ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Precipitation and Recovery of Metal Sulfides From Metal Containing Acidic Wastewater in a Sulfidogenic Down-Flow Fluidized Bed Reactor

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Received 10 April 2008; revision received 24 June 2008; accepted 7 July 2008

Published online 25 July 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/bit.22049

ABSTRACT: This study reports the feasibility of recovering metal precipitates from a synthetic acidic wastewater containing ethanol, Fe, Zn, and Cd at an organic loading rate of 2.5 g COD/L-day and a COD to sulfate ratio of 0.8 in a sulfate reducing down-flow fluidized bed reactor. The metals were added at increasing loading rates: Fe from 104 to 320 mg/L-day, Zn from 20 to 220 mg/L-day, and Cd from 5 to 20 mg/L-day. The maximum COD and sulfate removals attained were 54% and 41%, respectively. The biofilm reactor was operated at pH as low as 5.0 with stable performance, and no adverse effect over COD consumption or sulfide production was observed. The metals precipitation efficiencies obtained for Fe, Zn, and Cd exceeded 99.7%, 99.3%, and 99.4%, respectively. The total recovered precipitate was estimated to be 90% of the theoretical mass expected as metal sulfides. The precipitate was mainly recovered from the bottom of the reactor and the equalizer. The analysis of the precipitates showed the presence of pyrite (FeS_2), sphalerite (ZnS) and greenockite (CdS); no metal hydroxides or carbonates in crystalline phases were identified. This study is the first in reporting the feasibility to recover metal sulfides separated from the biomass in a sulfate reducing process in one stage.

Biotechnol. Bioeng. 2009;102: 91–99.

KEYWORDS: biofilm; cadmium; fluidized-bed reactor; iron; metal precipitation; metal sulfides; sulfate-reduction; zinc

Introduction

Environmental pollution by heavy metals is a topic of the utmost relevance due to its impact on public health, environment and, finally, on the economy. Some industrial sectors, for example, mining, semiconductor, metallurgical, electroplating and metal finishing-industries generate contaminated effluents with a variety of toxic metals (Sierra-Alvarez et al., 2007). The traditionally used methods for the treatment of acidic metal-containing wastewaters have been based on chemical neutralization and hydroxide precipitation of metals (Johnson, 2000; Kaksonen and Puhakka, 2007). The disadvantages of chemical treatment include the high cost of the chemical reagents and production of bulky sludge, which must be disposed of (García et al., 2001). Sulfate reduction has become a suitable alternative for the treatment of wastewaters that contain metals. This anaerobic process is carried out by sulfate reducing bacteria (SRB) that use sulfate as terminal electron acceptor for the oxidation of organic compounds and hydrogen (electron donors), resulting in the production of hydrogen sulfide (H_2S). Removal of metals by SRB is mainly due to the production of highly insoluble precipitates with biogenic H_2S as shown in Equations (1) and (2). Moreover, produced alkalinity increases the pH of the wastewater (Eq. 3):

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (1)$$

where CH_2O = organic matter (electron donor)

$$H_2S + M^{2+} \rightarrow MS(s) + 2H^+ \quad (2)$$

where M^{2+} = metal, such as Fe^{2+}

$$HCO_3^- + H^+ \rightarrow CO_2(g) + H_2O \quad (3)$$

Kaksonen and Puhakka (2007) and Hao (2000) reviewed different sulfate-reducing bioreactor configurations for metal precipitation; these configurations include single or separated unit processes. In single stage processes the heavy metals are precipitated as metal sulfides and are retained within the bioreactor combined with the biomass (Janssen

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et al., 2001; Kaksonen et al., 2003a,b), whereas in separated unit processes the biological sulfide production and chemical metal sulfides precipitation takes place in separate units allowing the recovery of valuable minerals (Bhagat et al., 2004; Sierra-Alvarez et al., 2007; Tabak et al., 2003); depending on the reactor type and process configuration, the existing systems exhibit benefits and drawbacks (Kaksonen and Puhakka, 2007).

Various high rate reactors have been applied for biological metal sulfide precipitation including up-flow anaerobic sludge bed reactors (de Vegt et al., 1998; Kaksonen et al., 2003b), anaerobic filters (Elliott et al., 1998; Jong and Parry, 2003), expanded bed reactors (Sierra-Alvarez et al., 2007), and fluidized bed reactors (Kaksonen et al., 2003a,b). Amongst the different fluidized bed reactors configurations, the down-flow fluidized bed reactor (DFFBR) is an appropriate alternative not only for metals precipitation, but also for their recovery. In this type of reactor a carrier support floats at the top of a liquid column and this support is fluidized by means of down-flow liquid recirculation (Celis-García et al., 2007). After inoculation, a biofilm develops over the support that still remains at the top of the reactor and maintains the metal sulfide precipitates separated from the biomass, which does not occur in conventional high rate sulfidogenic reactors. To date, metal sulfide precipitates and biomass are recovered in separate units.

The aim of this work was to evaluate the feasibility of a sulfidogenic DFFBR for the precipitation and recovery of metal sulfides from an acidic wastewater containing sulfate, iron, zinc, and cadmium in just one single stage. It was expected that the DFFBR configuration would allow the separation and recovery of metal sulfides from the biomass.

