

Evaluation of feed COD/sulfate ratio as a control criterion for the biological hydrogen sulfide production and lead precipitation

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Abstract

The ability of sulfate-reducing bacteria to produce hydrogen sulfide and the high affinity of sulfide to react with divalent metallic cations represent an excellent option to remove heavy metals from wastewater. Different parameters have been proposed to control the hydrogen sulfide production by anaerobic bacteria, such as the organic and sulfate loading rates and the feed COD/SO₄²⁻ ratio. This work relates the feed COD/SO₄²⁻ ratio with the hydrogen sulfide production and dissolved lead precipitation, using ethanol as carbon and energy source in an up-flow anaerobic sludge blanket reactor. A maximum dissolved sulfide concentration of 470 ± 7 mg S/L was obtained at a feed COD/SO₄²⁻ ratio of 2.5, with sulfate and ethanol conversions of approximately 94 and 87%, respectively. The lowest dissolved sulfide concentration (145 ± 10 mg S/L) was observed with a feed COD/SO₄²⁻ ratio of 0.67. Substantial amounts of acetate (510–1730 mg/L) were produced and accumulated in the bioreactor from ethanol oxidation. Although only incomplete oxidation of ethanol to acetate was observed, the consortium was able to remove 99% of the dissolved lead (200 mg/L) with a feed COD/SO₄²⁻ ratio of 1.5. It was found that the feed COD/SO₄²⁻ ratio could be an adequate parameter to control the hydrogen sulfide production and the consequent precipitation of dissolved lead.

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Keywords: Hydrogen sulfide; Sulfate-reducing bacteria; Lead precipitation; Ethanol; Acetate

1. Introduction

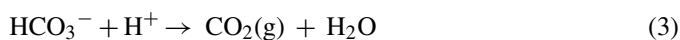
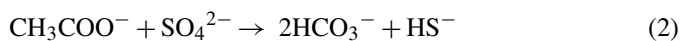
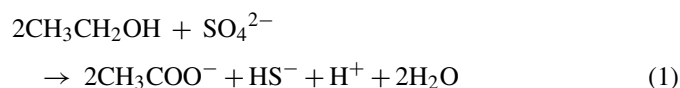
Mining industry, mineral processing, industrial effluents and landfill leachate are the principal sources of wastewaters containing high dissolved metal concentration. In the last two decades, the biological sulfate reduction to hydrogen sulfide has been proposed to remove metals from this type of wastewater. This process has been widely studied for the precipitation of heavy metals such as Cu, Ni, Zn, Fe, Cd, Hg, Mn, Co and Pb [1–7]. The main advantages of metal precipitation by hydrogen sulfide produced from the biological sulfate reduction include: (i) instantaneous metal-sulfide complex formation; (ii) low solu-

bility of the metallic sulfides compared to other metal complex compounds (hydroxides, carbonates, chlorides, etc.) and (iii) low sludge production.

Under anaerobic conditions, sulfate-reducing bacteria (SRB) can transform sulfate to hydrogen sulfide using simple substrates such as hydrogen, lactate, ethanol, acetate, methanol and propionate as electron donors [8–10]. Complex substrates, such as cellulose, starch, molasses and sewage [8,11,12] have also been used. The use of ethanol as primary electron donor has been proposed due to its easy availability and low cost [2]; furthermore, in accordance with White and Gadd [13], ethanol is a carbon source more effective than acetate in the stimulation of hydrogen sulfide production. Depending on the SRB species, ethanol can be incompletely oxidized to acetate (reaction (1)), or completely oxidized to CO₂ (reactions (1)–(3)) [9,14,15]. In the treatment of metal containing effluents, the abiotic metal

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precipitation (reaction (4)) is a function of the biogenic hydrogen sulfide production, which is related to ethanol and acetate oxidation as primary and secondary electron sources by sulfate reduction (reactions (1) and (2)).



where M^{2+} represents divalent metallic cations such as Pb^{2+} .

