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# Characterization, morphology and composition of biofilm and precipitates from a sulphate-reducing fixed-bed reactor

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**Abstract**

The characteristics of the biofilm and the solids formed during the operation of a sulphate-reducing fixed-bed reactor, fed with a moderately acidic synthetic effluent containing zinc and iron, are presented. A diverse population of δ-*Proteobacteria* SRB, affiliated to four distinct genera, colonized the system. The morphology, mineralogy and surface chemistry of the precipitates were studied by X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray (EDX). The XRD patterns observed are characteristic of amorphous solid phases. Peaks corresponding to crystalline iron sulphide, marcasite, sphalerite and wurtzite were also identified. SEM–EDX results confirm the predominance of amorphous phases appearing as a cloudy haze. EDX spectra of spots on the surface of these amorphous phases reveal the predominance of iron, zinc and sulphur indicating the formation of iron and zinc sulphides. The predominance of these amorphous phases and the formation of very fine particles, during the operation of the SRB column, are in agreement and can be explained by the formation pathways of metal sulphides at ambient temperature, alkaline pH and reducing conditions. Solids are precipitated either as (i) amorphous phases deposited on the bed material, as well as on surface of crystals, e.g. $Mg_3(PO_4)_2$ and (ii) as rod-shaped solids characterized by a rough hazy surface, indicating the encapsulation of bacterial cells by amorphous metal sulphides.


*Keywords:* Sulphate-reducing bacteria; Fixed-bed reactor; Amorphous phases; Zinc sulphides; Iron sulphides; *dsr*AB gene diversity

## 1. Introduction

Wastewater originating from mining and metallurgical industries is often acidic and typically characterized by a significant content of sulphates and soluble metals, such as Zn, Fe, Cu, Ni, Pb and Cd. Soluble metal- and sulphate-bearing wastewater treatment schemes are usually based on sulphate-reducing reactors recently developed in both pilot and full scale [1–8]. The operation of the sulphate-reducing reactors is based on (1) the exclusion of oxygen, (2) the presence of sulphates in the wastewater under treatment, (3) the establishment of a sulphate-reducing bacteria population, either indigenous or introduced, (4) a source of simple organic compounds to serve as carbon source for bacterial growth and (5) a way to retain metal sulphide precipitates.

In such reactor schemes, sulphate-reducing bacteria (SRB) [9] oxidize simple organic compounds, such as lactic acid [10], under anaerobic conditions, transforming the sulphates combined in the wastewater into hydrogen sulphide while generating bicarbonate ions:

$$2CH_3CH(OH)COOH + 3SO_4{}^{2-} \rightarrow 3H_2S + 6HCO_3{}^{-} \quad (1)$$

Hydrogen sulphide reacts with the present divalent soluble metals which are then sequestered from wastewater as insoluble metal sulphides in the form of various mineral phases [6,11,12]:

$$H_2S + Me^{2+} \rightarrow MeS_{(s)} + 2H^+ \quad (2)$$

where Me stands for metals such as Zn, Fe, Cu, Ni, Pb, Cd, etc.

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Bicarbonate ions react with protons to form $CO_2$ and water; thus, removing acidity from solution as $CO_2$:

$$HCO_3^- + H^+ \rightarrow CO_{2(g)} + H_2O \quad (3)$$

$H_2S$ and $HCO_3^-$ formed during sulphate reduction equilibrate into a mixture of $H_2S$, $HS^-$, $S^{2-}$, $CO_2$, $HCO_3^-$ and $CO_3^{2-}$. This mixture buffers the solution pH typically around neutral to slightly alkaline values [1]. The shift of the pH of the acidic solution towards neutral to slightly alkaline values may also lead trivalent metals, such as Al, Fe and Cr, to hydrolyze and precipitate as insoluble hydroxides or oxides. Highly soluble oxyanions, such as chromate, selenate, molybdate and uranyl-carbonate complexes, can also be successfully sequestered from wastewater when treated in sulphate-reducing reactors by reduction, thanks to the reducing, electron-rich environment of the reactor, and precipitated as hydroxides/oxides due to the alkalinity generated by the bacterial metabolism [13]. Metals complexed with ammonia or cyanide are also more readily precipitated as sulphides than as hydroxides [14].

