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TECHNICAL COMMUNICATION

A Kinetic Analysis of Microbial Sulfate Reduction in an Upflow Packed-Bed Anaerobic Bioreactor

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Abstract The effects of initial sulfate concentration on anaerobic sulfate reduction and sulfide generation kinetics were investigated in an up-flow bioreactor, using a consortium of sulfate-reducing bacteria (SRB) from water produced from a Brazilian oil reservoir. Redox potential and sulfate concentration were measured to indicate the growth and activity of the SRB throughout the experimental runs. The results of the batch regime indicate that sulfate conversion and sulfide generation are both first-order processes for initial sulfate concentrations of 1,000 and 3,500 mg/L. The kinetic constants for the sulfate conversion indicate that the enhanced initial sulfate content was initially inhibiting, but that the sulfide generation reaction is almost independent of the initial sulfate concentration, likely due to the presence of at least two in-series processes that are faster than the microbial conversion of the sulfate.

Keywords Acid mine drainage · Brazil · *Desulfovibrio vulgaris* · Sulfate reducing bacteria · Treatment

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Introduction

Wastewater from mining often contains elevated levels of sulfate and potentially toxic elements. The biological treatment of wastewater from mining using sulfate-reducing bacteria (SRB) is a relatively low-cost option that has received attention (Hammack et al. 1998; Lenz et al. 2002; Luo et al. 2008; Moosa and Harrison 2006; Nemati et al. 2001; Ros and Mejac 1991; Tsukamoto et al. 2004; Zamzow et al. 2006). Evaluation of the kinetics of sulfate consumption and sulfide generation for a specific SRB population is important in predicting the SRB activity.

Sulfate reduction by SRB follows the reaction below, where the electrons are generated by the oxidation of a source of carbon and energy, such as organic acids and alcohols (Lenz et al. 2002):

$$SO_4^{2-} + 8e^- + 4H_2O \rightarrow S^{2-} + 8OH^- \quad (1)$$

Kinetic studies have been performed with SRB in batch and continuous reactors (Okabe et al. 1992, 1995). Up-flow packed-bed bioreactors have been widely used in industrial microbiology, including the study of the anaerobic activity of SRB in oil fields, due to the large surface area available for the attachment of microorganisms and the reduced bioreaction time (Chen et al. 1994; Elliott et al. 1998; Jong and Parry 2003). High volumetric productivities have been reported in packed bed bioreactors compared to other configurations (Nagpal et al. 2000). In these reactive systems, the growth and activity of the SRB throughout the experimental runs have been assessed using plating, which typically requires 48–72 h.

In the bioremediation of acid mine drainage and other sulfate-laden waste-waters using SRB, sulfate reduction is a critical step (Baskaran and Nemati 2006). Studies with suspended mixed SRB consortia (fed with acetate and

ethanol) and lactate-fed immobilized SRB cells have shown that sulfate reduction reaction kinetics is influenced by the influent sulfate concentration (Lenz et al. 1995; Moosa et al. 2002, 2005; Oyekola et al. 2010).

As described in Eq. 1, during growth of the SRB, the pH increases due to the generation of OH^- ions, and the redox potential of the solution decreases due to the consumption of electrons. The shape of the redox potential curve is characteristic of the type of microorganism and generally, SRB can grow in the culture medium at an Eh range of −100 to −500 mV. The pH determines whether the dissolved sulfide occurs as S^{2-}, HS^-, or H_2S, while the redox potential determines whether the sulfide is found as SO_4^{2-}, S, HSO_4^-, etc. (Millero 1986). For an initial pH value of about 7.5, most of the sulfide content is HS^-. If the pH increases, the concentration of S^{2-} will increase. The species predominance is also a function of the solution redox potential. At a pH ranging from 7.5 to 8.0 and the range of redox potential values obtained during the experiments, the predominant sulfide form is HS^-. Temperature also affects sulfur speciation and is an important variable in most of microbiologic processes. Despite the fact that SRB are active at 6°C (Tsukamoto et al. 2004), SRB activity slows down at low temperatures and the optimal temperature is about 30–39°C. The kinetic tests presented in this study were performed at 32°C, because this temperature is close to the optimum value for SRB growth and can be found in some environments in the sulfide mines located in Brazil's arid region.

