



## Effect of sulfide concentration on the location of the metal precipitates in inversed fluidized bed reactors

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### ABSTRACT

The effect of the sulfide concentration on the location of the metal precipitates within sulfate-reducing inversed fluidized bed (IFB) reactors was evaluated. Two mesophilic IFB reactors were operated for over 100 days at the same operational conditions, but with different chemical oxygen demand (COD) to  $\text{SO}_4^{2-}$  ratio (5 and 1, respectively). After a start up phase, 10 mg/L of Cu, Pb, Cd and Zn each were added to the influent. The sulfide concentration in one IFB reactor reached 648 mg/L, while it reached only 59 mg/L in the other one. In the high sulfide IFB reactor, the precipitated metals were mainly located in the bulk liquid (as fines), whereas in the low sulfide IFB reactor the metal precipitates were mainly present in the biofilm. The latter can be explained by local supersaturation due to sulfide production in the biofilm. This paper demonstrates that the sulfide concentration needs to be controlled in sulfate reducing IFB reactors to steer the location of the metal precipitates for recovery.

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### 1. Introduction

Shortages in metal and mineral resources are expected in the next decades due to a growing demand by human consumption [1]. Therefore, metal-containing wastewaters can become a resource for metal recovery and reuse. Sulfide precipitation is an efficient method to remove and recover metals from wastewaters [2]. It is an attractive option over hydroxide and carbonate precipitation due to the lower solubility and faster reaction rates [3]. Biological sulfate reduction has become an attractive alternative method for the production of sulfide. During this process, sulfate reducing bacteria reduce the sulfate to sulfide in the presence of an organic electron donor or hydrogen [4].

Different reactor configurations have been tested for sulfate reduction and metal precipitation [5]. However, metal recovery cannot always be achieved in these reactors, since metals precipitate partly in the biomass, which hamper their recovery. A promising reactor configuration for metal sulfate reduction and metal removal in a single unit is the inversed fluidized bed (IFB) reactor [6]. This configuration is based on a floatable carrier material (on which the sulfate reducing bacteria biofilm is formed) which is fluidized downwards, whereas the metal sulfide precipitates settle and thus can be recovered at the bottom of the IFB [7].

From the standpoint of chemistry, supersaturation, which depends on the stoichiometry of the reactants, is a key factor in understanding the metal sulfide precipitation. Sulfate reduction has been studied to treat organic and inorganic sulfate-rich wastewaters [8], and the sulfide concentration varies greatly in these studies. Thus, these lead to different levels of supersaturation. Van Hille et al. [9] found that high supersaturation causes the rapid precipitation of copper sulfides, often resulting in the formation of fines and hydrated colloidal particles. On the other hand, Lewis and van Hille [10] found that low sulfide concentrations lead to the formation of aqueous sulfide clusters at high supersaturation points. These conclusions cannot be transferred directly to biological systems, where sulfide is not directly supplied to the reactor, but is produced by the biomass and hence, sulfide supply is linked to the biomass distribution over the reactor.

Bijmans et al. [11] investigated the effect of the sulfide concentration on the ZnS precipitation characteristics in a sulfate reducing gas lift reactor. At low sulfide concentrations (0.26 mg/L), larger ZnS particles were formed with better settling properties than at high sulfide concentrations (3.2–70.4 mg/L). These authors used only a small range of sulfide concentration (0.26–70.4 mg/L) and studied the evolution of the particle size of the metal precipitates in a consecutive reactor run. The aim of the present study was to evaluate the effect of a large difference in sulfide concentration (59 mg/L versus 501 mg/L) on the fate and recovery efficiency of heavy metals (Cu, Zn, Cd and Zn) in IFB reactors.

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## 2. Materials and methods

### 2.1. Source of biomass

The two IFB reactors were inoculated with 25 mL anaerobic sludge from a digester treating activated sludge from a domestic wastewater treatment plant (De Nieuwe Waterweg in Hoek van Holland, The Netherlands). The sludge contained 35.4 g volatile suspended solids (VSS) of the mixed liquor sample per liter of sludge (wet weight). The sludge was added to the reactor, which was kept on recirculation for one day to promote microbial attachment on the carrier material.

### 2.2. Carrier material

The carrier material consisted of 600 mL low-density polyethylene beads (Purell Pe 1810 E, Basell Polyolifins, The Netherlands) of 3 mm diameter. Prior to use, the surface of the polyethylene beads was roughened by abrasion with sand for approximately 15 min. Then, the polyethylene beads were rinsed to remove the sand.

### 2.3. Synthetic wastewater

The synthetic wastewater used for the reactor operation and batch experiments contained (mg/L):  $\text{KH}_2\text{PO}_4$  500,  $\text{NH}_4\text{Cl}$  200,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  2500,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  50 and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  2500. Lactate was used as electron donor. The pH of the medium was adjusted to 7.0 with NaOH. All reagents were of analytical grade.