The application of SRB-based systems to remove heavy metals from wastewater has been tested in single and two-stage configurations. In single-stage systems, biological sulfate reduction to hydrogen sulfide and metal precipitation steps occur simultaneously in the same reactor, whereas in two-stage configuration, the steps take place separately in different reactors [4].

Many studies have shown high metal removal efficiencies in single-stage systems under continuous operation [3,5,16–18]. However, in these systems special care is necessary to avoid the inhibition of the SRB with high hydrogen sulfide concentration. In addition, metal ion inhibition may occur with elevated dissolved metal concentrations. For instance, Sani et al. [19] observed a negative effect on *Desulfovibrio desulfuricans* of lead at concentrations greater than 3 mg/L.

Several parameters, such as the selection of the organic substrates, the feed $\text{COD}/\text{SO}_4^{2-}$ ratio and the organic and sulfate loading rates, have been proposed to control the biogenic hydrogen sulfide production [12,20,21]. However, there are few studies relating hydrogen sulfide production with the precipitation of dissolved lead.

The aim of this work was to evaluate the feed $\text{COD}/\text{SO}_4^{2-}$ ratio, using ethanol as sole carbon source, as the control criterion for hydrogen sulfide production by SRB to precipitate dissolved lead in an up-flow anaerobic sludge blanket (UASB) reactor.

2. Methods and materials

2.1. Microorganisms and reactor operation

An UASB reactor (5 L, $\emptyset 15$ cm) with a recirculation flow rate (F_R) of 2.5 L/h (Fig. 1) was used to evaluate the hydrogen sulfide formation, ethanol oxidation and lead removal. The UASB reactor was operated at a hydraulic retention time (HRT) of 4 days during the continuous flow experiment. The UASB was installed in a controlled temperature room at 30 °C and operated for 520 days. The pH was maintained automatically at 7.0 ± 0.5 by continuous titration with 0.5N NaOH.

The UASB was inoculated (10%, v/v) with granular sludge obtained from the wastewater treatment pilot plant located at Metropolitan Autonomous University-Iztapalapa (Mexico City), and previously adapted to sulfate reduction with ethanol

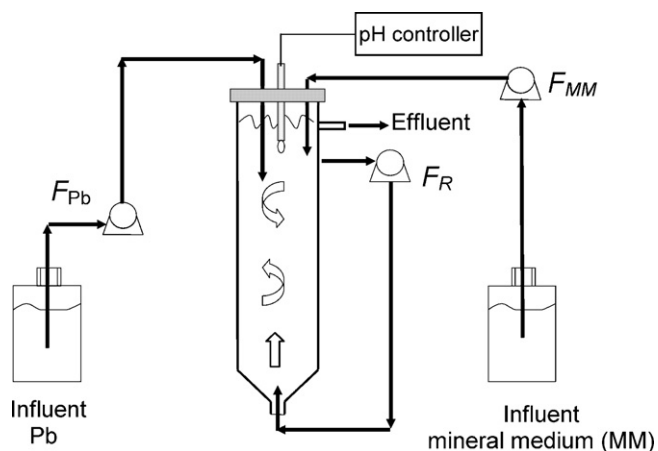


Fig. 1. Schematic diagram of the up-flow anaerobic sludge blanket (UASB) reactor.

as electron donor for 3 months in a batch reactor. The composition of the mineral medium was (g/L): 2.22 NaSO_4 , 0.28 NH_4Cl , 0.17 KH_2PO_4 , 0.01 CaCl_2 , and 0.15 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. The pH of the mineral medium was adjusted to 7 with NaOH 0.5N.