This paper investigates the activity of a mixed SRB culture including a strain of *Desulfovibrio vulgaris* ssp *vulgaris DP4*, which was collected from a Brazilian soured oil reservoir. An up-flow bioreactor packed with glass beads was used. The effects of the initial sulfate concentration on the anaerobic sulfate reduction and sulfide generation kinetics were investigated. Two initial sulfate concentration were used, respectively 1,000 and 3,500 mg/L.

Materials and Methods

Experimental Setup

The upflow packed-bed bioreactor used in this study was made using a 1 L graduated Pyrobras® (D:6 cm and H:32 cm) glass column with ten lateral sampling ports at 3.6 cm intervals. All sampling ports were sealed using a silicon septum. The bioreactor was then packed with 3.0 mm glass beads to provide a matrix for the establishment of the biofilm. The measured porosity of the stream bed was 40%. Following the packing with the carrier matrix, the bioreactor was sterilized in an autoclave for 30 min at 121°C. The glass joint at the top of the bioreactor was sealed with silicon sealant to make sure that there were no leaks from the bioreactor. Tygon tubing was used to transfer the medium into the bottom of the bioreactor and to remove the effluent from the top of the bioreactor. To guarantee an anaerobic environment inside the column, nitrogen was introduced continuously to the bottom of the column at a low flow rate, prior to and during the packing of the carrier matrix. A diagram of the experimental set-up is shown in Fig. 1. The bioreactor was maintained at 32°C using a box heated by a 25 W electric lamp and a variable AC voltage controller.

The solution was fed into the bottom of the reactor, close to the inferior part of the column with a peristaltic

