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Inhibition of sulfate reduction by iron, cadmium and sulfide in granular sludge

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ABSTRACT

This study investigated the inhibition effect of iron, cadmium and sulfide on the substrate utilization rate of sulfate reducing granular sludge. A series of batch experiments in a UASB reactor were conducted with different concentrations of iron (Fe^{2+}, 4.0–8.5 mM), cadmium (Cd^{2+}, 0.53–3.0 mM) and sulfide (4.2–10.6 mM), the reactor was fed with ethanol at 1 g chemical oxygen demand (COD)/L and sulfate to yield a COD/SO_4^{2-} (g/g) ratio of 0.5. The addition of iron, up to a concentration of 8.1 mM, had a positive effect on the substrate utilization rate which increased 40% compared to the rate obtained without metal addition (0.25 g COD/g VSS-d). Nonetheless, iron concentration of 8.5 mM inhibited the specific substrate utilization rate by 57% compared to the substrate utilization rate obtained in the batch amended with 4.0 mM Fe^{2+} (0.44 g COD/g VSS-d). Cadmium had a negative effect on the specific substrate utilization rate at the concentrations tested; at 3.0 mM Cd^{2+} the substrate utilization rate was inhibited by 44% compared with the substrate utilization rate without metal addition. Cadmium precipitation with sulfide did not decrease the inhibition of cadmium on sulfate reduction. These results could have important practical implications mainly when considering the application of the sulfate reducing process to treat effluents with high concentrations of sulfate and dissolved metals such as iron and cadmium.

1. Introduction

Sulfate reduction is an anaerobic biological process that can be used for metal precipitation and sulfate removal. This process is carried out by sulfate reducing bacteria (SRB); the generation of sulfide and alkalinity (Eq. (1)) is the key for its application to precipitate metals from solution as metal sulfides (Eq. (2)).

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

where CH_2O = electron donor (organic matter).

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

where M = metal.

Acid mine drainage (AMD) and the wastewaters from metal processing, mining and petrochemical industries contain high concentrations of sulfate and dissolved metals. Such characteristics make these effluents candidates for the application of biological sulfate reduction for metal precipitation, to minimize the environmental risk caused when these effluents are deposited in aquatic or terrestrial ecosystems [1]. The main advantage of the biological treatment over the chemical treatment of effluents with dissolved metals is the reduction of the bulky sludge that is generated, when hydroxides and carbonates are used to precipitate metals. Moreover, under anaerobic conditions metal sulfides are more insoluble than the corresponding hydroxides or carbonates according to the low solubility product constants of most metal sulfides. For example and as reference, the solubility product constants (K_{sp}) of iron carbonate and iron hydroxide are 2.0×10^{-11} and 7.9×10^{-16}, respectively, whereas the solubility product constant of iron sulfide is 7.9×10^{-19} ($T = 25\,^{\circ}C$, ionic strength = 0) [2], being more insoluble the metal compound with the lowest solubility product constant. Another aspect of AMD treatment to take into account is low pH, however the potential toxic effect of treating an effluent with low pH may be avoided using reactors with water recycling, as this will reduce direct contact between the acidic influent and the microorganisms [3].

The majority of the metals present in AMD are inhibitory or toxic (depending on their concentration) to anaerobic microorganisms including SRB, responsible of the sulfate reduction process. Heavy metals have the tendency to deactivate enzymes because they may react with functional groups, such as sulfhydril (−SH), and can replace cofactors such as Cu(II), Zn(II), Co(II), Ni(II) causing a negative effect over the growth (toxicity) or the metabolic activity of microorganisms (inhibition) [4].

The inhibitory effects of heavy metals, such as zinc and copper, on sulfate reduction have been widely studied [4–7]. Related to cadmium, for a mixed culture of SRB the concentration that

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inhibited sulfate reduction was 0.18 mM [5]; and for a coccus-like, Gram-positive SRB isolate a cadmium concentration of 0.174 mM yielded a sulfate consumption of only 16%, compared with the sulfate consumption obtained (26%) when manganese was present [8]. Cadmium showed to be highly toxic or inhibitory to sulfate reduction compared to other metals such as Zn, Cu, Ni or Mn [5,8]. In contrast, the information concerning the potential inhibitory effect at high concentrations of iron towards sulfate reduction is rather scarce, in spite of being the predominant element present in AMD. Therefore, a better understanding between iron and sulfate reduction is necessary to employ the biological sulfate reducing process effectively for the treatment of mining effluents with high concentrations of iron and sulfate [9].

To what extent SRB are able to tolerate the presence of metals, without the reduction of their metabolic capacity (sulfate reducing activity), highly depends on the metal and its concentration. Moreover is not only the dissolved metal which may cause inhibition, in experiments with zinc and copper it was found that the insoluble metal sulfides can inhibit the biological sulfate reduction process as well [4].

The objective of this work was to investigate the inhibitory effect caused by iron and cadmium in combination with sulfide on the sulfate reducing process of granular sludge. In addition, this study may contribute to a better understanding of metal inhibition towards the sulfate reducing process.