### 2.4. IFB reactors

The experiments were conducted in two IFB reactors (Fig. 1) constructed from a transparent polyvinyl chloride (PVC) pipe operated at room temperature (25 °C). Each reactor consisted of a column with a conical bottom of a total volume of 5 L (0.08 m diameter, 1 m height). The flow distributor and gas outlet were mounted in the removable cap covering the reactor. The influent was supplied by using a multichannel peristaltic pump (Watson-Marlow BV, The Netherlands) connected to the influent tank of each reactor. The expansion of the bed (30% of the reactor volume) was maintained by means of the recirculation flow using a magnetic drive pump (IWAKI MD-20R-22ON, Iwaki Holland BV, The Netherlands). The reactor was connected to an equalizer to maintain a constant liquid level in the reactor (Fig. 1). In addition, the equalizer functioned as a second settler from which metal precipitates were recovered as well.

### 2.5. Experimental design

#### 2.5.1. IFB reactor operation

Reactor 1 (R1) and reactor 2 (R2) were run for 109 and 103 days, respectively, at the same operational conditions but with different

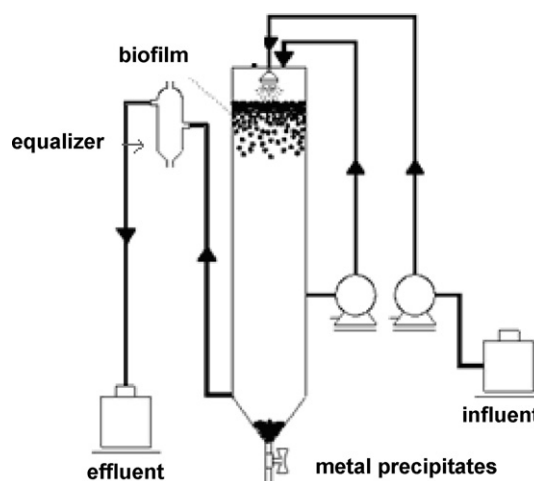


Fig. 1. Schematic representation of the IFB reactor set-up used in this study.

lactate concentrations (Table 1). R1 was operated at a chemical oxygen demand (COD) concentration and  $\text{COD}/\text{SO}_4^{2-}$  ratio (g/g) of 5 g COD/L and 5, respectively, whereas R2 was operated at 1 g COD/L and 1, respectively. After a start up period (I), Cu, Pb, Cd and Zn as chloride salts were added to the influent at a concentration of 5 mg/L each for 7 days (period II). In period III, no metals were added for 5 days to both reactors to ensure the complete removal of metals from the liquid phase prior to the increase of the metal concentration to 10 mg/L each (period IV to V).

From period I to IV, the HRT was maintained at 24 h, which was decreased to 9 h after day 76 for R1 and day 66 for R2 (period V) to test the robustness of the systems with respect to the metal removal efficiencies. The amount of COD, sulfate and dissolved metals removed were measured in the effluent during the whole reactor operation, whereas acetate and dissolved sulfide were started to be measured when the metal removal test started (period IV).

It should be note that the dissolved sulfide concentration was always maintained above the stoichiometric levels ( $[\text{S}]/[\sum \text{M}^{2+}]$  mol/mol) during the metal removal periods in order to ensure metal sulfide precipitation (confirmed by Visual Minteq Version 3.0, US EPA, 1999, <http://www.lwr.kth.se/English/OurSoftware/vminteq/index.html>) besides the presence of other possible precipitants contained in the synthetic wastewater. Iron was also considered in the  $[\text{S}]/[\sum \text{M}^{2+}]$  calculation since the medium contained rather high concentrations of this metal that easily precipitates as sulfide.

#### 2.5.2. Batch experiments

Batch experiments to quantify the sulfide production in the presence and absence of metals were performed at room temperature (25 °C) in serum bottles of 117 mL shaken at 100 rpm. The

**Table 1**  
Operational conditions of the two IFB reactors.

| Parameter                        | Experimental periods |                  |           |                    |                 |
|----------------------------------|----------------------|------------------|-----------|--------------------|-----------------|
|                                  | I                    | II               | III       | IV                 | V               |
| Characteristic                   | Start up             | Metal adaptation | No metals | Metal removal test | Decrease of HRT |
| Days                             |                      |                  |           |                    |                 |
| R1                               | 0–43                 | 44–50            | 51–56     | 57–76              | 77–109          |
| R2                               | 0–35                 | 36–42            | 43–48     | 49–66              | 67–103          |
| HRT (days)                       | 1                    | 1                | 1         | 1                  | 0.37            |
| Metals added <sup>a</sup> (mg/L) | No                   | 5                | No        | 10                 | 10              |

T = room temperature (25 °C), pH 7.

<sup>a</sup> Cu, Zn, Pb and Cd.

**Table 2**  
Effluent characteristics and removal efficiencies for sulfate reduction during the operation of the IFB reactors (mean  $\pm$  standard deviation).

