

Oxygen transfer and consumption in a thiosulfate oxidizing bioreactor with sulfur production

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ABSTRACT

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Aims: To evaluate the contribution of oxygen transfer and consumption in a sulfoxidizing system to increase the elemental sulfur yield from thiosulfate oxidation.

Methods and Results: A 10 l thiosulfate oxidizing bioreactor with suspended cells operating under microaerophilic conditions and a separated aerator with a variable volume of 0.8–1.7 l were operated with a consortium containing mainly *Thiobacillus* sp. that oxidizes several sulfide species to elemental sulfur and sulfate. From the gas–liquid oxygen balance, the k_{La} was estimated under different operation conditions. A k_{La} of around 200 h⁻¹ favoured elemental sulfur production and can serve as scale-up criterion. It was further shown that more than 50% of the oxygen fed to the system was consumed in the aerator.

Conclusions: The performance of the sulfoxidizing system can be improved by controlling oxygen transfer.

Significance and Impact of the Study: The proposed method for the k_{La} determination was based on the oxygen balance, which incorporates the oxygen concentrations measured in the liquid in steady state, reducing the interference of the response time in the traditional non-steady state methods. This approach can be used to optimize reactors where microaerophilic conditions are desirable.

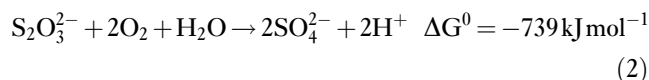
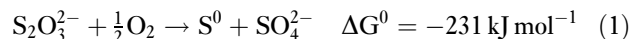
Keywords: elemental sulfur, k_{La} , microaerophilic conditions, oxygen transfer, recirculation reactor, *Thiobacillus*.

INTRODUCTION

Wastewater from refining operations in the oil industry may contain a number of undesirable contaminants including sulfides, ammonia and phenolic compounds. These pollutants have to be reduced to acceptable levels before being discharged.

Microbial species from the genus *Thiobacillus* have been studied to promote elemental sulfur production by partially oxidizing reduced sulfur compounds (Buisman *et al.* 1989; Janssen *et al.* 1997; Visser *et al.* 1997; Alcántara *et al.* 2004). Sulfur production (eqn 1) from the partial oxidation of thiosulfate, instead of the complete conversion

to sulfate (eqn 2), has favourable environmental implications as elemental sulfur can be removed by sedimentation. Furthermore, fewer chemicals are needed to neutralize the formed sulfate and lower energy consumption is required, as the oxidation to sulfur requires fourfold less oxygen.



According to the stoichiometry shown above, oxygen is the key parameter to control the extent of oxidation. In previous work (Alcántara *et al.* 2004; Velasco *et al.* 2004), a recycling reactor system (RRS) was proposed to enhance the formation of elemental sulfur and its continuous removal

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from the liquid phase. The main characteristic of the RRS is the physical separation of the aeration from the bioreactor. In the reactor, sulfur formation was favoured by the microaerophilic conditions and sedimentation and recovery were promoted by the induced laminar flow as shown by Velasco *et al.* (2004) where an ascending flow of 1.9 m h⁻¹ resulted in a Reynolds number of *c.* 80. Previous experiments indicated that the high oxygen concentration (>1 mg l⁻¹) in the aerator strongly promoted the complete oxidation (eqn 2). This results in the reduction of the sulfur yield (Velasco *et al.* 2004).

The global oxygen mass transfer coefficient k_{La} is a key parameter for bioreactor design in aerobic processes. It can be calculated from different experimental approaches including sulfite-oxidation and unsteady-state methods and their advantages and drawbacks are well known (Bailey and Ollis 1986).

The aim of this work was to evaluate the contribution of the oxygen transfer and consumption in the aerator of a RRS to increase the elemental sulfur (S⁰) yield from the oxidation of thiosulfate. A mass balance method based on the steady-state oxygen concentration was developed and a predictive k_{La} mathematical model was obtained.