2. Materials and methods

2.1. Bioreactor

Experiments were carried out in one lab-scale upflow anaerobic sludge blanket reactor (UASB) made of glass with a working volume of 840 mL. The UASB reactor was operated during 238 days under two different regimens: in batch for the determination of the kinetic parameters and inhibition experiments, and in continuous mode between the individual batch experiments. The duration of the batch assays was variable between 8 and 11 h, after each batch experiment the reactor was operated in continuous mode for at least 36 h. The rationale, for the operation in continuous mode after each batch assay, was to conduct each batch experiment under similar pseudo-steady state conditions. When operated in continuous mode the reactor was fed using a peristaltic pump, in batch mode the feed line was closed and the liquid contained in the reactor was recirculated at a flow of 44 mL/min equivalent to a superficial upward velocity of 1 m/h. All experiments were performed at ambient temperature 25 °C (±2 °C). Fig. 1 shows the experimental set-up.

2.2. Inoculum and basal mineral medium

The reactor was inoculated with 154 g of granular sludge to yield 20 g of volatile suspended solids (VSS) per liter of reactor. The sludge was obtained from a laboratory scale UASB reactor that was operated under sulfate reducing conditions at a chemical oxygen demand (COD) to sulfate (SO_4^{2-}) ratio of 0.66 (g/g).

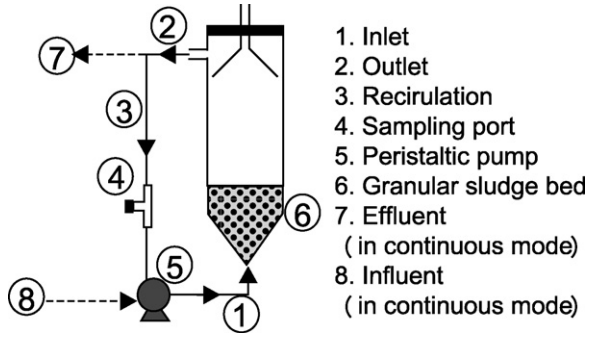

Fig. 1. Diagram of the UASB reactor set-up. Dashed line corresponds to the continuous mode configuration, when reactor operated in this mode there was not recirculation of the effluent.

The composition of the basal mineral medium was as follows (g/L): NH_4Cl (0.3), $CaCl_2{\cdot}H_2O$ (0.015), KH_2PO_4 (0.2), $MgCl_2{\cdot}6H_2O$ (0.098), KCl (0.25), yeast extract (0.02) and for the continuous mode operation 0.1 mL/L of trace elements solution as follows (g/L): $FeCl_2{\cdot}4H_2O$ (1.5), $MnCl_2{\cdot}4H_2O$ (0.1), EDTA (0.5), H_3BO_3 (0.062), $ZnCl_2$ (0.07), $NaMoO_4{\cdot}2H_2O$ (0.036), $AlCl_3{\cdot}6H_2O$ (0.04), $NiCl_2{\cdot}6H_2O$ (0.024), $CoCl_2{\cdot}6H_2O$ (0.07), $CuCl_2{\cdot}2H_2O$ (0.02), HCl 36% (1 mL/L).

2.3. Continuous flow reactor operation

The continuous operation was divided in four periods each one corresponds to the period when a set of batch experiments was performed: Period 1 corresponds to the kinetic parameters determination; period 2 to the experiments with sulfide; period 3 to the experiments with iron and period 4 to the experiments with cadmium. The reactor was fed with a synthetic wastewater that consisted of basal mineral medium (pH 5) supplemented with ethanol (1 g COD/L) and sulfate (2 g SO_4^{2-}/L as Na_2SO_4) to obtain a COD/SO_4^{2-} ratio (g/g) of 0.5, this ratio was chosen to avoid limitation of the electron acceptor (SO_4^{2-}) during the experiments. The hydraulic retention time was 10 h and the organic loading rate was constant at 2.5 g COD/L-d. Sulfide concentration was analyzed in the effluent; pH and COD were analyzed in the effluent and influent.

2.4. Batch assays

Batch experiments were conducted to obtain the maximum specific substrate utilization rate (q_{max}), the affinity constant (K_s) of ethanol oxidation, and to evaluate the inhibitory effect of sulfide, iron or cadmium on sulfate reduction with ethanol as electron donor. For this purpose, the feed flow of the reactor was discontinued and the liquid was recirculated by means of a peristaltic pump. Each batch experiment started with a shot of 140 mL at low pH (3–3.5), introduced into the reactor through the sampling port (Fig. 1). The content of the shot varied depending on the experiment to reach the initial concentrations according to Table 1. Each shot contained 14 mL of 10 times concentrated basal mineral medium, the corresponding amounts of ethanol, sulfate, and depending on the inhibition experiment different concentrations of sulfide, iron

Table 1
Concentrations of ethanol and sulfide, iron or cadmium added to the UASB reactor in the batch assays for the determination of the kinetic parameters (q_{max} and K_s) and the inhibition effect of sulfide, iron or cadmium on the sulfate reducing granular sludge.