|  | Experimental periods |                   |                  |                   |                   |
|--|----------------------|-------------------|------------------|-------------------|-------------------|
|  | I <sup>a</sup>       | II <sup>a,c</sup> | III <sup>a</sup> | IV <sup>a,d</sup> | V <sup>b,d</sup>  |
| <b>R1</b>  |                      |                   |                  |                   |                   |
| COD removal efficiency (%)                           | 15 ( $\pm$ 3)        | 22*               | 34*              | 27 ( $\pm$ 7)     | 27 ( $\pm$ 10)    |
| SO <sub>4</sub> <sup>2-</sup> removal efficiency (%) | 56 ( $\pm$ 24)       | 88( $\pm$ 3)      | 70*              | 76( $\pm$ 15)     | 74 ( $\pm$ 13)    |
| Acetate concentration (g COD/L)                      | ND                   | ND                | ND               | 1.39 ( $\pm$ 0.6) | 1.36 ( $\pm$ 0.4) |
| Sulfide concentration (mg/L)                         | ND                   | ND                | ND               | 212 ( $\pm$ 26)   | 648 ( $\pm$ 153)  |
| [S]/[ $\sum$ M <sup>2+</sup> ] (mol/mol)             | ND                   | ND                | ND               | 14.8              | 45.15             |
| Effluent pH  | 6.6 ( $\pm$ 0.2)     | 7.1 (0)           | 7.0 (0)          | 7.1 ( $\pm$ 0.1)  | 7 ( $\pm$ 0.2)    |
| <b>R2</b>  |                      |                   |                  |                   |                   |
| COD removal efficiency (%)                           | 35 ( $\pm$ 8)        | 48*               | –                | 68 ( $\pm$ 11)    | 53 ( $\pm$ 18)    |
| SO <sub>4</sub> <sup>2-</sup> removal efficiency (%) | 59 ( $\pm$ 14)       | 68 ( $\pm$ 15)    | 53*              | 17 ( $\pm$ 11)    | 38 ( $\pm$ 17)    |
| Acetate concentration (g COD/L)                      | ND                   | ND                | ND               | 0                 | 0                 |
| Sulfide concentration (mg/L)                         | ND                   | ND                | ND               | 59 ( $\pm$ 24)    | 44 ( $\pm$ 30)    |
| [S]/[ $\sum$ M <sup>2+</sup> ] (mol/mol)             | ND                   | ND                | ND               | 4.11              | 5.16              |
| Effluent pH  | 7.0 ( $\pm$ 0.1)     | 7.6 (0)           | 7.6 ( $\pm$ 0.1) | 7.5 ( $\pm$ 0.2)  | 7.5 ( $\pm$ 0.1)  |

HRT = <sup>a</sup>24 h and <sup>b</sup>9 h, influent metal conc. = <sup>c</sup>5 mg/L and <sup>d</sup>10 mg/L, \*average of two values, NR = not determined, [ $\sum$ M<sup>2+</sup>]: the sum of Zn, Cu, Pb, Cd and Fe molar concentrations.

bottles contained 5 mL of carrier material withdrawn from R1 at the end of the reactor operation and 112 mL synthetic wastewater.

**2.5.2.1. Sulfate-reducing activity.** The sulfate reducing activity (SRA) was determined in six serum bottles using lactate (1 g COD/L) as the substrate at a COD/SO<sub>4</sub><sup>2-</sup> ratio of 1 (Exp A). Prior to the experiment, the carrier material with the biofilm was stored at 4 °C. Therefore, the biofilm was activated to the experimental conditions of Exp A, but at 30 °C for 72 h. After this, the serum bottles were refilled with fresh synthetic wastewater containing lactate.

**2.5.2.2. Metal precipitation with active biofilm.** In order to study the effect of the metals on the sulfate reducing activity, three of the serum bottles used in Exp A, were refilled with fresh synthetic wastewater upon finishing (Exp B) and the other three were kept with the same medium (adding extra synthetic wastewater to cover the headspace) (Exp C). Cu, Zn, Cd and Pb were added to the serum bottles to an initial concentration of 10 mg/L.

The sulfide concentration was determined in Exp A, B and C approximately every 4 h and the metal concentration every hour during the initial 24 h of Exp B and C.

## 2.6. Analysis

Total suspended solids (TSS) and volatile suspended solids (VSS) in the biofilm are reported per gram of dry polyethylene and were determined according to standard methods [12] after detaching the biofilm from the polyethylene by successive washings with deionized water in an ultrasonic bath. COD was determined by the close reflux method [12]. Acetate was measured by gas chromatography (GC-CP 9001 Chrompack) after acidification of the samples with 5% concentrated formic acid and filtration through a 0.45  $\mu$ m nitrocellulose filter (Millipore). The gas chromatograph was fitted with a WCOT fused silica column, the injection and detector temperatures were 175 and 300 °C, respectively. The temperature of the oven was kept at 115 °C. The carrier gas was helium at 100 mL/min.

Sulfide was determined spectrophotometrically by the colorimetric method described by Cord-Ruwisch [13] using a spectrophotometer (PerkinElmer Lambda20). Sulfate was measured with an ion chromatograph (ICS-1000 Dionex with ASI-100 Dionex). The column (IonPac AS14n) was used in the ion chromatograph at a flow rate of 0.5 mL/min with an 8 mM Na<sub>2</sub>CO<sub>3</sub>/1 mM NaHCO<sub>3</sub> eluent, a temperature of 35 °C, a current of 35 mA, an injection volume of 10  $\mu$ L and a retention time of 8 min.

Metals were measured by flame (AAS PerkinElmer 3110) and furnace (AAS Solaar MQZe GF95) spectroscopy. Metal samples from batch experiments to determine the metal precipitation rate were measured in the liquid phase after diluting, acidifying with 5% HNO<sub>3</sub> and passing the sample through a 0.45  $\mu$ m nitrocellulose filter (Millipore). Metal samples from the bottom of the reactor and equalizer were analyzed after taking 50 mL of the liquid containing the precipitates and acidifying with 20% HNO<sub>3</sub> to ensure the complete dissolution of the metal precipitates. After this, the procedure for metal measurements mentioned above was followed.