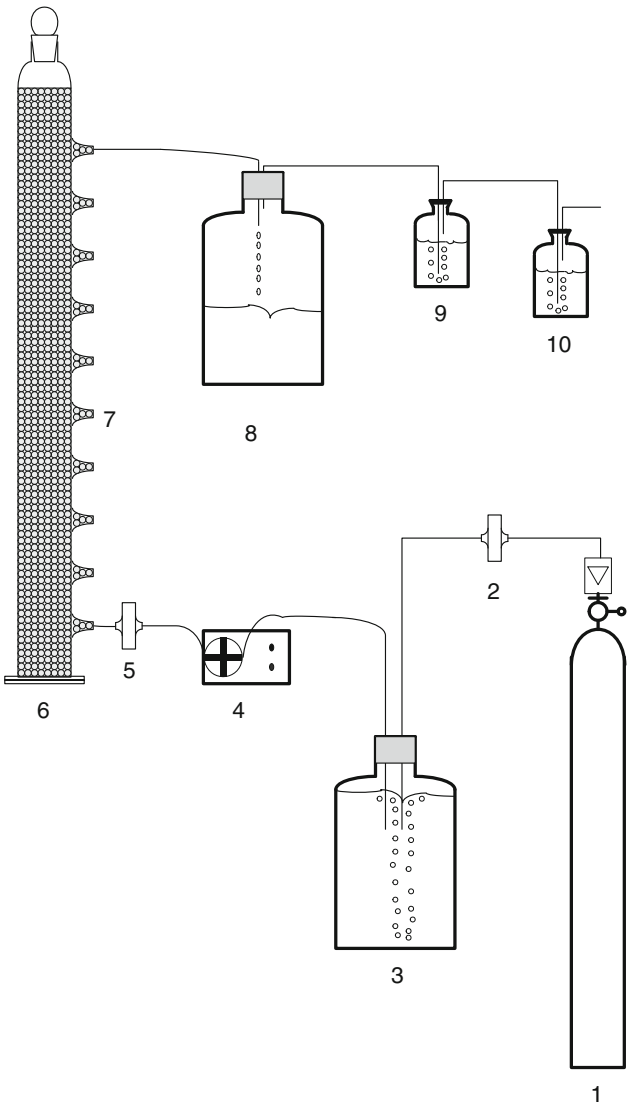

Fig. 1 Schematic diagram of the experimental set-up used for column kinetic studies. The numbers stand for: *1* nitrogen cylinder, *2* filter, *3* medium container, *4* peristaltic pump, *5* filter, *6* packed bed bioreactor, *7* sampling ports, *8* effluent container, *9* primary trap, *10* secondary trap

pump (Masterflex® L/S® model 77912-00) using polytetrafluorethylene (PTFE) tubing. The solution was then collected at the top of the column. The gas generated during the experiment was collected in a reservoir containing an alkaline 10% $ZnCl_2$ solution to precipitate ZnS. The pH of the trap solution was periodically measured and adjusted by adding pellets of NaOH. To make sure that no loss of H_2S occurred, the hydrogen sulfide (H_2S) of the effluent of the first container was transferred to a second trap containing an alkaline 5% $ZnCl_2$ solution. Solution turbidity was monitored for $ZnCl_2$ precipitation.

The liquid medium with sulfate was fed into the bottom of the bioreactor via Tygon tubing using a peristaltic pump. Before turning the pump on, all the tubing was washed with 70% ethanol solution to minimize the possibility of contamination. About two pore volumes of medium were pumped through the bioreactor to ensure that the medium completely filled the voids. The effluent stream left the top of the reactor and was collected in an effluent vessel. The pump was then switched off and the bioreactor was inoculated by injecting SRB enrichment into three sampling ports (bottom, medium, and top). The inoculation was performed from the bottom port to the top port to prevent the outflow of inoculum through the effluent tubing at the top and to assure the establishment of a uniform biofilm. Following the inoculation, the tubing at the top and bottom of the bioreactor was clamped to keep the bioreactor free from contamination. Samples were taken from the three ports (bottom, middle, and top) and analyzed for sulfate and sulfide.

Initially, the bioreactor was operated in batch mode, during which microbial activity was monitored by determining concentrations of sulfide, sulfate, and the redox potential. Once the conversion of sulfate was almost 100%, the bioreactor was switched to continuous mode by pumping medium into the bioreactor at a low flow rate (2.6 mL/min). Steady state conditions were assumed to be established when the sulfate conversion varied by less than 10% during a period of about 24 h, which had previously been determined to be equal to at least two residence times (Bernardez et al. 2008).

Microbial Culture and Medium

A mixed culture of SRB was enriched from the water produced from the oil fields from the Reconcavo Basin, Brazil and used as an inoculum. This culture was dominated by *Desulfovibrio* species. A volume of 100 μL of mix culture of SRB containing about 2.0×10^9 NMP/mL was inoculated and enriched in a culture medium inside the anaerobic chamber (Bactron VI, Shellab, Sheldon Manufacturing Inc.) at 38°C. The modified Postgate medium, in which sodium lactate is used as a potential carbon source and electron donor, was used for the growth of the SRB. This medium contained (per liter): agar, 2.0 g; KH_2PO_4, 0.5 g; NH_4Cl, 1.0 g; Na_2SO_4, 1.0 g; $CaCl_2$, 1.0 g; $MgCl_2.6H_2O$, 1.83 g; yeast extract, 1.0 g; ascorbic acid, 0.1 g; sodium thioglycollate, 0.013 g; sodium citrate, 6.38 g; sodium lactate 1.75 mL; NaCl 3.5%, resazurin, 2.0 mL 0.025% w/v, and $FeSO_4{\cdot}7H_2O$, 0.5 g. All the components were dissolved in deionized water and the pH was adjusted to 7.5–8.0 using 5 M HCl. After this, the solution was homogenized by agitation and later sterilized at 121°C for 30 min. It supports the growth of a wide spectrum of SRB, encouraging microbial diversity (Oyekola et al. 2010). In this medium, the formation of black precipitate indicates the formation of FeS.