Batch assay	Ethanol (g COD/L)	Iron (mM Fe^{2+})	Cadmium (mM Cd^{2+})	Sulfide (mM)
Kinetic parameters determination	0.26; 0.37; 0.55; 0.59; 0.69; 0.72; 0.80; 0.92; 0.96; 1.62; 2.8	NA	NA	NA
Inhibition with sulfide	1	NA	NA	4.7; 8.0; 10.2; 13.2
Inhibition with iron	1	4.0; 7.4; 8.1; 8.5	NA	NA
Inhibition with cadmium	1	NA	0.53; 2.14; 3.0	NA

NA: not added.

Table 2
Input data for the construction of the fraction diagrams shown in Fig. 5, using the free access software MEDUSA (www.kemi.kth.se/medusa).

Figure	Cd (mM)	CO_3^{2-} (mM)	Cl^- (mM)	H_2S (mM)	Eh (mV)	Ionic strength (M)	pH
5a	0.53	2.5	13	3.21	−180	0.060	6.59
5d	0.53	9.16	13	13.32	−200	0.060	7.04
5b	2.14	2.5	13	2.21	−180	0.066	6.13
5e	2.14	9.16	13	10.49	−230	0.065	6.58
5c	3	2.5	13	1.26	−180	0.068	6.09
5f	3	9.16	13	8.0	−200	0.068	6.58

or cadmium; the volume of the shot was completed to 140 mL with distilled water and the pH was adjusted between 3 and 3.5 with HCl, to maintain the metals in solution.

For the kinetic parameters determination (q_{max} and K_s), the initial concentration of ethanol in the reactor after adding the shot varied from 0.26 to 2.8 g COD/L; sulfate varied from 0.52 to 5.6 g/L to obtain a constant COD/SO_4^{2-} ratio of 0.5. For the inhibition experiments the carbon source (ethanol) and sulfate were fixed in 1 g COD/L and 2 g SO_4^{2-}/L, respectively. Several concentrations of sulfide, iron or cadmium were added to each shot to obtain the initial concentrations described in Table 1. Sulfide was added as $Na_2S{\cdot}9H_2O$, iron as $FeCl_2{\cdot}4H_2O$, and cadmium as $Cd(NO_3)_2{\cdot}4H_2O$. After 6 min of shot addition and vigorous mixing by means of high recirculation flow, the initial concentration of metals was determined. During the batch experiments liquid samples were collected through the sampling port, placed on the recycling line, at regular time intervals of 1–1.5 h. COD, pH, sulfide concentration, and dissolved iron or cadmium were analyzed in the liquid samples, including the initial samples taken after the shot was added.

2.5. Analytical methods

COD was determined by the closed reflux method according to standard methods [10]. Before COD determination, the sulfide present in the effluent samples (6 mL) was removed by adding a drop of concentrated HCl and flushing the sample during 10–15 min with air. Sulfide was determined by the iodometric method according to standard methods [10] using thiosulfate, and starch as indicator. VSS were analyzed according to standard methods [10], and pH was determined immediately after sample collection with a pH meter. Prior the determination of iron and cadmium the samples were filtered through a 0.22 μm Durapore® (Millipore) membrane syringe filter. Soluble iron concentration was measured with an atomic absorption spectrometer AAnalyst 400 (PerkinElmer) and soluble cadmium concentration was measured using an atomic absorption spectrometer PE 3110 (PerkinElmer).

2.6. Calculations

The ethanol oxidation rate was normalized to the total amount of biomass in the UASB reactor. Monod equation was used to obtain the kinetic parameters (q_{max} and K_s). A non-competitive inhibition model (Eq. (3)) was fitted to calculate the inhibition constant (K_I) for total dissolved sulfide (TS) and non-ionized sulfide (H_2S) according to Kaksonen [11].

$$q = \frac{q_{max} \cdot S}{(K_s + S)\left(1 + (I/K_I)\right)} \quad (3)$$

where q = specific substrate utilization rate (g COD/g VSS-d); q_{max} = maximum specific substrate utilization rate (g COD/g VSS-d); S = substrate concentration (g COD/L); K_s = affinity constant (g COD/L); I = inhibitor concentration (mM); K_I = inhibition constant (mM). q was calculated from the slope obtained from the COD profiles and the VSS content in the reactor.

The concentration of the non-ionized sulfide (H_2S), in the batch kinetic experiments was calculated from the total dissolved sulfide concentration using the following equation reported by Kaksonen [11].

$$H_2S = \frac{TS}{1 + 10^{(pH - pKa_1)}} \quad (4)$$

where TS is the total dissolved sulfide concentration and pKa_1 corresponds to 6.97, which is the first dissociation constant for H_2S (T = 25 °C) [12], the pKa_1 was corrected for the experimental ionic strength of 0.06 M, according to Stumm and Morgan [2], the pKa_1 value used for calculations was 6.87.

2.7. Chemical equilibrium diagrams

The chemical equilibrium diagrams shown in Fig. 5 were constructed using the free access software MEDUSA (www.kemi.kth.se/medusa). Input values were the concentrations of ligands (M) of: H_2S, CO_3^{2-}, and Cl^-; as well as Eh, pH, and ionic strength at a default temperature of 25 °C, for the initial and final conditions at the given cadmium concentration (Table 2).