Materials and Methods

Bioreactor Operation

The DFFBR consisted of a 2.5 L conical bottom poly-acrylate column, a flow equalizer and a device used as liquid–solid separator and water level adjuster (Fig. 1). The total liquid volume of the reactor including the water level adjuster, the flow equalizer and the recirculation lines was 3.34 L, the reactor was operated at ambient temperature (18–26°C). The DFFBR was inoculated with 600 mL of a sulfate-reducing biofilm developed over low-density polyethylene fine particles that were used as carrier support (500 μm mean diameter and apparent density of 400 kg/m^3). The biofilm was obtained from a similar laboratory scale DFFBR that treated a mixture of ethanol–lactate at a chemical oxygen demand (COD) to sulfate (SO_4^{2-}) ratio of 0.6 for over 225 days, at a pH between 7.0 and 6.0. The support was fluidized and maintained at half length of the column by flow recirculation; the recycle flow rate was 750 mL/min that resulted in a superficial velocity of 18.6 m/h. The recycle

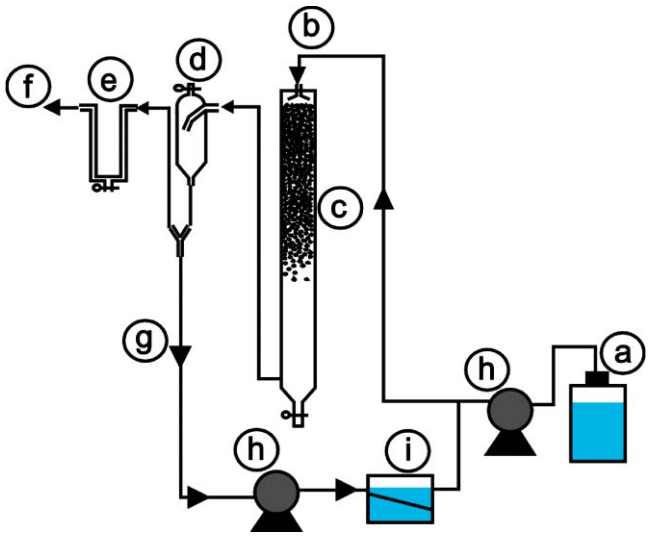

Figure 1. Experimental set-up of the down-flow fluidized bed reactor (DFFBR). The inner diameter and length of the column were 5.5 cm and 1.10 m, respectively. (a) Influent reservoir; (b) inlet; (c) fluidized bed; (d) liquid solid separator and water level adjuster; (e) water-lock; (f) outlet; (g) recirculation flow; (h) peristaltic pumps; (i) flow equalizer. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

ratio ensured completely mixed conditions in the DFFBR and a fluidization of 50% of the reactor's volume.

The reactor was fed with an acidic synthetic wastewater that consisted of mineral media containing (g/L): NH_4Cl (0.3), KH_2PO_4 (0.2), $MgCl_2 \cdot 6H_2O$ (0.12), KCl (0.25), $CaCl_2 \cdot 2H_2O$ (0.015), yeast extract (0.02) and 0.2 mL/L of trace metals solution according to Zehnder et al. (1980). The electron donor was a mixture of ethanol–lactate or ethanol and sodium sulfate was added as electron acceptor; during all the experiment the COD/SO_4^{2-} ratio was around 0.8. The DFFBR was operated continuously for 320 days under six main operational periods depending on the operational conditions (Table I). During the first 126 days (periods I–III) the reactor was operated without the addition of metals in the influent, whereas from days 127 to 318 (periods IV–VI) iron, zinc and cadmium were fed into the reactor as $FeCl_2 \cdot 4H_2O$, $ZnCl_2$ and $Cd(NO_3)_2 \cdot 4H_2O$, respectively. The metals were added one by one and their concentrations were gradually increased (Table I). The initial Zn and Cd concentrations were below the toxic values for SRB reported in the literature (Hao et al., 1994; Kaksonen et al., 2004). The amount of each metal (Fe, Zn, and Cd) fed to the reactor was used to estimate the theoretical production of metal sulfides according to the following stoichiometry: 1 g of Fe, Zn, or Cd would require 0.57, 0.49, or 0.28 g of sulfide to form the corresponding metal sulfide FeS, ZnS, or CdS. Then all the calculated theoretical mass of metal sulfides for each period and for each metal was summed to obtain the total mass of metal sulfides that should be recovered, which was a total of 156 g. The pH of the influent was adjusted between 5.0 and 6.0 to ensure a complete solubilization of the metals (Stumm

Table I. The operational conditions for the sulfate reduction and metal precipitation in the down-flow fluidized bed reactor operated at ambient temperature (18–26°C).

	Experimental periods					
Parameter	I	II	III	IV	V	VI
Operation days	0–55	56–86	87–126	127–177	178–258	259–318
Influent COD (g/L)	2.5	2.5	2.5	2.5	2.5	2.5
COD ratio ethanol/lactate[a]	2:1	1:0	1:0	1:0	1:0	1:0
HRT (day)	2	1.5	1	1	1	1
Organic loading rate (g COD/L-day)	1.25	1.66	2.5	2.5	2.5	2.5
Sulfate loading rate (g SO_4^{2-}/L-day)	1.5	2.25	3.0	3.0	3.0	3.0
Influent pH	5.0	5.0	6.0–5.0	6.0	6.0	6.0
Fe^{2+} loading rate (mg/L-day)	—	—	—	104	104–140	140–320
Zn^{2+} loading rate (mg/L-day)	—	—	—	—	20–40	40–220
Cd^{2+} loading rate (mg/L-day)	—	—	—	—	—	5–20

[a]Ratio was changed to 1:0 on day 37.

and Morgan, 1996). The performance of the reactor was evaluated for COD and sulfate removal, dissolved sulfide production and removal/precipitation of soluble Fe, Zn, and Cd as metal sulfides. The recovery of the metal sulfides precipitate in the system was conducted collecting the solids produced during periods of 7 days. The precipitate was dried at 105°C and then incinerated at 550°C for 1 h to eliminate all the volatile compounds. The ash content was used for the fixed solids balance; it was assumed that all the ashes after combustion of the solids at 550°C were metal sulfides.