Culture Medium for the Bioreactor

\A second modified Postgate medium was used in the bioreactor experiments. It was the previous modified Postgate medium without agar. The medium was prepared in a 2 L flask, autoclaved for 30 min at 121°C, cooled to room temperature and then purged with nitrogen for about 2 h before using it as a feed for the bioreactor. The initial concentration of sulfate used in the first kinetic test was the original amount of the modified Postgate medium, about 1,000 mg/L. In the second kinetic test, the initial concentration of sulfate was 3,500 mg/L; in this case, the amount of Na_2SO_4 and $FeSO_4{\cdot}7H_2O$ were proportionately enhanced in the culture medium to reach this concentration. The amount of the other components of the culture medium was the same. To maintain anaerobic conditions and to prevent contamination of the medium, nitrogen was purged periodically through the medium bottle during the experiment.

Sulfate Concentration

The sulfate concentration was measured by a turbidimetric method (Kolmert et al. 2000) that is based on the precipitation of sulfate ions as barium sulfate. Samples to be analyzed for sulfate was treated with an excess of zinc acetate dehydrate crystals to precipitate dissolved sulfide as zinc sulfide. Fixation of sulfide prevented oxidation to sulfate. Using 1.5 mL microcentrifuged tubes, 1.0 mL culture samples were stirred for 5 s with approximately 0.01 g of zinc acetate. The mixture was then centrifuged for 10 min at 6,000 rpm and at 4°C. Then, 50 μL of the supernatant was mixed with 950 μL of the conditioning fluid in a fresh microcentrifuge tube and stirred for 5 s. Then, approximately 0.01 g of barium chloride dehydrate crystals was added to the mixture, which was then stirred for 15 s; the relative absorbance was then immediately read at 420 nm using a UV/Visible spectrophotometer. The calibration standards were prepared using sodium sulfate and deionized water.

Sulfide Concentration

The dissolved sulfide concentration was measured using the turbidmetric method (Cord-Ruwish 1985) immediately after sampling to prevent its oxidation and volatilization. The assay is based on the precipitation of sulfide as a colloid copper sulfide. Using 1.5 mL microcentrifuge tubes, 50 μL of the culture samples was mixed with a 950 μL copper solution (5 mM $CuSO_4 \cdot 5H_2O$ and 50 mM HCl) and then stirred for 5 s. Then, the relative absorbance was measured immediately at 480 nm using a UV/Visible spectrophotometer. Calibration standards at 100, 200, 300, 400, 500, 600, and 700 mg/L were used for the analysis at higher sulfide concentrations.

Solution pH and Oxidation–Reduction Potential

The pH was measured with a Thermo Orion PerpHecT Meter (Model 330). The pH meter was regularly calibrated using pH 4 and 7 buffer solutions. Redox potential differences, ΔE_h, were measured ex-situ using an ORP electrode with an internal Ag/AgCl reference electrode from Cole-Parmer. The measurements were calibrated with ORP standard solutions (Analion) of 470 and 220 mV at 20°C.

Results and Discussion

Batch Operation of the Packed-Bed Bioreactor

In the first experiment, a liquid medium with sulfate content of 1,000 mg/L was introduced into the bottom of the bioreactor by a peristaltic pump and the bioreactor was inoculated by injecting 10 mL of SRB enrichment into the three sampling ports. Initially, the bioreactor was operated in batch mode, during which microbial activity was monitored by determining concentrations of sulfide and sulfate and the redox potential. After 7 days, a biofilm had formed