3. Results and discussion

3.1. Determination of the maximum specific substrate utilization rate (q_{max})

The value of the specific substrate utilization rate (q_{max}) and affinity constant (K_s) was determined at different ethanol concentrations from 0.26 to 2.8 g COD/L, the q_{max} value was used as reference for the inhibition experiments with sulfide, iron or cadmium. Fig. 2 shows the Monod type curve obtained from adjusting the experimental values, with a correlation of 0.74; the q_{max} was 0.25 g COD/g VSS-d and the affinity constant K_s was 0.18 g COD/L.

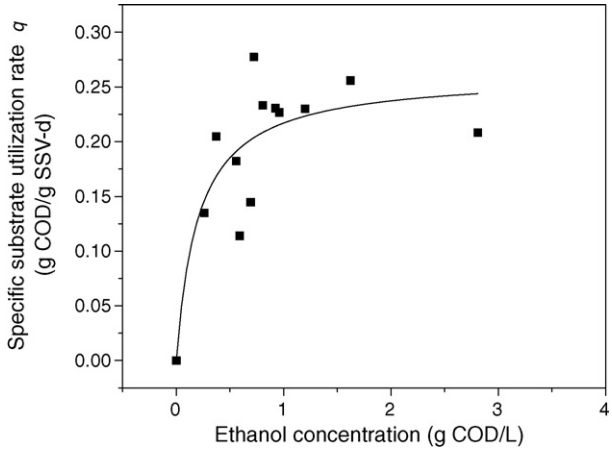

Fig. 2. Mathematical modeling with the Monod type equation of the specific substrate utilization rates with different concentrations of ethanol. Experimental values (■). The value of q_{max} = 0.25 g COD/g VSS-d (P < 0.0001) and K_s = 0.18 g COD/L (P > 0.05).

Once these parameters were determined, the inhibition experiments were started at a constant concentration of ethanol (1 g COD/L), a COD/SO_4^{2-} ratio of 0.5, and different concentrations of iron, cadmium or sulfide.

3.2. Batch assays with iron

Fig. 3 shows the profiles of residual iron, total dissolved sulfide concentration, COD consumption and pH at different iron concentrations added in the shot: 4.0, 7.4, 8.1 and 8.5 mM. It is important to note that the observed profiles are the result of different processes that were occurring at the same time. On one hand the metabolism of SRB produces sulfide and consumes COD, on the other hand the iron depletion profile was the result of the chemical precipitation of iron as FeS with the biogenic sulfide. More than 40% of the dissolved iron precipitated within 2 h after iron addition, at the same time sulfide reached its maximum concentration and, after 8 h of iron addition, more than 75% of iron precipitated in all the batches (Fig. 3a–d). At the end of the assays COD consumption was over 90% in all the experiments, which suggested a complete oxidation of the organic substrate. However at initial iron concentrations of 4.0 and 7.4 mM around 70% COD was consumed in the first 3 h (Fig. 3e–f), whereas less than 50% of COD was consumed in the first 3 h at initial iron concentrations of 8.1 and 8.5 mM (Fig. 3g–h). Most probably at the highest concentrations the iron added caused inhibition on the COD consumption rate and at the same time, the lower sulfide production caused low iron precipitation. For instance at the initial concentration of 4.0 mM, 83% of the iron precipitated in 2 h due to the higher sulfide concentration produced, that should correspond to a theoretical sulfide production of 11.4 mM (calculated from iron sulfide precipitation theoretically as FeS and the residual sulfide concentration), which at the equilibrium reached 8.1 mM, (highest sulfide concentration in Fig. 3a). In contrast, at the initial concentration of 8.5 mM Fe^{2+}, iron precipitation was only 64% (around 2 h) which should correspond to a theoretical sulfide concentration of 10.4 mM that at equilibrium was only 5.0 mM (highest sulfide concentration in Fig. 3d). These results pointed out that a direct iron precipitation associated to sulfide production occurred. In Fig. 3a and 3e correspondence between the COD consumed and the residual dissolved iron can be appreciated, however it is interesting to note that in the first 2 h while the maximum precipitation of iron is occurring, sulfide concentration is also the maximum. This could be explained by the equilibrium phase reached between the formed phase (FeS), the dissolved iron and sulfate reduction, according to the following equation:

$$2CH_2O + SO_4^{2-} + Fe^{+2} \rightarrow FeS + 2HCO_3^- + 2H^+ \quad (5)$$

Not all the produced sulfide reacts with the dissolved iron, when the equilibrium is reached sulfide is still produced because COD consumption is promoting sulfate reduction, this is why although iron is depleted sulfide accumulates according to Eq. (1), which promotes a pH increase (Fig. 3e–h).