Sulfate Reducing Activity Assays

The sulfidogenic activity is an important biological parameter that gives information about the microbial performance and the metabolic properties of the biofilm. The rate of sulfide production and the biomass adhered to the support were monitored at several times during the reactor operation. The sulfidogenic activity assays were performed in 70 mL serum bottles with 65 mL of basal medium without trace element solution (Visser et al., 1993), 5 mL of support (biofilm) recently withdrawn from the reactor as inoculum, ethanol as substrate (1 g COD/L), and sodium sulfate to obtain a COD/SO_4^{2-} ratio of 0.67; the pH was adjusted to 6.5 with $NaHCO_3$. All the serum bottles were sealed with rubber stoppers and aluminum crimps, and incubated at 30°C for a period of 3–4 days under shaking at 100 rpm. The activity was calculated from the slope of the sulfide production curves (sulfide concentration vs. time) and the content of volatile solids attached to the support in each bottle. The volatile solids attached to the support are reported as immobilized volatile solids (IVS) per volume of support (g IVS/L_S) and were quantified as volatile suspended solids (VSS) in the serum bottles at the end of the activity assays, after detaching the biofilm from the support by successive washings with deionized water in an ultrasonic bath.

Analytical Methods

For sulfate, COD, acetate, soluble metals and dissolved sulfide analyses the samples were filtered through 0.22 μm membrane (Millipore, Billerica, MA) syringe filters. COD was determined by the close reflux method (APHA, 1998). Sulfate and acetate concentrations were determined by capillary electrophoresis (Agilent 1600A, Agilent Technologies, Santa Clara, CA) using a fused-silica capillary with 72 cm of effective length and 50 μm of internal diameter, after centrifugation, dilution and filtration of the sample. The background electrolyte contained 5 mM 2,6-pyridine dicarboxilic acid and 0.5 mM cetyltrimethylammonium bromide as electro-osmotic flow modifier; the electrolyte pH was adjusted to 5.6 with 1 M NaOH. Before each injection the capillary was preconditioned during 4 min by flushing with the electrolyte. The sample was injected with a pressure of 50 mbar for 6 s. The applied voltage was set at −25 kV and the capillary temperature at 20°C. The detection was carried out with UV indirect detection using a diode-array detector; the signal wavelength was set at 350 nm with a reference at 200 nm. All chemicals were of high purity including the standards; deionized 18 MΩ-cm water was used for the sample and electrolyte preparation. The system control, data collection and analysis were done trough ChemStation B.01.03 (204) software (Agilent Technologies). Dissolved sulfide in the effluent was determined according to the iodimetric method (APHA, 1998), and in the activity assays was analyzed spectrophotometrically by the colorimetric method described by Cord-Ruwisch (1985). The alkalinity was analyzed by titrating recently withdrawn unfiltered samples with 0.1 M HCl to pH 5.8 according to standard methods (APHA, 1998), and the pH was determined in unfiltered samples using a pH electrode. VSS were quantified according to standard methods (APHA, 1998). The concentration of metals in solution was determined with a Perkin Elmer Analyst 400 atomic absorption spectrophotometer (Norwalk, CT), using 1,000 ppm standard solutions (Fisher Chemical, Pittsburgh, PA). The metal

sulfide precipitate was characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis. SEM samples were mounted in an aluminum holder using conductive double-sided adhesive carbon tape and recovered with gold. Samples were observed and analyzed in a Philips XL-30 electronic microscope (Almelo, The Netherlands) equipped with an energy dispersive X-ray analyzer, EDS (EDAX, Mahwah, NJ). The punctual microanalysis was done at counting times of 60 s and 20 kV, using the ZAF method and internal standards. Samples analyzed by XRD were dried at 105°C, grounded with a porcelain pestle in a porcelain mortar, and homogenized to a size <100 μm. The samples were placed in quartz zero background holders, and the identification of the crystalline phases that constituted the metal sulfides was done using a Rigaku DMAX-2200 X-ray diffractometer (Tokyo, Japan), diagrams were constructed in the 2θ range of 5–80°.

Results

COD and Sulfate Removal

Figure 2 shows the time course of the treatment performance of the reactor and the average treatment efficiencies obtained are summarized in Table II. During the first three periods the influent pH was maintained between 5.0 and 6.0, and changes in the composition of the carbon source and hydraulic retention time (HRT) were made. The performance of the sulfidogenic DFFBR was evaluated using an acidic synthetic wastewater that initially consisted of a mixture of ethanol–lactate as organic substrates (2:1, COD basis); afterwards the lactate concentration in the influent was decreased stepwise until ethanol was the only organic substrate in the feed by day 37. During period I the COD and sulfate removal efficiencies were on average 52% and 30%, respectively; sulfide reached a concentration of 230 mg/L. The influent pH of 5 was neutralized by the alkalinity produced by the organic substrates oxidation; effluent pH reached values of 6.9 the first 27 days of operation (Fig. 2B), however by day 40 the alkalinity dropped and the effluent pH reached values near to 6.0.

