjnej redukcji siarczynu (S - siarczany, L - mleczan sodu, A - octan)

Materials and methods

Nutrient medium

Selective nutrient medium for bacteria enrichment – Postgate's C has the following composition (per liter of distilled water): 0.5 g KH_2PO_4 , 1 g NH_4Cl , 4.5 g Na_2SO_4 , 0.2 g sodium acetate, 2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 1 g yeast extract, 0.1 g sodium thioglycollate, 0.1 g ascorbic acid, 0.5–1 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and resazurin (Postgate, 1984).

Growth and source of bacteria

The growth studies were performed in duplicate using a 20% inoculum. All cultures were stored in thermostat at temperature 30°C for 2–4 weeks.

Mixed culture of sulphate-reducing bacteria was obtained from mineral spring Gajdovka (Košice, Slovakia). This water is classified as potable, natural, mineralized water with pH 7–8 and strong H_2S odour.

Source of organic substrate and sulphates

Sodium lactate (60 % w/w) was used as a carbon source in an assay to determine the sulphidogenic metabolic potential. Experimental cultures were grown with different concentrations of sodium lactate (2 and 4 g/L).

Stock solution with sulphate concentration 2 g/L was prepared by dissolving K_2SO_4 (p.a. grade) in distilled water.

Experiments of sulphates removal

Elimination of sulphates from water was studied in 2 different experiments. First of them was carried out in 1L glass bottles using medium Postgate's C, with concentration of sodium lactate 2 and 4 g/L, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ dose 0.5 and 1 g/L and SRB inoculum 20%. The second study involved the usage of model solution instead of "classic" nutrient medium. 1L glass bottles were filled with 850 mL of model solution and 15% of SRB inoculum. Sodium lactate dose was 4 g/L. Trace elements according to Postgate's medium, sodium thioglycollate, ascorbic acid were added too. As a control for both experiments, same media were used, with lactate as carbon sources but without bacteria. The pH of solutions before experiments was adjusted at 7.5 ± 0.1 with 0.01 M NaOH and 0.01 M HCl.

The experiments took 14 days, they were realized at room temperature and sampling was carried out in selected time intervals in order to determine sulphates decrease and lactate utilization.

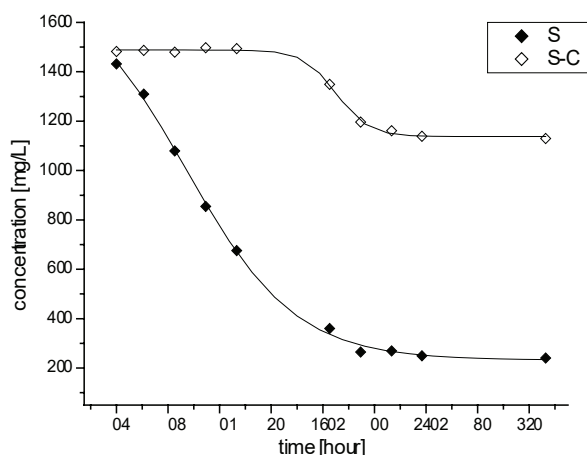


Fig. 3. Bacterial sulphate reduction in model solution (S – sulphates, S-C – sulphates in control sample)
Rys. 3. Bakteryjna redukcja siarczanu w roztworze modelowym (S – siarczany, S-C – siarczany w próbce kontrolnej)

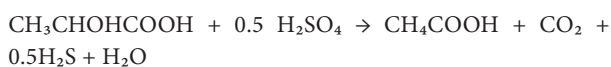
Methods

Growth of the SRB was monitored microscopically using an optical microscope Nikon Eclipse 400. Measurement of sulphate, lactate and acetate concentrations were made by the Dionex ICS-5000 Ion Chromatograph.

Results and discussion

The requirements for SRB development: a near-neutral pH, appropriate temperature, a reducing environment, a source of organic carbon, a source of sulphate and nutrients were fulfilled at the beginning of all experiments. In experimental cultures SRB occurrence was confirmed by light microscope, with predominance of genus *Desulfovibrio*. An attendant phenomenon was the formation of black precipitates at the bottom and the walls of the glass flasks during the first week after inoculation, which confirms expecting sulphate reduction and “FeS” formation. In addition, the smell of hydrogen sulphide was obvious.