## 2.7. Calculations

The metal precipitates that could not settle at the bottom of the reactor and equalizer due to their small size were defined as fines. It is important to consider these fines to prove the system not only for the removal of metals but also for the potential recovery. The dissolved metal concentration (excluding fines) was determined by passing the liquid samples through a filter (0.45  $\mu$ m) prior to acidification.

The metal removal efficiency in the down-flow FBR was defined as:

$$\text{Metal removal efficiency (\%)} = \frac{M_{\text{in}} - M_{\text{out,dissolved}}}{M_{\text{in}}} \times 100$$

where  $M_{\text{in}}$  = metal concentration in the feed (mg/L) and

$M_{\text{out,dissolved}}$  = dissolved metal concentration in the outlet (mg/L).

The results of the metals accumulated in the bottom of the reactor and equalizer during each experimental period were used to calculate the metal recovery efficiency. Then, mass balance calculations were done to determine the fate of the metals. The metal recovery in the IFB reactor was defined as:

$$\text{Metal recovery efficiency (\%)} = \frac{M_{\text{total}} - \sum_1^n (M_{\text{eq}} + M_{\text{b}})}{M_{\text{total}}} \times 100$$

where  $M_{\text{eq}}$  = metals (mg) from the equalizer in the sample,  $M_{\text{b}}$  = metals (mg) from the bottom of the reactor in the sample,  $M_{\text{total}}$  = total metals (mg) in the influent that entered the reactor over an operational period and  $n$  = number of samples in each operational period

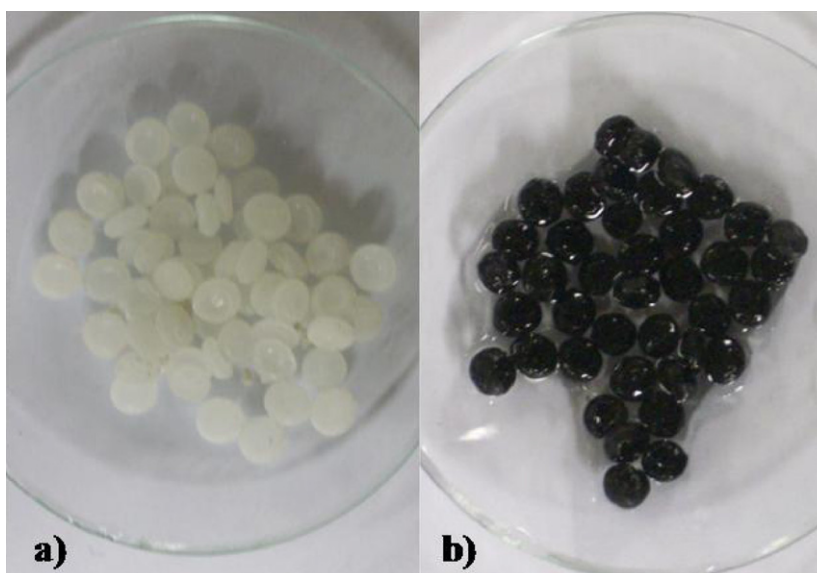


Fig. 2. Picture of the polyethylene beads a) before and b) after the biofilm formation in the IFB reactors.

### 3. Results

#### 3.1. IFB reactor operation

Table 2 compares the effluent characteristics of R1 and R2. The performance of both reactors during the start up period was characterized by instabilities in COD and sulfate removal. The gradual increase of the sulfate removal efficiency and the change in color of the polyethylene (Fig. 2) and the reactor liquids from brown to black confirmed that anaerobic conditions and a sulfate reducing biofilm developed. The pH of the effluent was lower than the influent pH (6.6) in R1, while the pH was maintained at 7.0 in R2.

In period II, sulfate removal efficiencies were on average 88% and 68%, and the average pH was 7.1 and 7.6 in R1 and R2, respectively, with no changes in the subsequent periods. The COD removal efficiency in R1 and R2 increased to 22% and 48%, respectively.

In period III, the sulfate removal efficiency decreased in both reactors, in R1 to 70% and in R2 to 53%, while the COD removal efficiency continued to increase to 34% in R1. In period IV, a recovery in the sulfate removal efficiency was observed in R1 (76%), while in R2 the sulfate removal efficiency dropped to 17%. The COD removal efficiency continued increasing to 68% in R2, while it decreased to 27% in R1. In this period, it was shown that part of the COD supplied was transformed to acetate in R1 (1.39 g COD/L), while the acetate concentration in R2 was below the detection limit. During period IV, the mean sulfide concentration in R1 and R2 was 212 mg/L and 59 mg/L, respectively. In the same period, polyethylene beads started to settle in both reactors, this was more pronounced in R2. This caused a failure of the recirculation pump on day 87 in R1 and on day 66, 81 and 101 in R2. The failure of the pump was also reflected in the lower sulfate removal efficiency by R2 on days 74, 88, and 102 (Fig. 3).