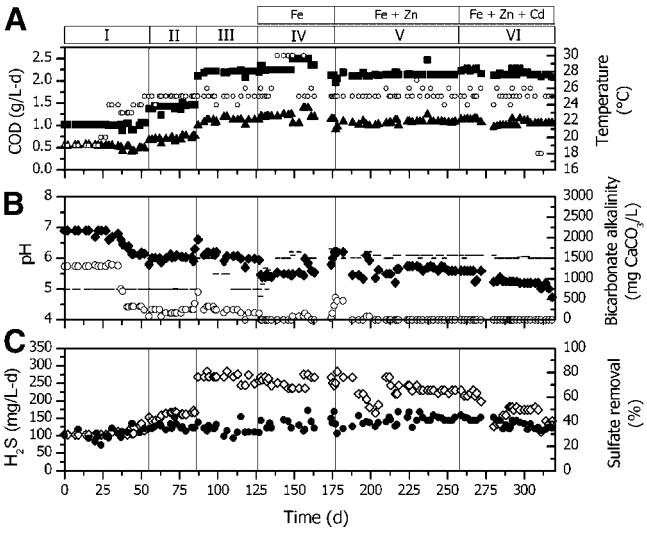

Figure 2. Performance of the sulfidogenic down-flow fluidized bed reactor. **A**: COD loading rate (■), COD removal rate (▲), temperature (○). **B**: Influent pH (–), effluent pH (◆), bicarbonate alkalinity (○). **C**: H_2S production rate (◇) and sulfate removal efficiency (●). See Table I for operational conditions.

In period II, the HRT was decreased to 1.5 days, with the consequent increase in the organic and sulfate loading rates. Under these conditions, the average COD removal efficiency remained around 50% and the sulfate removal efficiency slightly increased up to 36% that resulted in an average sulfide concentration of 250 mg/L; the effluent pH remained at 6.1. Further decrease of HRT to 1 day (period III) and the increase of organic and sulfate-loading rates to 2.5 g COD/L-day and 3 g SO_4^{2-}/L-day, respectively, allowed the COD and sulfate removal efficiencies to remain close to those values obtained during the previous operational period. Nonetheless, the maximum concentration of dissolved sulfide obtained during the reactor operation reached 284 mg/L; at the end of period III the dissolved sulfide concentration was 258 mg/L. At this point, the bicarbonate alkalinity of the system was negligible and consequently the effluent pH decreased to 5.4 (Fig. 2B). From day 127 and onwards, periods IV–VI, the reactor was operated with increasing metal loading rates at similar operational conditions as in period III (Table I). As shown in Figure 2 and Table II, with the addition of metals the COD and sulfate removal efficiencies remained around 50% and 35%, respectively, even at metal loading rates as high as 320 mg/L-day of Fe, 220 mg/L-day of Zn, and 20 mg/L-day of Cd during final operation in period VI. Because of the lack of alkalinity in the system and the acidity generated by the metal precipitation reaction (Eq. 2), the influent pH had to be increased to 6 by the addition of $NaHCO_3$ on day 139 and onwards. It is interesting to note that during period VI when the highest metal loading rates were applied to the system (days 270–318), the DFFBR operated at pH values as low as 5 with stable performance, highlighting the robustness of the system.

Iron, Zinc, and Cadmium Removal

Figure 3 shows the Fe, Zn, and Cd loading rates applied to the DFFBR and metal removal efficiencies as function of time at influent pH of 6. At the beginning of period IV (day 127), an initial Fe loading rate of 104 mg/L-day was applied to the reactor and was increased to 140, 160, and 320 mg/L-day on days 218, 294, and 309, respectively. A similar approach was applied for Zn and Cd; the initial loading rate of Zn was increased from 20 mg/L-day (day 178) to 220 mg/L-day (day 309); for Cd the loading rate was increased from 5 mg/L-day (day 259) to 20 mg/L-day (day 280). The efficiency of metal precipitation was over

Table II. Treatment efficiency during continuous operation of the down-flow fluidized bed reactor for sulfate reduction and metal precipitation (mean ± standard deviation).

Parameter	Experimental periods					
	I ($n=22$)	II ($n=16$)	III ($n=17$)	IV ($n=24^a$ or 20^b)	V ($n=31^a$ or 32^b)	VI ($n=31^a$ or 37^b)
Operation days	0–55	56–86	87–26	127–77	178–258	259–318
COD removal efficiency (%)	52 ± 3.3	50 ± 1.3	54 ± 1.2	50 ± 2.2	50 ± 1.4	50 ± 1.7
SO_4^{2-} removal efficiency (%)	30 ± 3.9	36 ± 2.5	33 ± 4.3	36 ± 4.8	41 ± 3.1	35 ± 1.5
Acetate concentration (g COD/L)	0.43 ± 0.1	0.96 ± 0.1	1.2 ± 0.3	1.2 ± 0.12	1.3 ± 0.2	1.4 ± 0.1
Sulfide concentration (mg/L)	230 ± 17.4	250 ± 28.3	268 ± 14.0	275 ± 14.2	230 ± 39.6	142 ± 15.0
Bicarbonate alkalinity (mg $CaCO_3$/L)	1,314 ± 19.3[c]; 274 ± 72[d]	254 ± 136	227 ± 129	0	0	0
Effluent pH	6.14 ± 0.02	6.1 ± 0.4	5.4 ± 0.5	5.8 ± 0.3	5.6 ± 0.2	5.2 ± 0.2