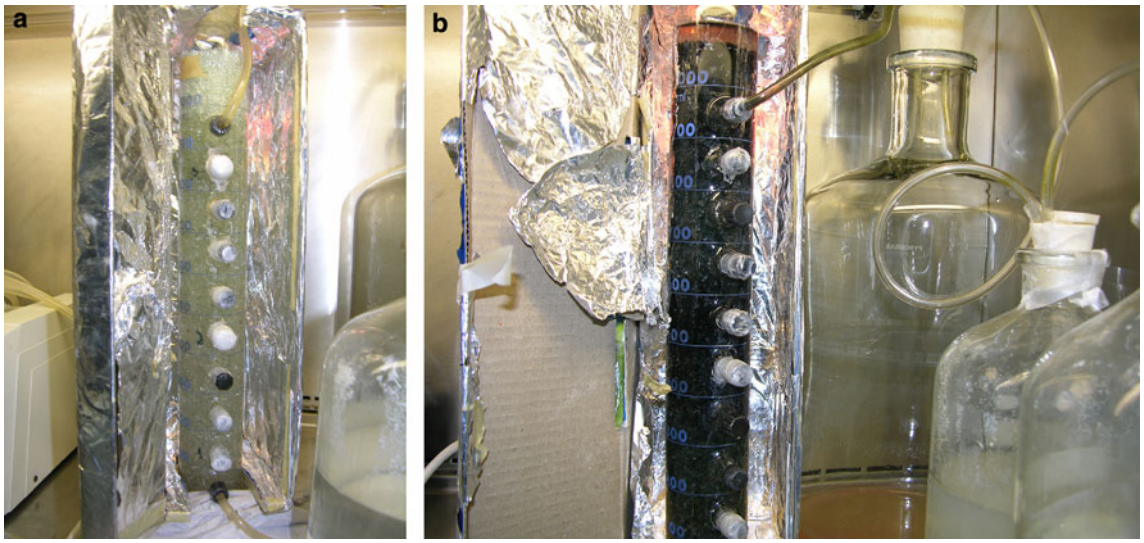

Fig. 2 Biofilm formation in the bioreactor; **a** after inoculation; and **b** after 7 days of operation

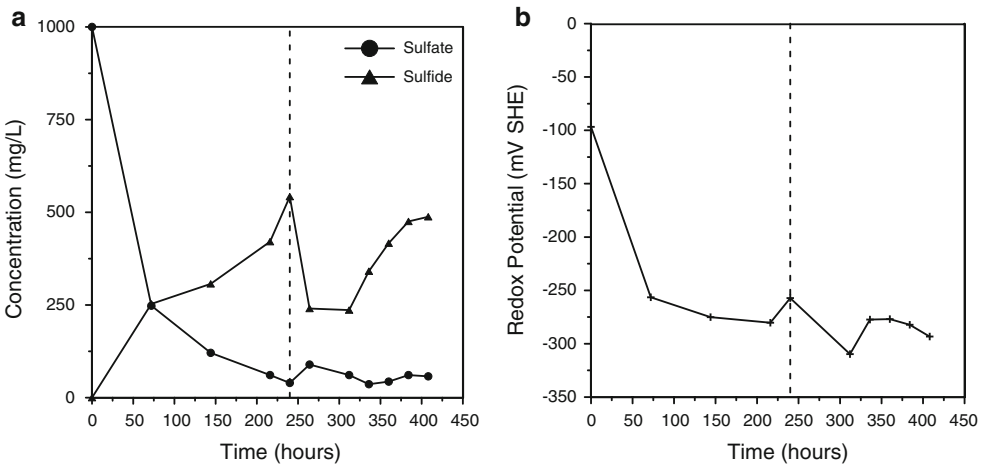

Fig. 3 Column kinetic experiment results at initial sulfate concentration equal to 1,000 mg/L: **a** sulfate and sulfide concentration time evolution, **b** solution oxidation–reduction potential time evolution. *Dashed line* indicates the end of the batch regime and the startup of the peristaltic pump for medium circulation