The specific substrate utilization rates (q_{Fe}) obtained from the COD depletion curves decreased from 0.44 to 0.19 g COD/g VSS-d

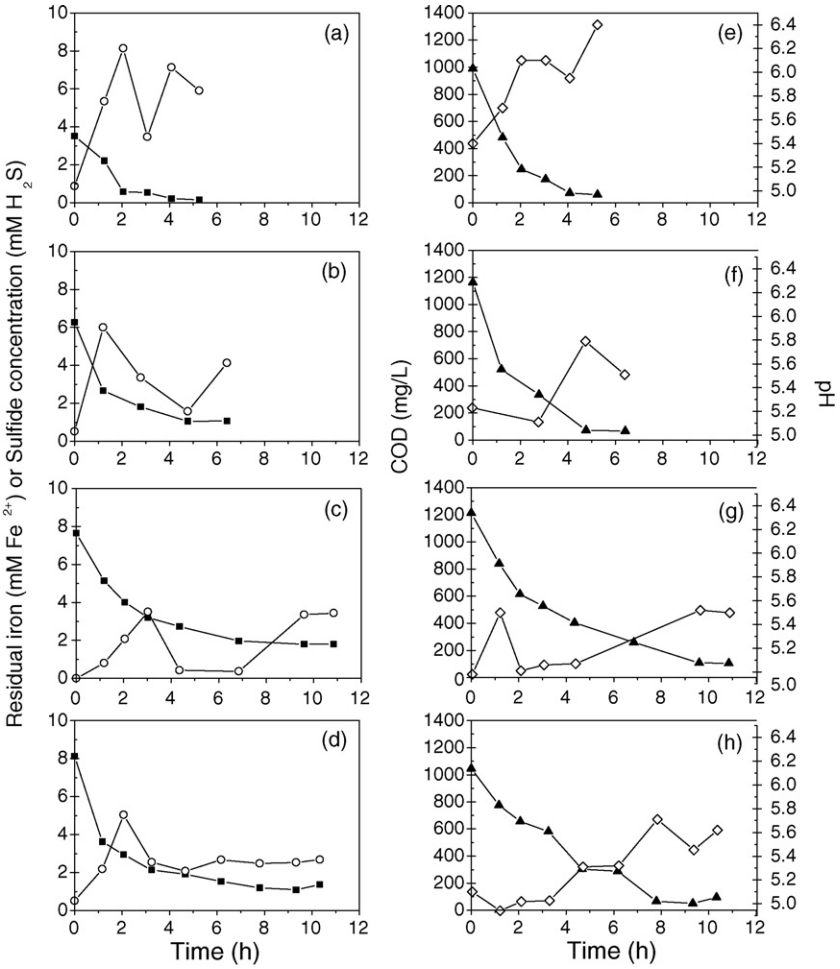

Fig. 3. Concentration profiles of residual iron (■), total sulfide (○), residual COD (▲), and pH (◊) for the following initial iron (Fe^{+2}) concentrations: 4 mM (a and e); 7.4 mM (b and f); 8.1 mM (c and g); 8.5 mM (d and h).

Table 3
Specific substrate utilization rates in g COD/g VSS-d obtained in the batch assays amended with iron (q_{Fe}), cadmium (q_{Cd}) and sulfide (q_{H_2S}) and the percentage of inhibition with respect to the maximum substrate utilization rate determined without the addition of metals or sulfide (q_{max} = 0.25).

Iron (mM Fe^{2+})	q_{Fe}	Inhibition[a] (%)	Cadmium (mM Cd^{2+})	q_{Cd}	Inhibition (%)	Sulfide (mM H_2S)	q_{H_2S}	Inhibition (%)
0	0.25	–	0	0.25	–	0	0.25	–
4.0	0.44	+76	0.53	0.21	16	4.7	0.19	24
7.4	0.40	+60	2.14	0.18	28	8.0	0.2	20
8.1	0.35	+40	3.0	0.14	44	10.2	0.19	24
8.5	0.19	24				13.2	0.1	60

[a] Plus sign indicates positive effect instead of inhibition.

as the iron concentration increased from 4.0 to 8.5 mM (Table 3); the dissolved iron concentration that caused 50% reduction (IC_{50}) on the specific substrate utilization rate was calculated from these data, in this case comparing the specific substrate utilization rates to the rate obtained when 4.0 mM of iron was added. The IC_{50} obtained was 8 mM and the apparent inhibition constant (K_I) determined with a non-competitive kinetic model was 12.7 mM. Concentrations equal or below 8.1 mM Fe^{2+}, rather than inhibit the specific substrate utilization rate had a favorable effect on it, compared to the value obtained without the addition of iron (q_{max} = 0.25 g COD/g VSS-d, Table 3). This may be explained as a combined effect between sulfide and iron. In the presence of sulfide, iron has no inhibitory effect on the microorganisms because it precipitates as iron sulfide, at the same time sulfide concentration was also reduced by the presence of iron, thus reducing the inhibition that sulfide may cause on the anaerobic microorganisms. Nonetheless, the addition of iron had a positive effect on sulfate reduction up to certain extent, because at an initial concentration of 8.5 mM, the specific substrate utilization rate decreased abruptly to 0.19 g COD/g VSS-d, equivalent to 24% inhibition compared with the q_{max} determined without the addition of metals or sulfide (0.25 g COD/g VSS-d). Most probably the iron concentration of 8.5 mM exceeded the sulfide protection mechanism, this is the sulfide produced was not enough to reduce the adverse effect of the metal. In anaerobic reactors the use of iron is a common practice to alleviate sulfide toxicity; iron is used to maintain low sulfide concentrations within the reactors [13–15].