In period V, the change of the HRT from 24 to 9 h did not vary the COD nor sulfate removal efficiencies in R1, while in R2 a partial recovery of the sulfate removal efficiency was observed (38%). The maximum sulfide concentration was reached in period V for R1 (648 mg/L), while only 44 mg/L of sulfide was produced on average in R2. In period V, it was confirmed by the mass balances (data not shown) that most of the COD consumed was used for sulfate reduction (>87%) in R1. Acetate production remained close to the value obtained in the previous period (1.36 g/L) and accounted for 43.4% of the COD consumed.

#### 3.1.1. Metal removal/recovery in the IFB reactors

Table 3 shows the metal removal efficiency of both R1 and R2 for the periods where the metals were added to the influent (II, IV and V). Metal removal efficiencies exceeded 91% in both reactors in period II, which further increased for both reactors and exceeded 95% in period IV. The Cu, Zn, Pb and Cd removal efficiencies in both reactors exceeded 98.4%, 96.5%, 96%, and 97.9%, respectively in period V, but were slightly higher in R2, in spite of the higher sulfide concentration in R1 compared to R2. Neither the increment of the metal concentration (period IV) nor the change in HRT affected the metal removal efficiency (period V).

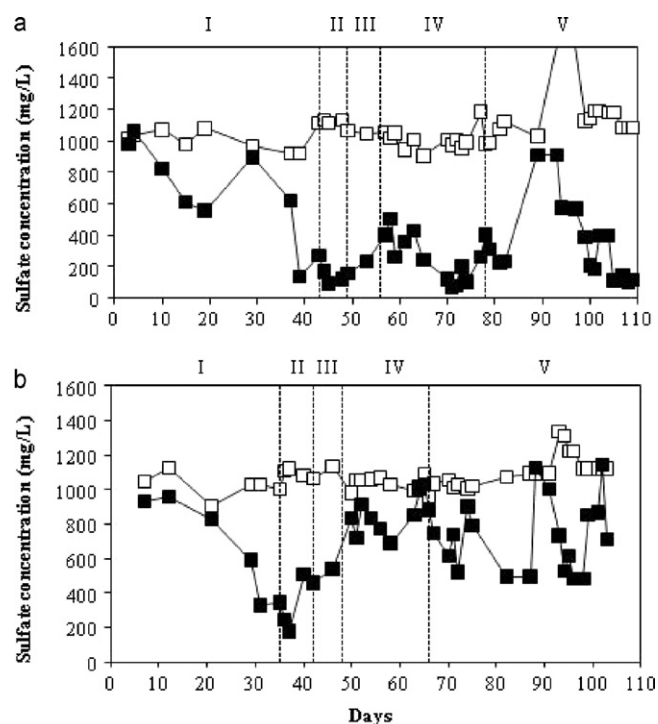


Fig. 3. Evolution of the sulfate concentration in the influent (□) and effluent (■) during reactor operation.



**Table 3**  
Metal removal efficiency and metal recovery in the IFB reactors in periods II, IV and V (mean  $\pm$  standard deviation).

| Period | Metal removal efficiency (%) |                    |                    | Metal recovery <sup>a</sup> (%) |      |      |
|--------|------------------------------|--------------------|--------------------|---------------------------------|------|------|
|        | II                           | IV                 | V                  | II                              | IV   | V    |
| R1     |                              |                    |                    |                                 |      |      |
| Cu     | 99.1 ( $\pm 1.3$ )           | 97.1 ( $\pm 2.9$ ) | 98.4 ( $\pm 3.1$ ) | 16.8                            | 17.2 | 49.4 |
| Zn     | 92.3 ( $\pm 4.2$ )           | 95.1 ( $\pm 4.4$ ) | 96.5 ( $\pm 3.8$ ) | 13.6                            | 16.5 | 43.6 |
| Pb     | 92.7 ( $\pm 4.9$ )           | 97.3 ( $\pm 1.9$ ) | 96.0 ( $\pm 5.1$ ) | 13.2                            | 21.4 | 57.9 |
| Cd     | 95.6 ( $\pm 1.7$ )           | 95.2 ( $\pm 2.5$ ) | 97.9 ( $\pm 3.0$ ) | 15.7                            | 17.2 | 46.3 |
| R2     |                              |                    |                    |                                 |      |      |
| Cu     | 99.1 ( $\pm 1.5$ )           | 96.7 ( $\pm 3.3$ ) | 99.9 ( $\pm 0.3$ ) | 4.1                             | 29.8 | 41.1 |
| Zn     | 91.3 ( $\pm 10.0$ )          | 95.6 ( $\pm 2.3$ ) | 98.6 ( $\pm 1.2$ ) | 2.7                             | 26.9 | 44.2 |
| Pb     | 92.4 ( $\pm 8.0$ )           | 96.1 ( $\pm 3.4$ ) | 99.2 ( $\pm 1.1$ ) | 3.5                             | 30.0 | 60.3 |
| Cd     | 96.5 ( $\pm 2.4$ )           | 95.3 ( $\pm 2.9$ ) | 99.7 ( $\pm 0.3$ ) | 2.9                             | 26.0 | 47.4 |

<sup>a</sup> Metal recovery from bottom of the reactor and equalizer.