The values for COD removal ($P < 0.0282$), sulfate removal ($P < 0.0001$), and sulfide concentration ($P < 0.0001$) showed significant differences between periods I and VI at a confidence interval of 95%.
[a]SO_4^{2-} removal efficiency, sulfide and acetate concentration.
[b]COD removal efficiency, alkalinity and pH.
[c]Substrate was ethanol/lactate (2:1, COD basis).
[d]Substrate was ethanol.

99.8% in periods IV and V; at the highest loading rate of each metal (period VI) the removal efficiencies were higher than 99.4% (Fig. 3). The soluble Fe, Zn, and Cd effluent concentrations at the end of period VI (last 9 days), were on average below 1, 0.8, and 0.2 mg/L, respectively.

The metal sulfides precipitate was recovered from the system during different periods. Table III shows the total mass of metals added during different periods of 7 days each, the theoretical metal sulfides mass expected, and the fixed solids content of the mass of metal sulfides precipitate recovered (in percentage). During the addition of metals, about 76–97% of the metal sulfides mass was actually recovered from the system, thus the DFFBR configuration allowed the precipitation and recovery of metal sulfides. At the end of the experiment we recovered 11.1 g of metal sulfides precipitate (as fixed solids) from the conical bottom of the reactor, and from the walls of the column and the flow equalizer.

The XRD diagrams and SEM-EDS images allowed us to verify the chemical composition of the metal sulfides precipitate recovered from the system (Fig. 4). According to the SEM-EDS elemental analysis of the metal precipitate samples, recovered from the system on day 292, the content (mean ± standard deviation, $n = 9$) of S, Fe, Zn, and Cd (wt%) was 43.5 ± 7, 37 ± 3.3, 14 ± 1.2, and 4.1 ± 1.9, respectively. XRD analysis confirmed that the metal crystals were predominantly pyrite (FeS_2), sphalerite (ZnS), and minor quantities of greenockite (CdS). Iron was present in major proportion followed by zinc, and cadmium was observed in minor proportion, iron monosulfides were also identified.

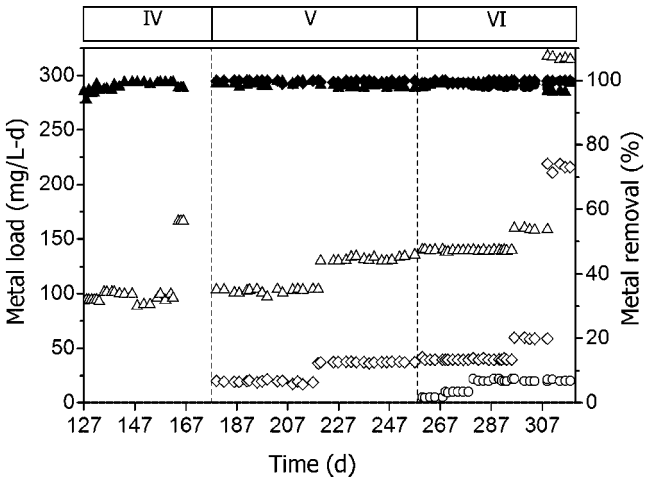

Figure 3. Soluble Fe (Δ), Zn (◇), and Cd (○) loading rates applied to the down-flow fluidized bed reactor, and the Fe (▲), Zn (◆), and Cd (●) removal efficiencies during reactor operation.

Sulfate Reducing Specific Activities of the Biofilm

The performance of the biofilm over the support was followed-up by electron microscope observations and through the sulfate reducing specific activity. The SEM images (results not shown) indicated microbial colonization of the support where cocci and vibrio shaped cells predominated.

The sulfate reducing specific activity was determined at different times during the reactor operation (Fig. 5). Before the addition of metals the sulfate reducing activity increased from 8.5 to 8.9 g COD-H_2S/L-day, indicating an enrichment of sulfate reducing microorganisms. The corresponding H_2S production rates were 17.7 and 20.5 mg H_2S/L-h, respectively. After the addition of metals, the sulfide production rate reached values up to 26 mg H_2S/L-h on day 153 and remained constant around 24 mg H_2S/L-h on days 224 and 293. However, sulfate reduction activity showed a reduction on day 153 to 6.9 g COD-H_2S/L-day. Regarding the solids attached to the support as IVS, it can be appreciated that before the addition of metals the immobilized

Table III. Mass balance of the metal sulfides precipitate recovered from the down-flow fluidized bed reactor on a 7-day basis period.