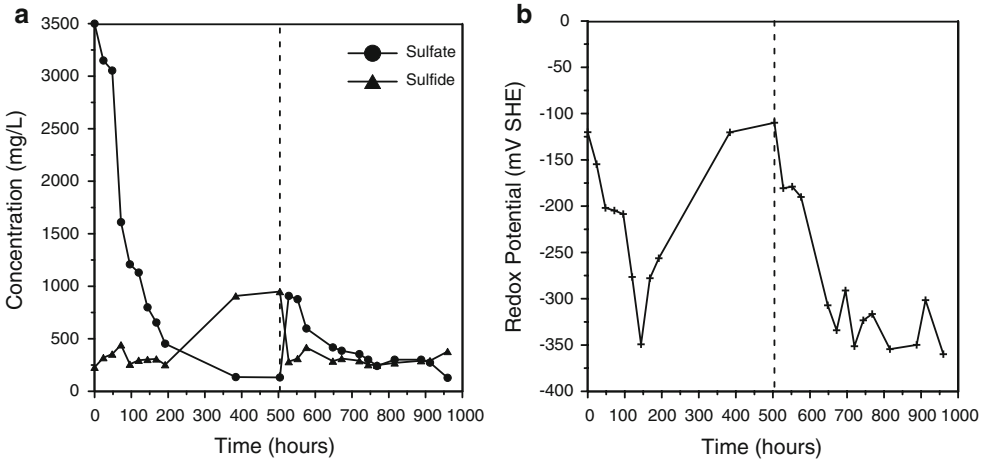

Fig. 4 Column kinetic experiment results at initial sulfate concentration equal to 3,500 mg/L: **a** sulfate and sulfide concentration time evolution, **b** solution oxidation–reduction potential time evolution. *Dashed line* indicates the end of the batch regime and the startup of the peristaltic pump for medium circulation

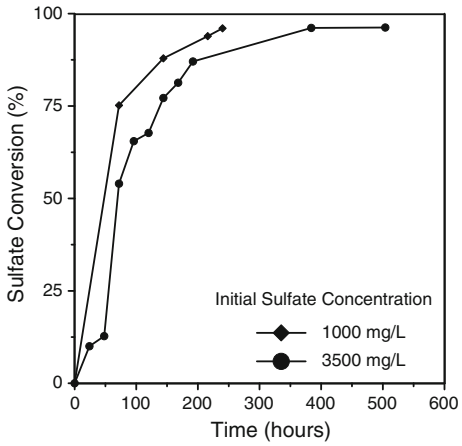

Fig. 5 Sulfate conversion evolution for the batch regimes in the column experiment

all along the bed of the bioreactor. After stabilization of the sulfate conversion, the peristaltic pump was switched on. Figure 2a, b illustrates the process of biofilm formation. The profiles of sulfate and sulfide concentrations as a function of time are shown in Fig. 3a. The sulfate concentration decreased exponentially to about 50 mg/L, and the produced sulfide content increased, reaching a maximum value of about 500 mg/L. At the beginning of the experiment, the environment pH was relatively constant at 7.5. A conversion of sulfate of about 80% was obtained over a period of 7 days. During this period, a gradual decrease in the solution redox potential was observed, as shown in Fig. 3b.

The measured sulfide content represents only the S^{2-} ion and cannot account for HS^{-}, H_2S, and other species generated by the ionic equilibrium, which explains the apparent sulfur mass imbalance. The same observation was reported in a previous study (Baskaran and Nemati 2006). The redox potential value of about −300 mV was the threshold for optimal sulfate reduction. It was also noted that ZnS precipitation was not observed in the traps; thus, H_2S was not volatilized during the experiment.

The effect of enhanced initial sulfate content on the process kinetics was determined by feeding a solution with an initial sulfate concentration of 3,500 mg/L into the bottom of the bioreactor. Figure 4a shows the profiles of residual sulfate and produced sulfide concentrations as a function of time. During batch mode operation, the sulfate content decreased from 3,500 to 130 mg/L (a conversion of about 96%) and the sulfide concentration increased to 949 mg/L. At the beginning of the experiment, the pH of the solution was relatively constant at 7.5. The solution redox potential profile (Fig. 4b) was analogous to the previous cases, and again, no ZnS precipitation was observed in the traps during the experiment.