Apart from the obvious advantage of simultaneous substantial reduction of the concentrations of metals and sulphates effected by the treatment of wastewater in sulphate-reducing reactors, sulphide precipitation has several additional potential advantages. Metal sulphides are generally less soluble than their corresponding metal hydroxides; allowing quantitative metal precipitation [15,16]. Metal sulphides are also more compact, have faster settling velocities and exhibit better thickening and dewatering characteristics than the corresponding hydroxide sludge [17]. Metal sulphide volume is 6–10 times lower than metal hydroxide/gypsum sludge from conventional neutralization [8]. Despite the advantages mentioned above, the objectionable odour and toxicity of $H_2S$ as well as the fate of the sludge produced are issues often addressed for technology improvement.

The study of the composition and the characteristics of the solids produced by the operation of a sulphate-reducing reactor is necessary in order to better understand the main mechanisms involved and their relative importance for (i) operating conditions definition, (ii) efficiency improvement and (iii) safer operation. Moreover, the information acquired by the study of the solids composition and their characteristics is necessary for the decisions to be taken about the fate of the sludge produced, dealing with options such as further treatment of the sludge for metal recovery or disposal of the untreated sludge [18].

The present paper summarizes the results obtained from the study of the morphology and composition of the biofilm and the precipitates formed during 1 year of continuous operation of a sulphate-reducing fixed-bed reactor treating metal-bearing, sulphate-rich simulated wastewater.

## 2. Materials and methods

### 2.1. Sulphate-reducing fixed-bed reactor

The sulphate-reducing fixed-bed reactor, operating in upflow mode, is presented in Fig. 1. The biofilm was established on porous sintered glass spheres with average diameter of 1 cm and 1500 $m^2$/L specific surface area. This material has the advantage of a very large surface area, available for biofilm development.

Sludge from the anaerobic digestion tank of a wastewater treatment plant (Municipal Wastewater Treatment Plant, Metamorphosi, Athens) was used as inoculum for the development of a mixed, sulphate-reducing culture.

The reactor was fed with a variation of Postgate's medium (DSMZ, *Desulfovibrio* medium, Medium 63). This nutrient

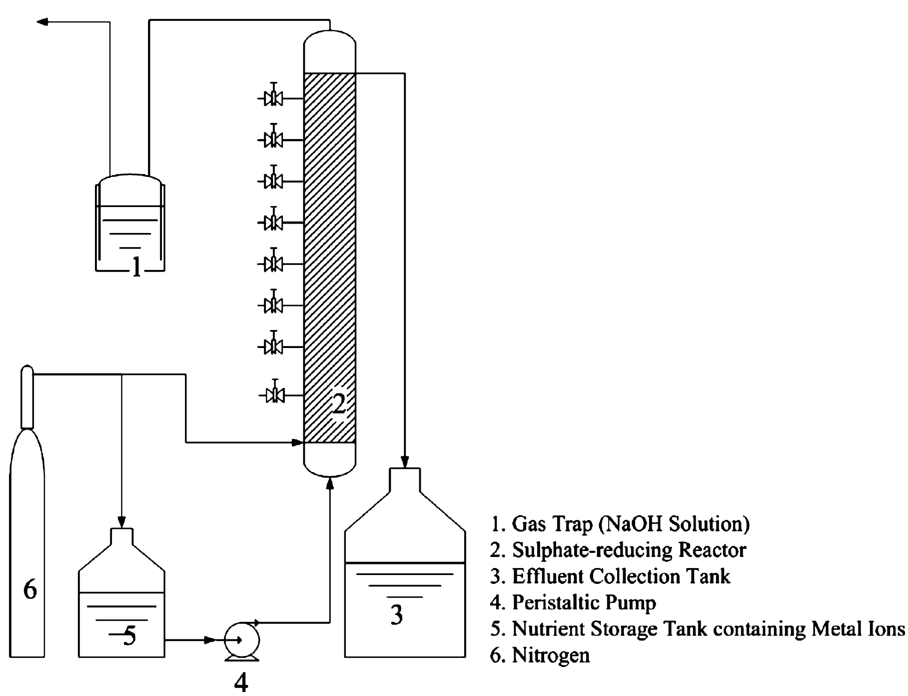


Fig. 1. Sulphate-reducing fixed-bed reactor. Height: 1 m, diameter: 9.5 cm, bed height: 82 cm and head space (necessary for gas collection and removal): 18 cm.