Desulfovibrio belongs to the incomplete oxidizers and can use lactate as an electron donor and carbon source. Sulphate reduction using lactate can be described as follows:



Two moles of lactate are oxidized per mole of sulphate reduced by *D. desulfuricans*, and this stoichiometric ratio is not temperature dependent (Liamleam and Annachhatre, 2007).

Study of sulphates elimination caused by bacterial activity in nutrient medium without modification ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ dose 0.5 g/L) illustrates Figure 1. We can see that bacteria reduce sulphates in medium until the complete lactate consumption. After this time (about 10 days of experiment duration) the process was stopped. The amount of acetate production corresponds to the quantity of oxidized lactate. The reduction of sulphates was about 50%. Changes of sulphates, lactate and acetate concentration in abiotic controls are negligible. Samples without bacterial cultures were “inactive”.

On Figure 2 are experimental results from modified nutrient medium, i.e. sodium lactate dose was doubled in order to achieve more effective sulphate elimination. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ dose in this case was 1 g/L. Initial concentration of total sulphates in solution 2000 mg/L was lowered to 400 mg/L (80% reduction) when 4 g/L of sodium lactate was oxidized completely. This process took 14 days. For total sulphate reduction is necessary to use even higher lactate dosage and prolong the experiment duration, respectively.

The influence of SRB on the sulphate removal process in experiment with model solution was studied in the next step. The results verified by measuring of sulphate concentration decrease are shown on Figure 3. The initial concentration 1450 mg/L declined to the 240 mg/L within 14 days. This value meets the water quality requirements for the discharging into the recipient. Initial sodium lactate amount was 4 g/L. The concentration of sulphates in control sample without bacteria was reduced a little, probably because of some chemical reaction in solution. The measurements of sodium lactate and acetate concentration during experiment with model solution were not performed, they will be studied in the next stage.

Conclusion

The purpose of this work was to investigate sulphate-reducing bacteria utilization for the removal of high levels of sulphates from water. These results refer to the need of sufficient organic substrate amount for the growth of sulphate-reducing bacteria with respect to initial sulphate concentration. SRB eliminated about 84% of sulphates from model solution within 14 days and final concentration achieved a value allowed to be discharged – below 250 mg/L. Presented theoretical knowledge as well as our experimental results from sulphate elimination using SRB allows to note that their natural metabolic activity can be used in environmental technology for treatment of industrial waste water with excessive content of sulphates.

Acknowledgements

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Usuwanie zanieczyszczeń z wody za pomocą bakterii

Wysokie stężenie siarczanów i metali w wodach jest często konsekwencją działalności antropogenicznej i przemysłu. Zasady biologiczno-chemicznych metod usuwania zanieczyszczeń obejmują różne procesy. Najszerszym szlakiem metabolicznym bakterii redukujących siarczany – ogólna redukcja dyssymilacyjna – jest całkowita redukcja siarczanu do siarkowodoru. Znane są dwie główne grupy metaboliczne, w zależności od tego, czy mogą utleniać octan. Jedna grupa wykorzystuje mleczan, fumaran, propionian, kwas masłowy, pirogronian i związki aromatyczne, które zwykle utleniają do octanu, podczas gdy druga grupa utlenia octan do CO₂ i H₂O. Siarczan jest redukowany do H₂S poprzez szereg reakcji pośrednich. Produkt końcowy tej reakcji – siarkowodor – może reagować z jonami metali, tworząc nierozpuszczalne siarczki i metale lub redukować rozpuszczalne metale toksyczne, często do postaci mniej toksycznych lub mniej rozpuszczalnych. W ten sposób bakterie redukujące siarczany są wykorzystywane do bio-eliminacji siarczanu i metali z wody.

Słowa kluczowe: zanieczyszczenie, siarczany, bakterie, redukcja