Continuous Operation of the Packed-Bed Bioreactor

The time the pump was turned on is indicated in Figs. 3a and 4a by the dashed vertical lines. In all cases, there was a transient decrease in dissolved sulfide concentration due to the effects of dilution. As the biomass within the bioreactor adjusted to changes in the feed, the sulfide and sulfate concentrations returned to their original value. Figures 3a and 4a show sulfide conversions of greater than 95% by the end of the tests. Figures 3b and 4b show how the redox potential evolved, reaching a value of about −300 mV.

A Kinetic Model for the Batch Operation

To evaluate the effect of the sulfate concentration on sulfate consumption, the sulfate conversion evolution for the two cases was contrasted; the conversion was defined by:

$$\chi(t) = \frac{C_{SO_4^{2-}}(0) - C_{SO_4^{2-}}(t)}{C_{SO_4^{2-}}(0)} \times 100 \quad (2)$$

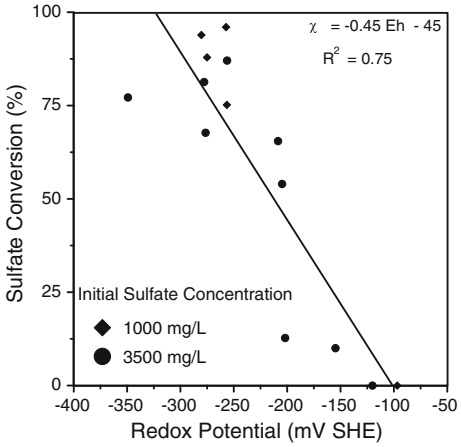

Fig. 6 Relationship between the reduction–oxidation potential and sulfate conversion

where $C_{SO_4^{2-}}(t)$ is the sulfate content at time t and $C_{SO_4^{2-}}(0)$ is the initial sulfate concentration (Fig. 5). It can be seen that sulfate conversion was inhibited at the enhanced initial sulfate content, yet in both cases, 95% of the sulfate was converted between 10 and 15 days. Figure 6 shows the relationship between the solution reduction–oxidation potential and the sulfate conversion for the batch regime. The significant agreement between these variables is convenient to process monitoring, since redox potential can be easily and quickly measured.

Assuming that the bioreactor is operating as a batch reactor of constant volume, the apparent kinetic models for sulfate consumption and sulfide production are proposed as follows:

$$\frac{dC_{SO_4^{2-}}}{dt} = -k_{SO_4^{2-}} C^{\alpha}_{SO_4^{2-}} \quad (3)$$

$$C_{SO_4^{2-}}(0) = C_{SO_4^{2-},0} \quad (4)$$

$$\frac{dC_{S^{2-}}}{dt} = k_{S^{2-}}\left(C_{S^{2-},\infty} - C_{S^{2-}}\right)^{\beta} \quad (5)$$

$$C_{S^{2-}}(0) = 0 \quad (6)$$

Table 1 Kinetic parameters for the sulfate conversion for the batch regimes in the column experiments

$C_{SO_4^{2-},0}$ (mg/L)	$k_{SO_4^{2-}}$ [(mg/L)$^{1-\alpha}$/h]	α	$k_{S^{2-}}$ (L/h)	β	$C_{S^{2-},infty}$ (mg/L)
1,000	-11.1×10^{-3}	1.1	3.0×10^{-3}	1.0	600.0
3,500	-5.7×10^{-3}	1.1	3.0×10^{-3}	1.0	1,000.0

where $C_{SO_4^{2-}}$ is the dissolved sulfate concentration, $k_{SO_4^{2-}}$ is the sulfate consumption kinetic constant, α is the sulfate consumption reaction order, $C_{SO_4^{2-},0}$ is the initial dissolved sulfate concentration, t is time, $C_{S^{2-}}$ is the dissolved sulfide concentration, $k_{S^{2-}}$ is the sulfide generation kinetic constant, β is the sulfide generation reaction order, and $C_{S^{2-},\infty}$ is the final dissolved sulfate concentration.