2.2. Evaluation of hydrogen sulfide production at different molar feed $\text{COD}/\text{SO}_4^{2-}$ ratios

To obtain the maximum hydrogen sulfide production in the continuous flow UASB reactor, different feed $\text{COD}/\text{SO}_4^{2-}$ ratios were evaluated. The sulfate concentration (1500 mg/L) was maintained constant during the whole experiment and the ethanol concentration was varied in the medium (475, 720, 1080, 1440 and 1800 mg/L) to obtain feed $\text{COD}/\text{SO}_4^{2-}$ molar ratios of 0.67 (corresponding to the stoichiometric ratio), 1.0, 1.5, 2.0 and 2.5, respectively. Each ratio was evaluated for about 25 days.

2.3. Acetate consumption rate

To determine the acetate consumption rate, the UASB reactor was operated in batch regime with an initial acetate concentration of 500 mg/L as sole carbon source ($\text{COD}/\text{SO}_4^{2-}$ ratio of 0.67). The initial sulfate concentration was 1500 mg/L. The acetate oxidation was followed for 4 days, corresponding to one HRT in a continuous flow experiment. The batch test was repeated twice.

2.4. Evaluation of lead precipitation in the continuous flow UASB

A feed $\text{COD}/\text{SO}_4^{2-}$ ratio of 1.5 was used to determine the efficiency of lead precipitation. A solution of lead nitrate ($\text{Pb}(\text{NO}_3)_2$) was fed to the UASB reactor for 380 days. Feed lead concentrations from 20 to 200 mg/L, with increments of 20 mg/L, were evaluated. The solution of lead nitrate was previously adjusted to a pH of 5.0 with 0.5N nitric acid. To avoid the abiotic precipitation of soluble lead with sulfate and phosphate, the mineral medium and the lead solution were separately fed to the reactor using a peristaltic pump. The flow rate for both solu-

tions (F_{Pb} and F_{MM}) was 1.25 L/day corresponding to a HRT of 4 days.

2.5. Analyses

Duplicate samples of sulfate, dissolved sulfide and dissolved lead were measured from the influent and effluent streams. Before each analysis, the samples were filtered through a 0.45 μm nitrocellulose membrane syringe filters. Sulfate concentration was determined at 420 nm in accordance with the EPA-9038 method (SW-846, 1996). Dissolved sulfide was measured at 480 nm according with the Cord-Ruwish method [22]. Both sulfur compounds were measured using a Lambda II Spectrometer (PerkinElmer, USA).

Ethanol and acetate were determined by gas chromatography (Hewlett Packard 5890, Series II, USA) after adding 50 μL of HCl (50%) in 1000 μL of sample. The GC was equipped with a 10 m \times 0.53 mm (i.d.) capillary column and a flame ionization detector. The injector and detector temperatures were 130 and 150 $^{\circ}\text{C}$, respectively; the oven temperature program was 80 $^{\circ}\text{C}$ for 1.5 min, then the temperature was increased to 120 $^{\circ}\text{C}$ at a rate of 50 $^{\circ}\text{C}/\text{min}$ and 5 min at 120 $^{\circ}\text{C}$. Nitrogen was used as carrying gas (5 mL/min).

The dissolved lead concentration was measured by atomic absorption spectrometry (SpectraAA 200-VARIAN, Australia) by direct aspiration according to the EPA-7420 method (SW-846, 1996). The oxidation–reduction potential (ORP) was measured with a platinum probe and an Ag/AgCl reference electrode (EW-27018-40, Cole Parmer, Vernon Hills, IL, USA).

3. Results and discussion

3.1. Hydrogen sulfide production at different molar feed $\text{COD}/\text{SO}_4^{2-}$ ratios

Fig. 2 shows the effect of different feed $\text{COD}/\text{SO}_4^{2-}$ ratios on the ethanol and sulfate concentrations, besides the production of hydrogen sulfide measured as dissolved sulfide and acetate during the continuous operation of the UASB reactor. These results indicate that when the feed $\text{COD}/\text{SO}_4^{2-}$ ratio increased, hydrogen sulfide production was improved, whereas ethanol was incompletely oxidized to acetate. Negligible gas production was observed in the system.