Operation days (mg)	Total mass of metals added (mg)	Theoretical mass of metal sulfides expected[a]	Mass of metal sulfides recovery (%)
127–133	2,408	3,785	97
179–185	2,804	4,369	86
220–226	3,908	6,071	80
259–265	4,258	6,583	80
269–275	4,331	6,665	82
279–285	4,572	6,981	86
294–300	5,529	8,444	79
309–315	12,676	19,371	76

Mass of metal sulfides recovery percentage was calculated from the fixed solids content of the precipitate.
[a]Theoretical mass of sulfides expected was calculated from the mass of metal added on the 7-day period and the corresponding mass of sulfide that would be required to form the corresponding metal sulfide FeS, ZnS, or CdS.

biomass reached values of up to 1.4 g IVS/L_S; after the addition of metals, the IVS determined in the biofilm at day 153 increased up to 2.2 g IVS/L_S. This increase in the IVS content could be attributed to an overproduction of exopolymeric substances (EPS), and in order to remove the EPS from the biofilm, the samples withdrawn from the reactor on days 224 and 293 were washed with 0.1 M EDTA solution prior to the VSS determination to complex EPS and

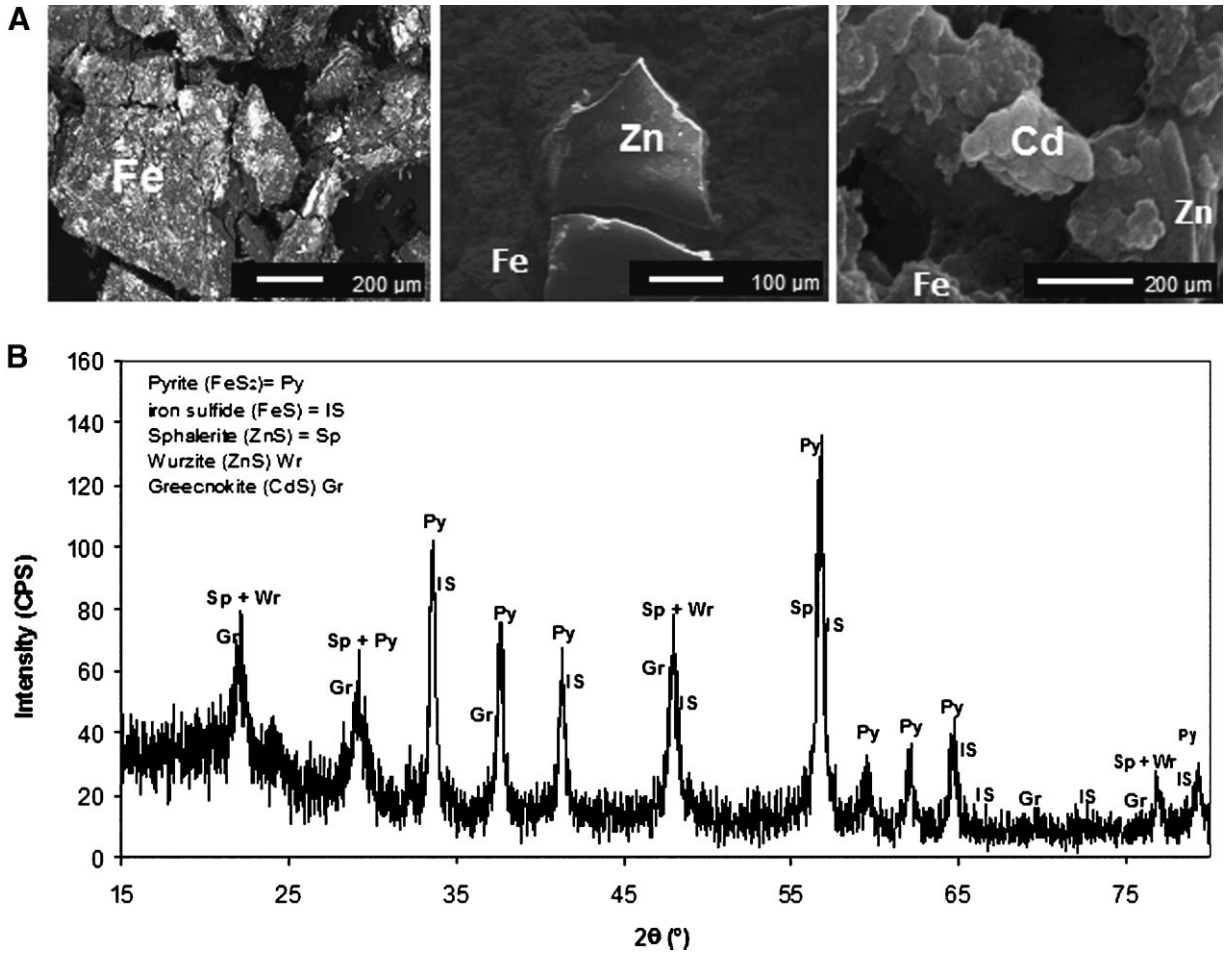

Figure 4. **A**: Scanning electron microscopy images of the metal precipitates recovered from the down-flow fluidized bed reactor during the addition of Fe (**left panel**); Fe and Zn (**middle panel**); Fe, Zn, and Cd (**right panel**). **B**: X-ray diffraction diagram of the precipitate.