Equations 3 and 5 were numerically integrated using the initial conditions given by Eqs. 4 and 6 and the fourth order Runge–Kutta method. The kinetic parameters ($k_{SO_4^{2-}}$, $k_{S^{2-}}$, α, β, $C_{S^{2-},\infty}$) were evaluated by curve fitting using nonlinear least square. Figure 7a, b shows the resulting curve fittings for the sulfate and sulfide solution concentration for the two cases.

Table 1 summarizes the kinetic parameter estimation. The results indicate that sulfate conversion and sulfide generation are both first-order processes. The kinetic constants for sulfate conversion indicate an inhibition at the enhanced initial sulfate concentration. Sulfate consumption kinetic inhibition of *Desulfovibrio* activity has been previously observed (Okabe et al. 1995). On the other hand, the kinetic constants for sulfide generation indicate that it is virtually independent of the initial sulfate concentration. This result indicates that after the microbial conversion of the SO_4^{2-} to S^{2-}, at least two in-series processes take place. First, in accordance with chemical ionic equilibria, most of the S^{2-} converts to HS^-; afterwards, some of the HS^- converts to H_2S. The kinetic of these processes seems

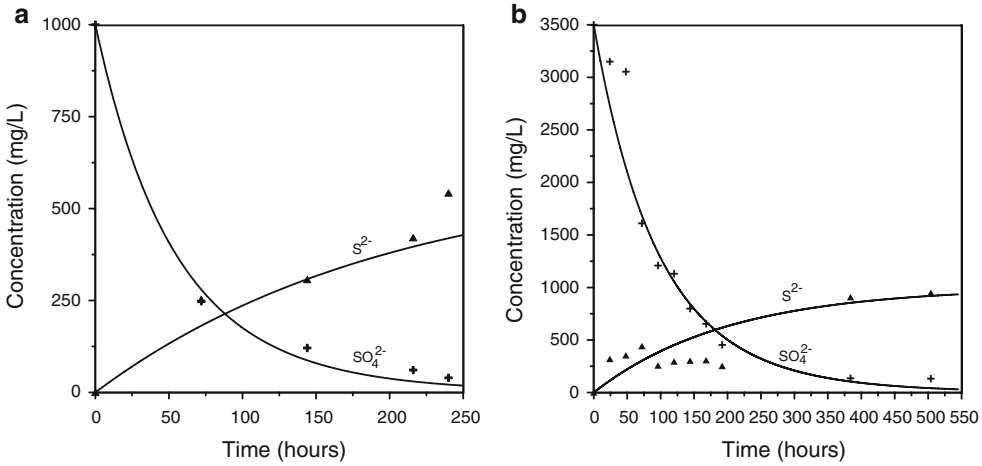

Fig. 7 Kinetic model fitting for sulfate and sulfide conversion for the batch regime in the column experiment; initial sulfate concentration: **a** 1,000 mg/L; **b** 3,500 mg/L

faster than the microbial conversion of the sulfate (Millero 1986).

Conclusion

This study investigated the activity of a mixed SRB culture collected from a Brazilian soured oil reservoir. The effects of the initial sulfate concentration (1,000 and 3,500 mg/L) on the anaerobic sulfate reduction and sulfide generation kinetics were investigated in an up-flow bioreactor packed with glass beads. Redox potential measurements were also used to monitor the growth and the activity of the SRB throughout the experimental runs. The results indicate that sulfate conversion and sulfide generation are both first-order processes. The kinetic constants for sulfate conversion indicate an initial inhibition at an initial sulfate concentration of 3,500 mg/L. In contrast, the kinetic constants for sulfide generation indicate that this reaction is almost independent of the initial sulfate concentration. This might be due to the presence of at least two in-series processes that are faster than the microbial conversion of the sulfate.

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