The lowest hydrogen sulfide production of 145 ± 10 mg S/L with a sulfate reduction of $38.2 \pm 1.3\%$ was obtained with a feed

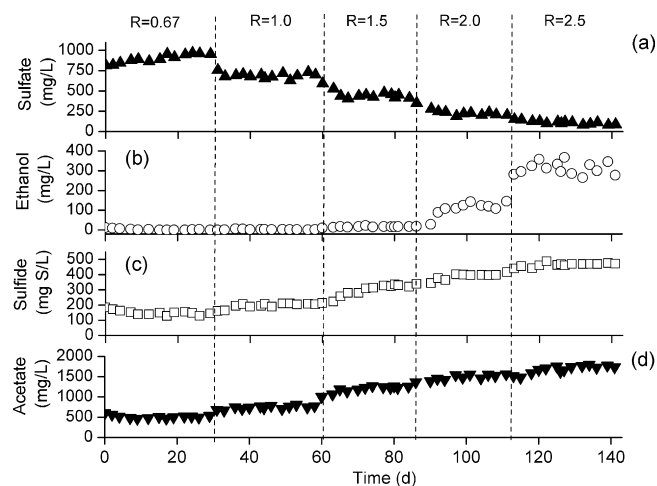


Fig. 2. Effect of different feed $\text{COD}/\text{SO}_4^{2-}$ ratios on: (a) effluent sulfate, (b) effluent ethanol, (c) sulfide production and (d) acetate production in the UASB during 142 days, where $R = \text{feed } \text{COD}/\text{SO}_4^{2-}$ ratio.

$\text{COD}/\text{SO}_4^{2-}$ ratio of 0.67 (stoichiometric ratio for the complete ethanol oxidation to CO_2) (reactions (1) and (2)). When the ratio was increased to 2.5, the hydrogen sulfide production improved up to a maximum of 470 ± 7 mg S/L with sulfate reduction of $94.0 \pm 1.2\%$ (Fig. 2). This result was similar to that obtained by Damianovic and Foresti [23], who reported sulfate removal efficiencies over 91% at $\text{COD}/\text{SO}_4^{2-}$ ratios equal to or higher than 2.5 for sulfate concentrations up to 1960 mg/L and a mixture of volatile fatty acids as carbon source in a horizontal-flow anaerobic reactor.

From Table 1, the lowest ORP values were observed when the highest concentrations of dissolved sulfide were reached. Since at a feed $\text{COD}/\text{SO}_4^{2-}$ ratio of 2.5 most of the sulfate in the influent was converted to hydrogen sulfide, higher feed $\text{COD}/\text{SO}_4^{2-}$ ratios were not evaluated (Fig. 2a). Choi and Rim [24] reported that at $\text{COD}/\text{SO}_4^{2-}$ ratios exceeding 2.7, a negative effect on the SRB activity can be observed because methanogenic archaea (MA) can compete with SRB for hydrogen and acetate. El Bayoumy et al. [20] suggested that $\text{COD}/\text{SO}_4^{2-}$ ratios between 1.5 and 2.25 were enough to attain the highest hydrogen sulfide production when lactate or acetate was used as carbon and electron sources.

Molar sulfide yield from sulfate (hydrogen sulfide produced/sulfate consumed) increased at feed $\text{COD}/\text{SO}_4^{2-}$ ratios higher than 1.5 (Table 1), suggesting that at lower feed

Table 1

Molar dissolved sulfide and acetate yields and redox potential at different feed $\text{COD}/\text{SO}_4^{2-}$ ratios for steady state conditions in continuous flow experiment without Pb

$\text{COD}/\text{SO}_4^{2-}$ ratio	$Y_{\text{Sulfide/sulfate}}^a$ (%)	$Y_{\text{Sulfide/ethanol}}^a$ (%)	$Y_{\text{Sulfide/available electrons}}^b$ (%)	$Y_{\text{Acetate/ethanol}}^a$ (%)	Redox (mV)
0.67	73 ± 5	42 ± 2	61.7	81 ± 4	-279 ± 4
1.0	75 ± 3	41 ± 2	63.2	85 ± 3	-314 ± 3
1.5	87 ± 4	42 ± 2	68.7	88 ± 3	-343 ± 5
2.0	90 ± 1	42 ± 1	67.4	87 ± 3	-359 ± 4
2.5	97 ± 1	43 ± 1	66.6	87 ± 3	-383 ± 3

^a Mol produced/100 mol consumed.