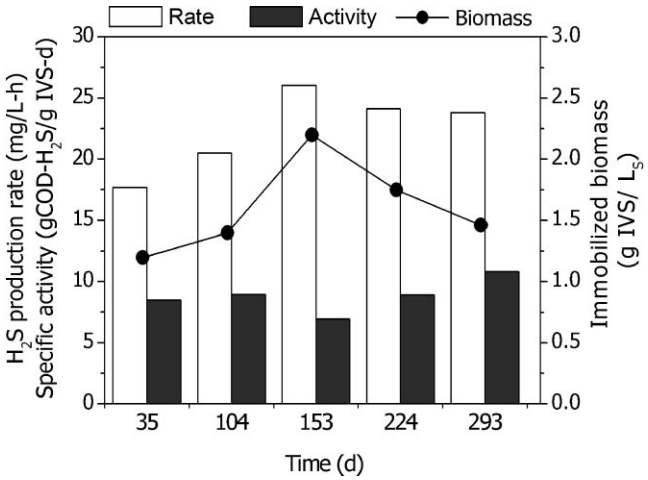

Figure 5. H_2S production rate, specific activity and the biomass adhered to the support at different times during continuous reactor operation.

remove them from the sample, as proposed by Martinez et al. (2000). The value of IVS was 1.46 g IVS/L_S on day 293, which was around the value of IVS obtained before the addition of metals on day 104.

Discussion

Sulfate Reducing Process

The performance of the reactor in periods I–III corroborated that sulfate reduction continued with no problems at initial pH around 5. The decrease of the HRT from 2 to 1 day, did not affect sulfate removal efficiency that remained above 30%, the corresponding sulfate removal rates were 0.4 (period I), 0.81 (period II) and 0.99 g SO_4^{2-}/L-day (period III). After the addition of metals the highest sulfate reduction rate was obtained in period V (1.2 g SO_4^{2-}/L-day), in periods IV and VI sulfate reduction was close to the rate obtained in period III. However, sulfate reduction rates in biofilms tend to be higher, for instance Celis-García et al. (2007) achieved up to 5.3 g SO_4^{2-}/L-day in a similar reactor fed with a mixture of lactate, propionate, and butyrate (12:1:1, respectively in COD basis). Whereas the highest sulfate reduction rates reported by Nagpal et al. (2000) and Kaksonen et al. (2003a) were 6.3 and 2.0 g SO_4^{2-}/L-day for fluidized-bed reactors fed with ethanol and lactate, respectively. In terms of COD, removal was not complete throughout the experiment mainly due to the accumulation of acetate, which amounted to almost half of the COD in the feed (Table II). This acetate accumulation was most probably due to the presence of sulfate reducers that incompletely oxidize the substrate to acetate and CO_2 as shown in Equations (4) and (5); members of the genus *Desulfovibrio* carry out this type of reactions. The presence of *Desulfovibrio* in the inoculum used for this experiment was confirmed by molecular phylogenetic analysis (Celis et al., submitted).

$$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O \quad (4)$$

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2HCO_3^- \quad (5)$$

On the other hand, Nagpal et al. (2000) reported the accumulation of acetate in the operation of a fluidized bed fed with ethanol, at HRT between 55 and 18.5 h acetate represented about half of the COD supplied as ethanol; however in their experiment, sulfate was reduced between 90% and 68% in the same HRT range. The accumulation of acetate has not only been observed in biofilm reactors, and it seems that is an inherent problem of sulfidogenic reactors in which the presence of methanogens or other acetate-consumers is negligible. Incapacity of acetate-utilizing sulfate reducers to compete for sulfate has been proposed as the main reason for the predominance of sulfate reducers that use substrates incompletely (Nagpal et al., 2000). Nevertheless, studies concerning the competition between acetate-utilizing sulfate reducers and SRB that oxidize the substrate incompletely have to be done more profoundly for biofilms.

In the introduction we mentioned that the alkalinity produced by the biological oxidation of substrates would increase the pH of the effluent and thus contribute to the neutralization of the acidic wastewater. Contrary to the results expected, we did not observe an increase in the alkalinity, in fact the bicarbonate alkalinity dropped after we suppressed lactate in the feed, the incomplete oxidation of the substrate was the main reason of this drop and the subsequent absence of alkalinity after day 140 (period IV onwards). From Equation (4) we can see that the incomplete oxidation of ethanol yields acetate, sulfide, protons, and no CO_2 (bicarbonate) is produced; in addition the precipitation of metals also yields protons (Eq. 2), this is most probably the reason why the effluent pH was lower than the influent pH in periods IV–VI (Fig. 2B). This study further showed that in spite of the low pH within the reactor (near 5.0), sulfate reduction proceeded satisfactorily because no change in COD removal was noticed. Moreover, no clear change was seen in the sulfate reduction rate of the biofilm (Fig. 5). This is the first report of sulfate reduction and metal precipitation at pH as low as 5.0 using a non-acidophilic bacteria consortium. Kimura et al. (2006) reported sulfate reduction and Zn precipitation at pH 3.8–4.2 using a mixed culture of acidophilic bacteria isolated from an enrichment culture of acidic sediment. Other studies report the treatment of acidic wastewater at an inlet pH of 5.0 or even at 2.5, however the pH in the reactor (effluent) rises to around 7.0 (Jong and Parry, 2006; Kaksonen et al., 2003b), which is mainly due to the alkalinity produced. On the other

hand, sulfate reduction proceeded satisfactorily in an acidifying UASB reactor, after 70 days of continuous operation at 55°C and controlled pH of 5 sulfate removal amounted to 70%, however no metal precipitation was assayed in this work (Lopes, 2007).

