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# Toxic effects of dissolved heavy metals on *Desulfovibrio vulgaris* and *Desulfovibrio* sp. strains

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**Abstract**

Biological treatment of metal-containing wastewaters with sulphate-reducing bacteria (SRB) is an attractive technique for the bioremediation of this kind of medium. In order to design a suitable engineering process to address this environmental problem, it is crucial to understand the inhibitory effect of dissolved heavy metals on these bacteria. Batch studies were carried out to evaluate the toxic effects of several heavy metal ions [Cr(III), Cu(II), Mn(II), Ni(II) and Zn(II)] on two cultures of SRB (*Desulfovibrio vulgaris* and *Desulfovibrio* sp.). The experimental data indicate that SRB show different responses to each metal. At the highest metal concentration tolerated for each metal, the precipitation levels for *D. vulgaris* were as follows: 24.7%-15 ppm Cr(III), 45%-4 ppm Cu(II), 60%-10 ppm Mn(II), 96%-8.5 ppm Ni(II) and 9%-20 ppm Zn(II). The corresponding values for *Desulfovibrio* sp. were: 25.5%-15 ppm Cr(III), 71%-4 ppm Cu(II), 66.2%-10 ppm Mn(II), 96.1%-8.5 ppm Ni(II) and 93%-20 ppm Zn(II). Results obtained in batch studies will be taken into account for the subsequent design of a sulphate-reducing bioreactor to reduce levels of heavy metals present in different types of contaminated media.


*Keywords:* Sulphate-reducing bacteria; *Desulfovibrio vulgaris*; *Desulfovibrio* sp.; Heavy metals; Tolerance; Bioprecipitation

## 1. Introduction

The presence of heavy metals in the environment represents a serious threat to the environment and human life. Current and past mining activity, as well as various industrial discharges, have contributed large quantities of acid wastewaters to the environment [1]. These waste streams usually contain high levels of sulphate and dissolved metals. The most widely used method to treat such effluent is chemical neutralization followed by the precipitation of metals. This method is expensive and generates large amounts of residual sludge. Biological treatment of these acidic and metal-containing wastewaters is an attractive alternative. The main advantage of these systems over chemical neutralization is that large volumes of sludge are not generated and the metal precipitates in the form of insoluble compounds such as oxides or sulphides. Among the biological treatment methods, the selective precipitation of metals with biologically produced $H_2S$ has been proposed as a possible process [2,3].

Sulphate-reducing bacteria (SRB) are heterotrophic microorganisms that require strictly anaerobic conditions and a redox potential of less than −200 mV. The main organic carbon/energy substrates utilized by the fastest growing organisms (*Desulfovibrio* species) are low molecular mass organic acids, such as lactic or acetic acid, and alcohols, such as ethanol [4,5]. The pattern of carbon dissimilation is essentially the same in all cases in that the organic substrate is oxidized either completely to $CO_2$ or to some intermediate compound [6]. Under anaerobic conditions, SRB carry out the oxidation of simple organic compounds by using sulphate as a terminal electron acceptor—the sulphate is reduced to sulphide. The generation of sulphide produces reducing conditions, removal of acidity and the precipitation of metals from solution as sulphides. This property makes these bacteria suitable for the removal of acidity and metals from contaminated effluents [7]. The method consists of two stages: (1) the production of $H_2S$ by SRB and (2) the precipitation of metals by the biologically produced $H_2S$, a reaction that produces insoluble metal sulphides that can be easily separated from a

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solution [2,7,8].

$$\underset{(e^- \text{ donor})}{\text{Organic matter}} + \underset{(e^- \text{ acceptor})}{SO_4^{2-}} \rightarrow 2\,CH_3COO^- + HS^- + HCO_3^- \quad (1)$$

$$Me^{2+} + HS^- \rightarrow MeS \downarrow + H^+ \quad (2)$$

Numerous heavy metals are toxic to microorganisms – including SRB – due to their capacity to deactivate enzymes by reacting with their functional groups, to denature proteins, and to compete with essential cations. The ability of this group of bacteria to immobilize heavy metals depends on the concentration of the metal in solution, which can cause a reduction in the metabolic activity or can even be toxic to the bacteria (causing death). This fact led to several studies that focused on determining the inhibitory effect of heavy metals on different cultures of sulphate-reducing bacteria with the aim of applying these microorganisms in metal reduction treatment processes [9,10]. The reported toxic concentrations of heavy metals to sulphate-reducing bacteria range from a few ppm (mg/L) to as much as 100 ppm [11]. Hao et al. [12] studied the toxic concentrations of several heavy metals for a mixed culture of sulphate-reducing bacteria: Zn (25–40 ppm), Pb (75–80 ppm), Cu (4–20 ppm), Cd (>4–20 ppm), Ni (10–20 ppm) and Cr (60 ppm). Utgikar et al. [11] reported that the effect of heavy metals on SRB can be stimulatory at lower concentrations and toxic/inhibitory at higher concentrations.