^b Available electrons were calculated from the ethanol converted to acetate ($2e^-/\text{mol}$) and to CO_2 ($6e^-/\text{mol}$).

Table 2
Ethanol and acetate oxidation rates in continuous flow and batch experiments without Pb

Experiment	Ethanol consumed (mg/(L d))	Acetate produced (mg/(L d))	Acetate consumed (mg/(L d))	Sulfate consumed (mg/(L d))	Dissolved sulfide produced (mg/(L d))
Ethanol ^a	120 ± 7	126 ± 6	29 ± 2	146 ± 9	37 ± 2
Acetate ^b	0.0	0.0	25 ± 2	52 ± 5	14 ± 1

^a Data from continuous experiment (initial ethanol concentration of 450 mg/L).

^b Data from batch experiments (initial acetate concentration of 500 mg/L).

COD/SO₄²⁻ ratios other sulfur species may have been produced. This corresponds to the conditions where the ORP was higher (Table 1) favoring the formation of more oxidized forms such as polysulfides, thiosulfate and elemental sulfur, which may be formed abiotically [25]. These results showed that at feed COD/SO₄²⁻ ratios higher than 1.5, the fraction of available electrons transferred to sulfate during the hydrogen sulfide production, was not affected by the incomplete ethanol oxidation.

On the other hand, molar sulfide yield from ethanol was around 42% and its value was independent of the feed COD/SO₄²⁻ (Table 1), indicating that hydrogen sulfide production increased as a function of ethanol consumption. A similar behavior was observed for the sulfide yield from available electrons with a value of approximately 65%.

Ethanol removal was close to 100% at feed COD/SO₄²⁻ ratios between 0.67 and 1.5. At higher ratios (2 and 2.5), ethanol was detected in the effluent, reaching an oxidation of 91 and 84%, respectively (Fig. 2b). This accumulation of ethanol can be attributed to the sulfate-limiting conditions (Fig. 2a).

Substantial concentrations of acetate (510 to 1730 mg/L) were observed from ethanol oxidation during steady state conditions with all tested feed COD/SO₄²⁻ ratios (Fig. 2d). The acetate concentration increased proportionally to the feed COD/SO₄²⁻ ratio; but the acetate yield from ethanol (86% ± 3) did not change significantly (Table 1). The incomplete oxidation of ethanol to acetic acid decreased the pH (reaction (1)) and chemical neutralization was necessary during the 142 days of operation.

The acetate accumulation in the bioreactor indicated a poor acetotrophic activity, probably due to the low concentration of this type of bacteria. Lens et al. [8] reported that the acetate oxidation to CO₂ is mainly associated to the methanogenic activity, when organic material is available at feed COD/SO₄²⁻ ratios higher than 0.67. However, in the present study substantial acetate accumulation in the system was observed, indicating that methanogenesis was negligible. Some studies [5,9] report that in ethanol supplemented systems, methane production is not observed, although it is well established that during the anaerobic treatment of sulfate-containing wastewater acetotrophic sulfidogenic bacteria and acetotrophic MA compete for hydrogen and acetate [8,24,26]. The absence of methanogenic activity indicated a possible inhibition of MA by the high hydrogen sulfide concentration in the system (Fig. 2c). Lens et al. [8] reported that hydrogen sulfide toxicity was higher for MA than for SRB. Visser et al. [26] report that the inhibitory effect of hydrogen sulfide concentration depends on many factors, including

the bacterial species, the biomass form (granular or suspended sludge) and pH. In our study the lowest dissolved sulfide concentration was around 145 mg S/L, which has been reported to be inhibitory for MA [27].