Table 3 also shows the percentage of metals that had accumulated at the bottom of the reactor and equalizer, expressed as the metal recovery efficiency. In period II, only less than 5% of the metals were recovered in R2, while in R1 up to 17% of the metals could be recovered. In period IV, the metal recovery increased in both reactors, the highest values were obtained in R2 (29.8%, 26.9%, 30.0%, and 26.2% for Cu, Zn Pb and Cd respectively). The recovery continued to increase in both reactors in period V achieving values of 41.1–60.3%. In general, the difference in the sulfide concentration and the operational conditions of both reactors did not result in any relevant variation in metal recovery.

### 3.1.2. Location of metal precipitation in the biofilm

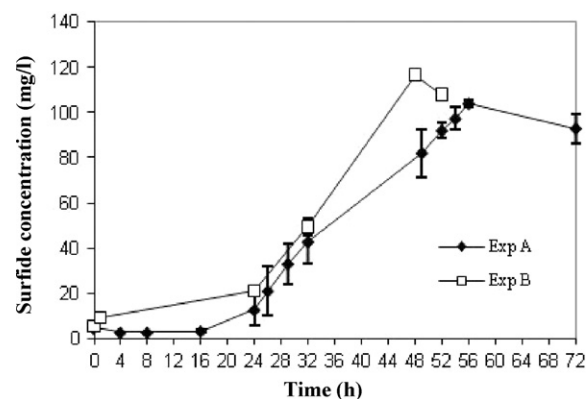
It was assumed that the metals which were not present in the effluent, bottom of the reactor and equalizer were present in the reactor as fines or adsorbed or precipitated in the biofilm. At the end of the reactor operation, metals, TSS and VSS in the biofilm were analyzed on polyethylene bead samples located at the top and bottom of the polyethylene bed (Table 4).

The TSS and VSS in the biofilm were different at the top and at the bottom of both fluidized beds. The TSS was especially high at the bottom of the R2 polyethylene bed. No differences in the metal concentration were observed between beads sampled at the top and the bottom of the fluidized bed in R1. In general, higher metal concentrations in the biofilm were found in R2. Moreover, the differences in metal concentration between the top and the bottom were significantly higher in R2.

## 3.2. Batch experiments

### 3.2.1. Sulfate reducing activity of the biofilm

Fig. 4 compares the sulfide production in the absence (Exp A) and presence of metals (Exp B). The sulfide started to increase after a lag phase of 16 h for Exp A, while no lag phase was observed for Exp B. Prior to the addition of metals (Exp A), the SRA was 0.16 mg S<sup>2-</sup>/mg VSS h, which increased to 0.21 mg S<sup>2-</sup>/mg VSS h after the addition of metals (Exp B). These results confirm that the metal concentration itself was not directly inhibitory to the biofilm.



**Fig. 4.** Sulfide production in the absence (Exp A) and in the presence (Exp B) of metals in the batch experiments with the carrier material withdrawn from R1.

### 3.2.2. Metal precipitation with active biofilm

Fig. 5 compares metal precipitation when sulfide is formed by the sulfate reducing biofilm during the batch experiment (Exp B) and when sulfide is already produced by the sulfate reducing biofilm at the start of the experiment (Exp C). In Exp B, the metal concentration decreased within the first hour, even when sulfide was not yet accumulating in the medium. After 24 h, the sulfide concentration was 30.9 mg/L, at this sulfide concentration Pb could not be detected in the liquid phase anymore (Fig. 5). The sulfide concentration continued increasing with time, whereas the metal concentration reached steady state after 32 h. Metals remained in the liquid phase at 1 mg/L for Cu and 0.3 mg/L for Cd and Zn.

In Exp C, almost all the Cu, Cd and Pb precipitated within the first hour, while 6.7 mg/L of the Zn remained in the liquid phase. After 24 h, Cd and Pb were not found in the liquid phase anymore, while 1.2 mg/L of Zn and 2.1 mg/L of Cu remained in the liquid phase with slight variations during the subsequent 48 h. After 52 h, Cu and Zn had further decreased and only 0.9 mg/L Zn remained in the liquid phase.

**Table 4**  
TSS, VSS and metal concentration in the biofilm of both IFB reactors at the end of the experiment (average of duplicate samples). Samples taken at the top and the bottom of the polyethylene bed.

|    |        | TSS (mg/g <sub>polyethylene</sub> ) | VSS (mg/g <sub>polyethylene</sub> ) | Metal concentration (mg/g <sub>polyethylene</sub> ) |     |     |     |
|----|--------|-------------------------------------|-------------------------------------|---|-----|-----|-----|
|    |        |                                     |                                     | Cu  | Pb  | Zn  | Cd  |
| R1 | Top    | 2.7                                 | 0.8                                 | 0.4   | 0.2 | 0.5 | 0.3 |
|    | Bottom | 6.8                                 | 1.4                                 | 0.4   | 0.2 | 0.5 | 0.3 |
| R2 | Top    | 4.8                                 | 0.8                                 | 0.6   | 0.3 | 0.8 | 0.7 |
|    | Bottom | 35.6                                | 4.6                                 | 1.5   | 0.8 | 1.8 | 1.4 |