In recent years, several studies have evaluated the precipitation of heavy metals in real wastes (mine waste piles, acid mine drainage) by sulphate-reducing bacteria in batch and continuous systems. Kim et al. [7] described batch and column studies that were conducted to evaluate the feasibility of inoculating mine waste piles with SRB in order to neutralize the acidic supernatant and decrease the heavy metal levels. Batch incubation led to a decrease in the dissolved concentration of Cd, Cu, Ni and Zn in the supernatant to undetectable levels. Furthermore, continuous flow column experiments gave metal removal efficiencies greater than 99% for Cd, Cu and Zn and 87% for Ni. Foucher et al. [2] proposed a process that used SRB to treat acid mine drainage on the laboratory pilot scale. In this system, a fixed-bed bioreactor was used in conjunction with a gas-stripping column. Cu and Zn could be selectively recovered at pH 2.8 and 3.5, respectively. Ni and Fe could also be removed at pH 6.0 by sulphide precipitation. Sulphate reduction and metal precipitation (as a sulphide) are significant aspects of some successful large-scale processes for the biotechnological removal of metals. In some cases, this process is combined with a prior metal solubilization step [5]. Bioleaching using sulphuric acid, produced by sulphur-oxidizing bacteria, was followed by the precipitation of leachate metals by sulphate-reducing bacteria [13].

The purpose of the work described here was to study the tolerance of two cultures of sulphate-reducing bacteria (*Desulfovibrio vulgaris* and *Desulfovibrio* sp.) that occur in mining environments [14] to several heavy metal ions [Cr(III), Cu(II), Mn(II), Ni(II) and Zn(II)]. These metallic ions were selected due to they are present in a real contaminated effluent from the zone. The study was carried out by following bacterial growth and sulphate uptake. Moreover, the ability of SRB to precipitate these heavy metals in an artificially contaminated solution was evaluated by measuring the decrease in the dissolved metal concentration. This work involved an initial batch study, which forms part of a wider research programme focused on the application of this process in continuous mode to remove or reduce heavy metals present in real contaminated effluent that occurs in the industrial zone around Cadiz.

## 2. Materials and methods

### 2.1. SRB cultures

The bacterial strains used in this study were *D. vulgaris* (ATCC 29579) and *Desulfovibrio* sp. (ATCC 49975). These cultures were maintained in modified Postgate B medium (9 mL) (Table 1) in 10 mL sealed glass bottles. Medium was sterilised before pour it into the bottles at 121 °C during 20 min and allowed to cool down to room temperature. Ten percent (v/v) of inoculum was then added to the Postgate B medium. Bottles were sealed immediately in order to give the anaerobic conditions that are promoted by reducing compounds (ascorbic acid, thioglycolic acid). SRB cultures were incubated at 30 °C for 24 h. The formation of ferrous sulphide, which was detected as a black precipitate, indicate that bacterial growth had taken place and the bottles were then stored at 4 °C.

### 2.2. Medium and cultivation conditions

Experiments with heavy metals were carried out using the modified nutrient Postgate C medium (Table 1), which contains a high sulphate concentration. This medium does not contain Fe(II) to allow the evaluation of the precipitation of other metal under investigation.

The medium was adjusted to pH 7.5 ± 0.5 and was placed into 50 mL Pyrex glass bottles. These vessels were capped with crimped aluminium butyl rubber stoppers and sterilised in an autoclave [15]. The bottles were allowed to cool down to room temperature and they were spiked with metal solutions that had previously been sterilised by membrane filtration (pore size 0.22 μm). Chromium, copper, manganese, nickel and zinc sulphate standard solutions were used to obtain several concen-

Table 1
Composition of Postgate B and Postgate C media (g/L) for maintenance and metal experiments of sulphate-reducing bacteria

| g/L | Postgate B | Postgate C |
|---|---|---|
| $KH_2PO_4$ | 0.5 | 0.5 |
| $NH_4Cl$ | 1.0 | 1.0 |
| $Na_2SO_4$ | – | 4.5 |
| $CaSO_4{\cdot}2H_2O$ | 1.26 | – |
| $MgSO_4{\cdot}7H_2O$ | 2.0 | 0.06 |
| Sodium lactate | 3.5 | 6.0 |
| Yeast extract | 1.0 | 0.25 |
| Ascorbic acid | 0.1 | – |
| Thioglycolic acid | 0.1 | – |
| $FeSO_4{\cdot}7H_2O$ | 0.5 | – |