An acetate oxidation rate of 25 ± 2 mg/(L d) was achieved when acetate was used as sole carbon source (in the absence of ethanol) in a batch experiment (Table 2). This result confirms that the system had a low acetotrophic bacterial activity as compared to acetate production from the incomplete oxidation of ethanol thus provoking acetate accumulation. The acetate oxidation rate in batch experiments (without ethanol) was similar to the value observed in continuous operation with ethanol (25 ± 2 mg/(L d) and 29 ± 2 mg/(L d), respectively) (Table 2), indicating that acetate oxidation was the rate limiting step in the sulfidogenic ethanol oxidation, as it has been previously demonstrated [2,5,9].

Madigan et al. [14] reported that dissimilatory SRB can be divided in two broad physiological groups: (i) SRB that utilize lactate, pyruvate, ethanol, and certain fatty acids (*Desulfovibrio*, *Desulfomonas*, *Desulfobulbus*, *Desulfomonile*, *Desulfobotulus*, *Desulfomicrobium*, *Desulfotomaculum* and *Desulfobacula*); and (ii) SRB specialized in the oxidation of fatty acids, especially acetate (*Desulfonema*, *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, *Desulfobacterium*, *Desulfacinum* and *Desulforhabdus*). Both bacterial groups have been commonly reported to grow in media containing ethanol as electron donor [15]. According with our results, we suggest that the consortium can be classified in the first physiological group. Also, Nagpal et al. [9] reported that ethanol was converted to acetate by *D. desulfuricans* without a substantial CO₂ production.

The incomplete oxidation of ethanol was compensated by adding an excess of ethanol (up to 1800 mg/L) to obtain the highest hydrogen sulfide production at a feed COD/SO₄²⁻ ratio of 2.5, with a sulfate reduction of 94 ± 1% (Fig. 2). Complete oxidation of ethanol to CO₂ can generate three times more hydrogen sulfide as compared to the incomplete oxidation to acetate (reactions (1) and (2)). Kaksonen et al. [5] showed that the stoichiometric COD/SO₄²⁻ ratio of 0.67 was adequate to attain around 60% of sulfate reduction with an initial sulfate concentration of 2000 mg/L in a fluidized-bed reactor inoculated with SRB capable to completely oxidize ethanol to CO₂.

3.2. Hydrogen sulfide production and lead precipitation

For lead precipitation studies, a feed COD/SO₄²⁻ ratio of 1.5, and a sulfate concentration of 1500 mg/L were used. Under these conditions, based on the results depicted in Fig. 2,

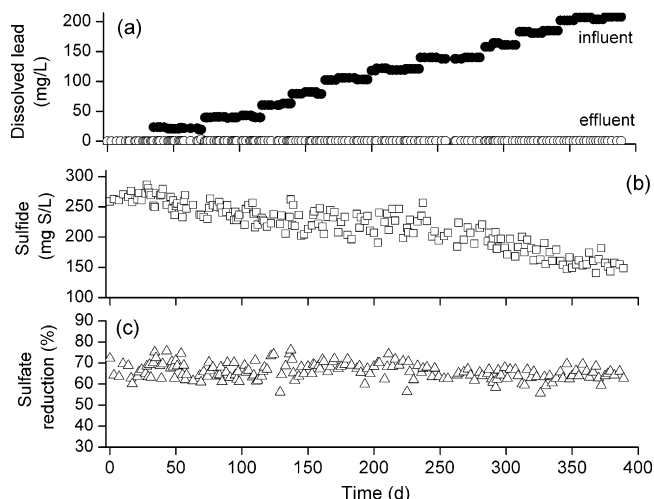


Fig. 3. UASB reactor performance during 380 days at a feed COD/SO₄²⁻ ratio of 1.5: (a) dissolved lead removal, (b) residual dissolved sulfide and (c) sulfate reduction.

a dissolved sulfide concentration about 330 mg S/L could be expected, which is enough to precipitate dissolved lead concentrations up to 200 mg/L. Kaksonen et al. [2] reported 99.8% of Zn (240 mg/L) and Fe (57 mg/L) removal and residual dissolved sulfide concentration of around 300 mg/L after metal precipitation.