Considering the application of the sulfate reducing process to treat AMD with high concentrations of iron, the question was: up to what extent iron could be beneficial to the anaerobic process? The iron concentration in an AMD could be up to 91 mM [16], this study showed that at a concentration of 8.5 mM Fe^{2+} the substrate utilization rate decreased by more than 50%, compared to the substrate utilization rate obtained when an iron concentration of 4.0 mM was added. This means that AMD with iron concentrations higher than 8.5 mM need to be diluted to reduce the risk of inhibition of the sulfate reducing process by iron. Although iron in an AMD is present as Fe^{3+} it can be easily reduced to Fe^{2+} due to the highly reducing conditions reached by biogenic sulfide production [17,18].

3.3. Batch assays with cadmium

Fig. 4 shows the profiles of cadmium, sulfide, COD concentration and pH during the assays. The cadmium concentrations added were: 0.53, 2.14 and 3.0 mM. The COD consumption for all the experiments with cadmium was less than 80% (Fig. 4d–f); at a cadmium concentration of 3.0 mM the COD removed was only 44.7% which pointed out that the process was strongly inhibited. The residual cadmium after the shot addition (about 5 or 6 min after) was less than 0.1 mM, which means that more than 99% of cadmium precipitated immediately after its addition. However, an inhibitory

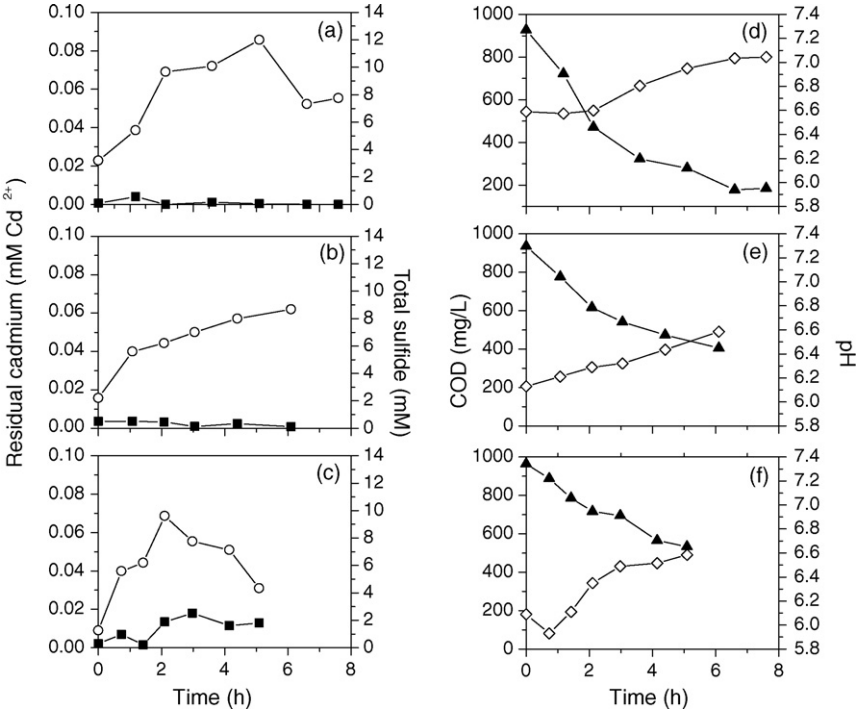

Fig. 4. Profiles of the residual cadmium concentration (■), the sulfide concentration (○), residual COD concentration (▲), and pH (◊) for the following initial concentrations of cadmium (Cd^{2+}): 0.53 mM (a and d); 2.14 mM (b and e); 3.0 mM (c and f).

effect over sulfate reduction still occurred. It is remarkable that cadmium reached equilibrium with sulfide rapidly due to its solubility product constant value as CdS, 1.0×10^{-27}, in comparison with the solubility product constant value of FeS that is 7.9×10^{-19} [2].

Table 3 shows the specific substrate utilization rates obtained with the concentrations of cadmium added and the percentage of inhibition over the sulfate reducing process. When a cadmium concentration of 0.53 mM was added 16% inhibition was observed, at a cadmium concentration of 2.14 mM the inhibition increased to 28% and when 3.0 mM Cd^{2+} was added the substrate utilization rate was inhibited 44%, compared to the value obtained without cadmium (q_{max}). The strong inhibition observed was evident when compared to the effect of iron (Table 3). The COD consumption was less than 80% at initial cadmium concentrations of 0.53 and 2.14 mM, whereas with the cadmium addition of 3.0 mM only 47% of COD was removed after 6 h, in spite of the extremely low dissolved cadmium concentrations. Hao et al. [5] observed a similar trend in batch tests using different amounts of zinc, cadmium or copper to evaluate metal toxicity over a SRB enriched culture, although the dissolved concentration of the afore mentioned metals was negligible sulfate reduction was inhibited; the authors proposed that non-dissolved metal forms were responsible for inhibiting the sulfate reduction process. This could be explained by the well-known biomass capacity to adsorb heavy metals; several compounds produced by microorganisms such as organic acids, alcohols and extracellular polymeric substances among others play an important role in the uptake of soluble and insoluble metal species [19]. In experiments performed in sulfate reducing biofilms White and Gadd [20] found the accumulation of cadmium as insoluble sulfide mainly in the superficial layer of the biofilms. The maximum concentration of cadmium tested by Hao et al. [5] was 0.18 mM at which sulfate reduction of a SRB enrichment culture was not detected. In the present study the maximum concentration of cadmium tested was 3.0 mM, at which 44% reduction in sulfate reducing activity was observed; this high tolerance could be due to the exopolymeric substances present in granular sludge. The granular sludge showed high tolerance to cadmium, but contrary to the inhibition experiments with iron, the effect of metal detoxification was not observed, pointing out that cadmium has a strong effect on the sulfate reducing process.