Metal Sulfides Recovery

The present study is the first to report both the possibility of metal sulfides recovery separated from the biomass and a fixed solids balance of the precipitate in one stage sulfate-reducing process for acidic wastewater treatment. In previous studies, metal precipitation and sulfate reduction took place in separate reactor units (de Vegt et al., 1998; Sierra-Alvarez et al., 2007) or took place in the same reactor but the precipitate was mixed with the biomass (Kaksonen et al., 2004) and it is not easy to recover it. In our study up to 97% of the precipitate was mainly recovered from the bottom of the reactor and the equalizer, and the biomass remained at the top of the reactor. However, not all the precipitate could be recovered, and when Zn and Cd were added to the reactor the recovery was below 90% (Table III). This could be due to the formation of colloidal precipitate, fine particles with poor settling properties, and to the low pH in the reactor, which may increase the solubility of the metal sulfides. In experiments done in a chemostat, Bhagat et al. (2004) observed that after precipitation of iron with a gas containing H_2S, the concentration of iron in solution increased due to the low pH value (around 2.0). On the other hand, Esposito et al. (2006) found that the efficiency of ZnS precipitation decreased at pH 5.0, in a chemostat fed with $ZnSO_4$ and Na_2S. Thus, although the concept of recovering the precipitates in the bottom of the DFFBR was feasible, the precipitate recovery balance (Table III) showed that the reactor configuration needs to be modified to enhance the recovery of the particles. A wider conical bottom could reduce the down-flow velocity and maybe allow the formation of larger particles. Once the reactor was stopped, we found 19.4 g of precipitate accumulated in the bottom of the reactor and in the wall. The ash content of the precipitate was 57%, thus 11.1 g were fixed solids. The total amount of metal sulfides that should be recovered at the end of the operation was estimated to be 156 g. From the ash content of recovered precipitate during the three operational periods with the addition of metals, the total amount of fixed solids was estimated to be 130.3 g of solid metal sulfides. Adding the 11.1 g of fixed solids found at the end of operation, the total metal sulfides, as fixed solids, recovered amounted to 141.4 g. The difference between the experimental recovery and the theoretical metal sulfides amount expected is 14.6 g, and therefore, 90% of metal sulfides were recovered from the DFFBR.

The construction of the Pourbaix diagrams (not shown) for each metal predicted that at pH equal or minor than 6.0, the metals would be dissolved and would not precipitate as carbonates or hydroxides in the feed. For all metals over 98% precipitated at a HRT of 1 day and pH 6.0 (Fig. 3) and the presence of FeS, ZnS, and CdS was corroborated by XRD analysis and SEM-EDS. The precipitate was composed by metal sulfides only, no metal hydroxides or carbonates crystalline phases were found as predicted by the Pourbaix diagrams. It is important to note that in the majority of the works related with metal precipitation through sulfate reduction, the precipitation of metals is calculated from the soluble metals concentration in the influent and effluent of reactors, and thus the precipitation efficiencies are over 98% (Bhagat et al., 2004; Kaksonen et al., 2004), even at low (8°C) and high (69°C) temperatures (Sahinkaya et al., 2007), which is in accordance with the results presented in Figure 3.

Biofilm Performance

The biofilm was responsible of the sulfate reducing process as showed by the sulfate-reducing rates. The inhibitory concentrations to SRB reported for Zn and Cd are in the range of 13–65 and >4–112 mg/L, respectively (Kaksonen and Puhakka, 2007). The Cd and Zn loading rates of 20 and 220 mg/L-day, respectively, did not affect the sulfate reducing rates of the biofilm mainly due to the hydraulic characteristic (recirculation) of the reactor. The formation of EPS most probably helped the biofilm to tolerate the presence of toxic metals (Kaksonen and Puhakka, 2007).

To compare the biofilm performance with other types of microbial aggregates, the specific sulfate reducing activity value was considered. Before metal addition (day 35) the sulfate reducing specific activity was 8.4 g COD-H_2S/g IVS-day and increased to 10.8 g COD-H_2S/g IVS-day, at day 293 when Fe, Zn, and Cd were already added. These results indicated that the biofilm had a higher sulfate reducing activity when comparing the values obtained with those of sulfidogenic granular sludge (0.45–2.1 g COD-H_2S/g VSS-day) with volatile fatty acids as electron donors (Omil et al., 1996; Visser et al., 1993) and with that of 0.92 g COD-H_2S/g IVS-day reported for a sulfidogenic biofilm developed in a DFFBR using volatile fatty acids as electron donors (Celis-García et al., 2007).

Conclusions

The DFFBR showed to be suitable for the precipitation and recovery of metal sulfides produced during its continuous operation. In the present study the DFFBR operated at influent pH values as low as 5, and no inhibitory effects on the SRB were observed as indicated by the performance of the reactor. Nonetheless, influent pH values below 5 may hinder the sulfidogenic activity of the SRB, providing that there is not enough alkalinity produced by the system. Consequently, a complete oxidation of the organic substrate by SRB, and neutralization of the water by the alkalinity produced, is essential if the pH of the wastewater is 5 or less.

Even when more research is needed to know the limits of the DFFBR system (e.g., metal levels in the influent and final effluent, minimum allowable HRT, etc.), the DFFBR has a

potential application for the treatment of effluents that contain metals which react immediately with the produced sulfide and form insoluble metal precipitates that can be recovered easily at the bottom of the reactor, separated from the biomass.

This research was financially supported by the project SEP-CONACyT-46506. The authors acknowledge the technical assistance of Dulce Partida Gutiérrez and Guillermo Vidriales Escobar.

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