Fig. 3a, shows the lead precipitation during the continuous operation of the UASB reactor with different feed lead concentrations. During 380 days, the dissolved lead concentration was gradually increased from 20 to 200 mg/L. It was observed that the dissolved lead concentration remained below 0.2 mg/L in the effluent throughout the study, indicating that the lead removal efficiency was >99% independently from the feed concentration. The effluent dissolved sulfide concentration decreased, as expected (Fig. 3b), due to the reaction of dissolved sulfide with lead (reaction (4)).

From a mass balance, a difference not attributable to the lead precipitation was observed between the theoretical (calculated by sulfate conversion) and the measured residual dissolved sulfide (Fig. 4). In addition, this difference was higher when the

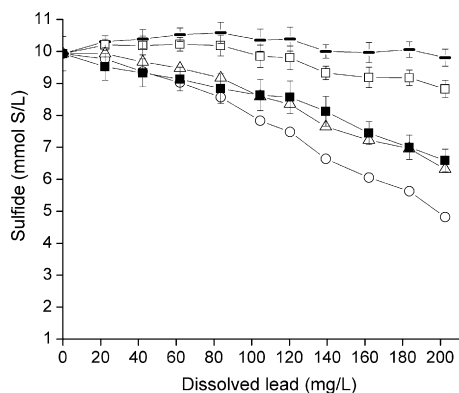


Fig. 4. Sulfide balance. Comparison of experimental residual dissolved sulfide (■) vs. theoretical dissolved sulfide considering no lead removal (●), lead removal (□), lead removal and nitrate conversion of 60% (△) and 100% (○), evaluated according to reaction (5).

inlet lead concentration increased. This result suggests that a fraction of the produced hydrogen sulfide was oxidized to other sulfur compounds by a chemical and/or biological process. A probable explanation is the biological oxidation of the excess of dissolved sulfide to sulfur or sulfate through an autotrophic denitrification process favored by the presence of nitrate (from lead nitrate) and the microbial diversity found in the wastewater anaerobic sludge [28–31]. The excess of dissolved sulfide (sulphide/nitrogen ratio > 5), may have promoted the partial oxidation to elemental sulfur (observed on the wall of the UASB as a white layer) according to the following reaction [28]:



Although the denitrification process was not evaluated in this work, its effect on dissolved sulfide consumption was estimated. Fig. 4 shows that the residual dissolved sulfide would be equivalent to the theoretical value considering 60% nitrate removal and the sulfide removal ratio according to reaction (5).

As ethanol was almost completely consumed ($98 \pm 2\%$) and the acetate yield from ethanol remained relatively constant ($87 \pm 4\%$) during this period (data not shown), it is suggested that heterotrophic denitrification was negligible and that autotrophic denitrification was probably the main pathway for sulfide oxidation. Reyes-Avila et al. [28] observed sulfide and nitrate removal under batch conditions with acetate. They suggested that the specific sulfide consumption rate increased as the acetate oxidation rate decreased due to the accumulation of nitrite and elemental sulfur. Low nitrite reduction rates were found when acetate was used as substrate.