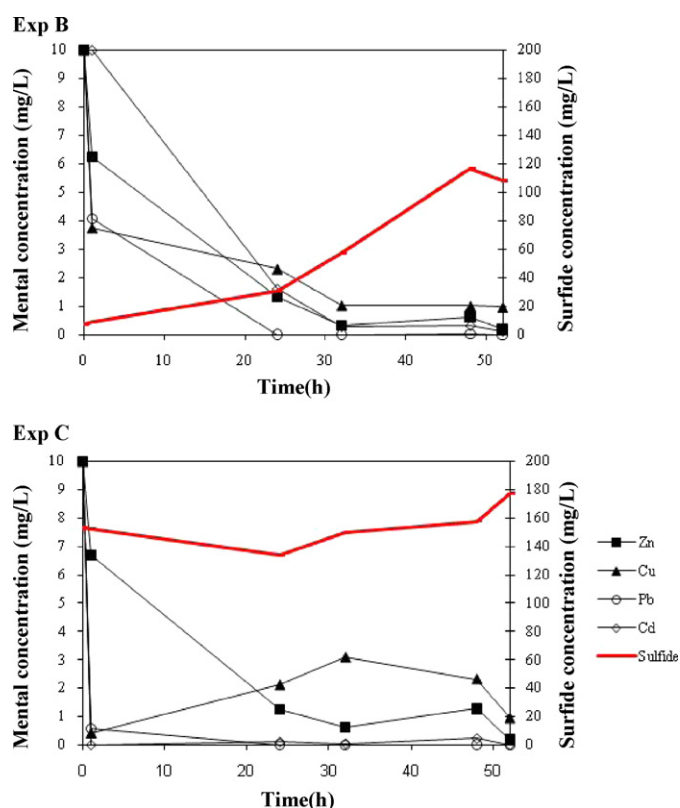


Fig. 5. Evolution of the metal concentration in time when sulfide is formed by the biofilm (Exp B) or when sulfide (152 mg/L) is present in the liquid phase from  $t = 0$  onwards (Exp C) in the liquid phase (top) and in the biofilm (bottom).

#### 4. Discussion

This paper shows that the sulfide concentration in the reactor mixed liquor controls the location of the metal precipitates in an IFB reactor. Moreover, the results suggest that the recovery of the metal precipitates at the bottom of the IFB reactors is independent of the sulfide concentration, and hence, other mechanisms determine the settling properties of the sulfide precipitates, e.g. agglomeration. To the best of our knowledge, the present study is the first to report the location of metal sulfide precipitation as a function of the sulfide concentration in an IFB reactor treating a multimetal wastewater.

##### 4.1. Location of metal sulfide precipitation

Table 4 shows that the metals are partly immobilized in the biofilm. Bijmans et al. [14] suggested that biofilms might function as nucleation seeds, enhancing the crystal growth for metal sulfides. Other authors have induced the precipitation of heavy metals on the sand surface in fluidized bed reactors (FBRs) using sulfide or carbonate [9,15,16]. Zhou et al. [15] observed that when the ratio

of carbonate to metals (Cu, Ni and Zn) was low, metal precipitation was coated on the sand surface, while at high ratios (6:1 and 3:1) the precipitation occurred in the bulk solution. These findings are in agreement with this study: in R2 the sulfide concentration in the bulk liquid was much lower than in R1. Therefore, supersaturation, and thus precipitation of metal sulfides, mainly occurred within the biofilm in R2, where the sulfide is produced. In R1, in contrast, a larger fraction of the supersaturation, and thus precipitation, occurred in the bulk liquid, due to the much higher sulfide concentration in the reactor mixed liquor, resulting in a lower metal content in the biofilm (Table 4 and Fig. 6).

Metals might also be immobilized in biofilms due to sorption onto the microorganisms and/or on their extracellular polymeric substances (EPS) [17–19]. However, this mechanism was apparently not the case in this study as the sulfide concentration was always maintained above the stoichiometric value to precipitate the four metals as metal sulfides in both reactors. In addition, the metal sulfide formation rate is extremely fast compared to the adsorption mechanisms reported in the literature [2,20]. Wang et al. [20] showed that CdS was formed at the cell surface because the cadmium sulfide formation rate is extremely fast ( $K_{\text{CdS}} = 3.7 \times 10^5 \text{ s}^{-1}$ ) compared to the slow rate of sulfide transport ( $K_{\text{diff}} = 1000 \text{ s}^{-1}$ ) on the bacterial cell surface.

##### 4.2. Metal recovery

In both reactors, the metal removal and recovery did not vary with the sulfide concentration or the different operational conditions. Metal removal efficiencies were on average 95% for the four metals tested (Table 3), while metal recovery from the bottom of the reactor and equalizer was less than 50% in both reactors (Table 3). In previous studies, similar metal removal efficiencies have been achieved in biological reactors treating wastewaters with more than two metals in a single unit [7,21–26], although, the recovery of metals is often not reported. Gallegos-Garcia et al. [7] reported 76–97% recovery of Fe, Zn, and Cd in an IFB at initial metal concentrations between 5 and 320 mg/L and sulfide concentrations over 140 mg/L. These results differ considerably from this study. The difference could be related to the treatment of the samples. Gallegos-Garcia et al. [7] as well as other authors [21] have reported the metal recovery assuming that the TSS concentrations equal the metal sulfide composition. However, in this study it was shown that the TSS also contained salts from the mineral medium that contribute to the weight (observed in the results of the biofilm composition, Table 4). Therefore, this study reports the recovery of the metals based on the direct measurement of metals after acidification of the recovered solids (Table 3).