From the fraction diagrams of cadmium built with the initial and final redox potential conditions of the batch assays (Fig. 5), it was corroborated that at initial concentrations of cadmium of 0.53 and 2.14 mM all the cadmium added immediately reacted with sulfide and formed CdS (Fig. 5a and b), in none of these experiments the pH was higher than 7.5. Thus, most probably, the inhibition was caused by the non-dissolved metal form. In contrast, in the batch assay with cadmium concentration of 3.0 mM (Fig. 5c), at the beginning of the experiment (pH 6.1) some cadmium soluble species such as free ion (Cd^{2+}) and complexed with chloride ($CdCl^{+}$) could have been present, however as the fraction diagram shows (Fig. 5c) the predominant form of cadmium could have been as $CdCO_3$ (47%) fol-

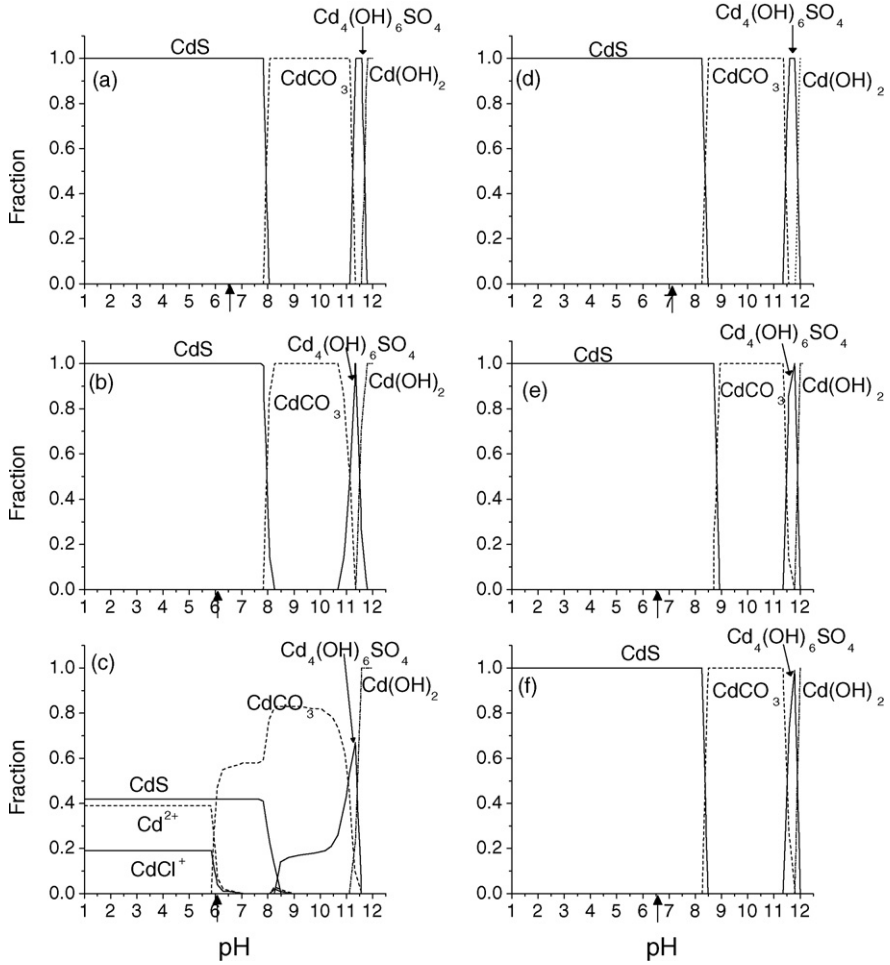

Fig. 5. Fraction diagrams of cadmium species for the initial and final redox potential (Eh in mV) in the batch experiments amended with the following initial concentrations of cadmium (Cd^{2+} in mM): 0.53 mM (a) initial Eh −180 and (d) final Eh −200; 2.14 mM (b) initial Eh −180 and (e) final Eh −235; 3.0 mM (c) initial Eh −180 and (f) final Eh −200. Arrows on *X*-axis show the initial and final pH, respectively. More details of these diagrams are presented in Table 2.

lowed by CdS (42%). As the sulfide concentration increased, with the course of the experiment, the $CdCO_3$ phase was not stable anymore and at the end of the experiment (Fig. 5f) the predominant specie of cadmium at pH 6.6 was mainly CdS. Thus, the experimental data (Fig. 4c) are in accordance with the thermodynamic equilibrium (Fig. 5c and f) where the carbonate phase is shifted by the sulfide phase. However, at the initial condition (pH 6.1) the carbonate phase is potentially more bioavailable than the sulfide phase [21] which could provoke a more important reduction on the sulfate reducing activity. The precipitation of cadmium as carbonate (if occurred) or sulfide, apparently did not contribute to the reduction of cadmium inhibition on the granular sludge.