Although several studies [17,19] have demonstrated the inhibitory effects of dissolved metals on SRB, in this study, sulfate reduction (Fig. 3c) and ethanol oxidation remained approximately constant (68 ± 7 and $98 \pm 2\%$, respectively) during all the experiment, indicating that feed dissolved lead concentrations and the precipitated lead sulfide (PbS, with a solubility product constant of 3×10^{-28}) had no influence in the activity of SRB. It has also been reported than the particles of metal sulfide can serve as support for biomass, improving its retention inside the reactor [5]. To avoid lead inhibition at feed concentrations higher than 200 mg/L, the feed COD/SO₄²⁻ ratio can be increased from 1.5 to 2.5 allowing a high production of hydrogen sulfide thus increasing the lead removal capacity.

On the other hand, the excess of hydrogen sulfide must be controlled. Kolmert et al. [32] recommend that hydrogen sulfide produced by SRB should not greatly exceed the metal ion concentration to avoid the dissolved sulfide inhibition. Some studies have proposed diverse control parameters for the optimum growth of SRB and hydrogen sulfide production. According to El Baoumy et al. [20] organic, nitrogen, phosphorus and sulfate loading rates are the main factors affecting the SRB growth. De Lima et al. [12] suggested a strict control of the available organic load when SBR grow in a continuous bench-scale bioreactor for hydrogen sulfide production.

For a given sulfate concentration in wastewater, the feed COD/SO₄²⁻ ratio can be an useful control for hydrogen sulfide production during the metal precipitation process. The feed

COD/SO₄²⁻ ratio can be selected depending on the concentration of heavy metals and pH of the wastewater. According to the hydrogen sulfide production observed in this study, corresponding to a sulfate concentration of 1500 mg/L (Fig. 2c), a feed COD/SO₄²⁻ ratio of 0.67 could be expected to produce enough hydrogen sulfide to precipitate heavy metals from landfill leachate, usually containing metal concentrations below 1 mg/L. In case of acid mine drainage, which contains heavy metals in the range of 10–1000 mg/L, and supported in the previous results, we can suggest that a feed COD/SO₄²⁻ ratio equal to or higher than 1.5 could be needed. In general, high metal removal efficiencies (about 90%) have been obtained by SRB processes in studies evaluating several divalent cations such as Cu, Co, Cd, Fe, Mn, Ni and Zn [5,16,17]. Kaksonen et al. [5] reached Zn and Fe precipitation rates over 600 and 300 mg/(L d), respectively, in a fluidized-bed reactor. Ma and Hua [16] obtained a Cd removal efficiency of 99.5% (100 mg/L at HRT of 1 h, corresponding to 2400 mg Cd/(L d)) using SRB in a fluidized bed reactor with synthetic wastewater. Jong and Parry [33] reported removal efficiencies greater than 99.5% for Cu, Zn and Ni at initial concentrations of 10 mg/L, using a bench-scale up-flow anaerobic packed bed reactor filled with silica sand with HRT of 16.16 h; they also obtained a sulfate reduction rate about 475 mg/L d. It is important to highlight that precipitation rates are dependent on metal species involved [34]. For lead, El Bayoumy et al. [17] reported 100% removal efficiencies of 40 mg Pb/L at HRT of 20 h by SRB, in an up-flow anaerobic fixed-film reactor.

The lead removal efficiency (>99%) observed in the present study and the performance reported in the studies mentioned above indicate that the biological sulfate reducing process to remove dissolved metals in a single stage system can be potentially used to treat wastewater from mining industry, mineral processing, industrial effluents and landfill leachate.

4. Conclusions

The feed COD/SO₄²⁻ ratio can be an useful parameter to control hydrogen sulfide production in the metal precipitation process when ethanol is used as sole electron donor and carbon source. Although incomplete oxidation of ethanol to acetate was observed, hydrogen sulfide production of 330–470 mg S/L was attained at feed COD/SO₄²⁻ ratios between 1.5 and 2.5. Due to incomplete ethanol oxidation chemical pH neutralization was needed. Excess hydrogen sulfide obtained at a feed COD/SO₄²⁻ ratio of 1.5 was adequate to reach a dissolved lead removal efficiency of >99% with inlet lead concentrations up to 200 mg/L without detectable inhibition of SBR.

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