##### 4.2.1. Formation of fines

The difference between the metal removal and recovery efficiency (Table 3) can be partly explained by the accumulation of metals in the biofilm (Table 4), but also by the formation of small precipitates ( $<0.45 \mu\text{m}$ ), known as fines, which do not settle at the

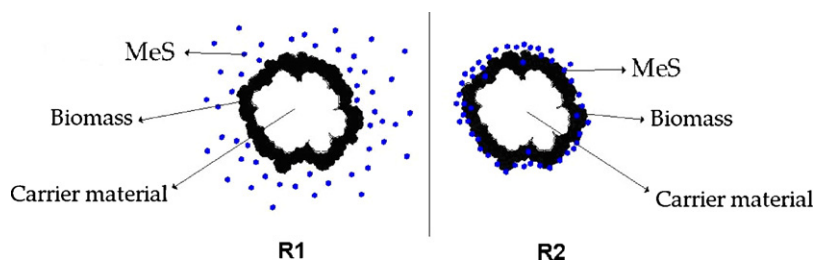


Fig. 6. Location of the metal sulfide (MeS) precipitate formation, depending on the sulfide concentration: MeS precipitation outside the biofilm at high sulfide concentrations as in R1 and MeS precipitation in the biofilm at low sulfide concentrations as in R2.

bottom of the reactor and leave the IFB reactor with the effluent. These fines did not contribute to the metal concentrations in the effluent, as samples were filtered through a 0.45  $\mu\text{m}$  filter prior to analyses.

The formation of fines is attributed to high levels of supersaturation [16] and high nucleation rates [27] of the metal sulfides. This was observed in the batch experiments (Fig. 5): when the sulfide was slowly produced (Exp B), the precipitation rate could be quantified, whereas when sulfide was already present (Exp C), metal precipitation occurs too fast to monitor the metal precipitation kinetics of Cu, Pb and Cd. Note that the ZnS precipitation is slower due to the higher solubility product compared with the solubility products of the other three metals tested [28].

#### 4.2.2. Effect of agglomeration

The metal recovery gradually increased in both reactors during reactor operation (Table 3), despite the differences in operational conditions including sulfide and acetate production (Table 2). Both parameters influence the size of the particles, which affects the settling properties of the metal precipitates for recovery. However, this contribution is of lesser importance since the size range of the particles is still very small to allow fast settling (for review see [29]).

Fines in the reactor can form agglomerates, that later settle and dewater better. Precipitation occurs through several steps: nucleation, crystal growth, and eventually agglomeration [30]. Large particles can be produced if the supersaturation is optimum to promote crystal growth, and the residence time of the crystals is long enough to promote agglomeration [31]. In the precipitation process of metal sulfides, which have an extremely low solubility [28], the formation of fines is predominant over crystal formation. Therefore, agglomeration is an important mechanism for the settling of the precipitates. Biological processes can contribute to the agglomeration mechanism. It has been shown that the presence of extracellular proteins promotes the aggregation of metal sulfide nanoparticles [32]. Further research using Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) coupled to X-ray diffraction (XRD) and dewatering tests are required to relate the morphology of the produced solids in the IFB reactor with substances present in sulfate reducing bioreactors, for example, extracellular proteins or nutrients added for bacterial growth.

#### 4.2.3. Reactor operation

A period of approximately 40 days was necessary to form a sulfate reducing biofilm on the polyethylene beads in an IFB reactor using lactate as electron donor, independent of the  $\text{COD}/\text{SO}_4^{2-}$  ratio. A similar time was necessary in the study of Celis et al. [6], who used a lactate:ethanol mixture, a  $\text{COD}/\text{SO}_4^{2-}$  ratio of 0.6 and operated in batch mode for 45 days.

In R1, the production of acetate by incomplete oxidizers was probably more favorable than in R2 due to the excess of COD [33]. This acetate also explains why the pH level did not increase in R1 (Table 2), since the incomplete oxidation of lactate generates protons [21]. Contrary to R1, in R2, acetate was not detected during the reactor operation and the pH increased in the effluent by  $\text{H}_2\text{S}$  and  $\text{CO}_2$  production (Table 2). This suggests that different microbial populations developed in both reactors.

The comparison of Exp A and Exp B in the batch experiments (Fig. 5) confirmed that the metal concentration itself was not directly inhibitory to the biofilm. The inhibitory concentrations to SRB reported for Cu, Zn, Pb and Cd are in the range of 6–100, 13–65, 25 to >80 and >4–112 mg/L, respectively [5]. The inhibitory effects of metals, however, depend on the experimental conditions [34], e.g. time exposure, which differed in batch experiments and continuous reactor operation.

## 5. Conclusion

Several studies with sulfate reducing bioreactors to treat metal containing streams have mainly concentrated on maximizing the sulfate reduction rate. In the present study, it was shown that the sulfide concentration is important, not only to precipitate metals but also to steer the location where the precipitates are formed. Moreover, a sulfide concentration in excess is not desirable since it causes residual pollution problems in the effluents, and may impose a sulfide removal post-treatment step.

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