3.4. Batch assays with sulfide

To quantify the inhibition caused by sulfide, a set of batch experiments was performed with the addition of 4.7, 8.0, 10.2 and 13.2 mM of total dissolved sulfide. At sulfide concentrations equal or lower than 10.2 mM, 90% of COD was consumed in about 8.5 h; on the contrary at the highest sulfide concentration (13.2 mM) only 83% of COD was consumed after 11 h. The specific substrate utilization rates (q_{H_2S}) calculated from the depletion curves of COD amended with sulfide were normalized with the specific substrate utilization rate without sulfide (q_{max}) and are presented in Table 3. The specific substrate utilization rate diminished from 0.25 g COD/g VSS-d (q_{max}) to 0.1 g COD/g VSS-d at the highest total dissolved sulfide concentration of 13.2 mM which accounted for 60% inhibition of the sulfate reducing activity. A threshold was observed at 10.2 mM, where the sulfate reducing activity was reduced only by 24% with respect to the control. The pH of the assays amended with sulfide remained around neutral values (6.2–6.6) and according to several authors at neutral pH values the inhibitory effect of sulfide is mainly caused by the non-ionized sulfide (H_2S); due to its neutral character the molecule penetrates into the cytoplasm and reacts with disulfide bridges [6,11,22,23]. Thus, non-ionized sulfide concentration is a parameter to take into account when pH is around neutral values.

In view of this the IC_{50} value was calculated to be 12.4 mM of total dissolved sulfide and 9.1 mM as H_2S. In the experiments with iron and cadmium the highest concentration of either total dissolved sulfide or H_2S was not higher than these values, pointing out that the inhibition observed in the experiments with cadmium and iron was due to the presence of metals. It is worth to note that in the experiments without iron the produced total dissolved sulfide reached concentrations around 15.6 mM, whereas in the experiments with iron the maximum concentration of total dissolved sulfide was 8.1 mM and in the experiments with cadmium the maximum concentration was 12 mM which is very close to the IC_{50} determined for total sulfide. Most probably the inhibitory effect when cadmium was added is related to the combination between the inhibition caused by sulfide and by the metal itself.

3.5. Performance of the UASB reactor operated in continuous mode

Fig. 6 shows the performance of the UASB reactor during continuous operation between the batch experiments. The COD removal was over 85% and the effluent pH remained almost constant around 7.0. Total dissolved sulfide presented a wide variation, between 2 and 6 mM, but never was higher than 10 mM. The pseudo-steady states reached in terms of COD removal pointed out that the observed variations in the specific substrate utilization rates in batch experiments were due to the different concentrations of the compounds used. Thus, the reactor was robust because in despite of all the disturbances occasioned by the batch experiments the reactor performance reached pseudo-steady states that did not interfere

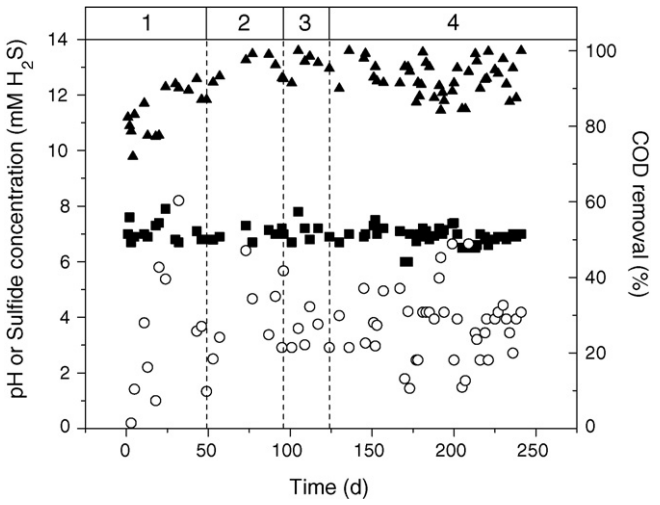

Fig. 6. Performance of the UASB reactor operated in continuous flow. Periods 1, 2, 3 and 4 correspond to the batch assays done with ethanol (q_{max} and K_s determination), sulfide, iron or cadmium, respectively. COD removal percentage (▲), total dissolved sulfide effluent concentration (○) and effluent pH (■).

in the results observed during the batch experiments. The sulfide variation may be attributed to the different conditions applied to the UASB reactor in the batch assays.

4. Conclusions

The results showed that iron has an important control effect over the toxicity caused by sulfide to sulfate reducing granular sludge. In contrast to cadmium, iron was always present in solution and therefore it is available for the consumption of the biogenic sulfide. In the case of cadmium and in accordance to the fraction diagrams the non-dissolved metal was responsible of the inhibition observed. Thus, the precipitation of cadmium as sulfide, apparently did not contribute to the reduction of cadmium toxicity, causing a 44% reduction of the sulfate reducing activity which may be associated to a combined inhibitory effect of sulfide and cadmium concentrations.

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