MATERIALS AND METHODS

Micro-organisms

A chemoautotrophic sulfoxidizing consortium, composed mainly of *Thiobacillus* sp. that uses sulfide, thiosulfate, carbon disulfide and elemental sulfur as energy sources was used. It was obtained from the chemostat culture described by Alcántara *et al.* (2004). The mineral medium (Sublette 1987) used in the experiments contained (g l⁻¹): Na₂HPO₄ (1.2), KH₂PO₄ (1.8), MgSO₄·7H₂O (0.1), NH₄Cl (0.5), CaCl₂ (0.03), MnSO₄ (0.02), FeCl₃ (0.02), NaHCO₃ (4.0) and Na₂S₂O₃·9H₂O (20.0) which corresponded to 9 g l⁻¹ of S₂O₃²⁻. The final pH was adjusted to 7.

Analyses

Thiosulfate and sulfate were measured by HPLC (Water-Millipore model 600, no. 600; Waters, Milford, MA, USA), using a Photo Diode Array detector, at 308 nm and a Chrompack Ionospher A column (200 × 3 mm; Varian Inc., Palo Alto, CA, USA). Potassium hydrogen phthalate (0.04 mol l⁻¹ pH 4) was used as mobile phase. Sulfur content in the suspension and sediment was measured by the method reported by Bartlett and Skoog (1954). Biomass was estimated by the Lowry method. The oxygen liquid concentration was measured using a polarographic probe (Applisens, model Z01005902020; Applikon, Schiedam, the Netherlands).

Recycling reactor system

The studies were performed in the system depicted in Fig. 1. It had been in operation for more than 3 years and it was under steady state conditions when the oxygen studies were performed. The RRS consisted in a continuous reactor with recycling that allowed the formation and recovery of elemental sulfur. The operational conditions were pH 5.0 and 30°C. The reactor had 0.14-m internal diameter and a 10-l volume (V_A). The aerator had a 0.06-m internal diameter and a variable volume (V_B) of 0.8–1.7 l. The volume of the aerator is also represented dimensionless, (V_B^*), as its fraction with respect to the volume of the reactor. The streams, feed flow (F) and recycling flow (R), were pumped up-flow with variable speed pumps. The air flow (F_{air}) to the aerator was regulated and measured with a rotameter (0.5 LPM; Cole Parmer, Vernon Hills, IL, USA).

Different oxygen load rates (Q_{O_2}) to RRS were established by varying R according to eqn 3. Equation 4 depicts the thiosulfate load rate ($Q_{S_2O_3}$). Consequently, in a continuous system, the O₂/S₂O₃²⁻ molar ratio and the extent of thiosulfate oxidation could be theoretically controlled. The O₂/S₂O₃²⁻ load ratio is defined as $R_{mt} = Q_{O_2}/Q_{S_2O_3}$.

$$Q_{O_2} = \frac{R \cdot ([O_2]_B - [O_2]_A)}{V_{total}} \quad (3)$$

$$Q_{S_2O_3}^{2-} = \frac{F \cdot ([S_2O_3^{2-}]_{in} - [S_2O_3^{2-}]_B)}{V_{total}} \quad (4)$$

The stoichiometric molar ratio R_{me} is defined by eqns 1 ($R_{me} = 0.5$) and 2, ($R_{me} = 2.0$). Additionally other loads are defined as they are helpful to compare the performance of the RRS. The oxygen load rate, referred to reactor volume, expressed in mg O₂ l⁻¹ h⁻¹, is defined by eqn 5.

$$Q_{O_2 reactor}^A = D_R^A \cdot ([O_2]_B - [O_2]_A) \quad (5)$$

The oxygen load rate referred to aerator volume, expressed in mg O₂ l⁻¹ h⁻¹, is defined by eqn 6

$$Q_{O_2 reactor}^B = D_R^B \cdot ([O_2]_B - [O_2]_A) \quad (6)$$

Estimation of the volumetric oxygen transfer coefficient (k_{La})

The k_{La} determinations were made with a F of 0.21 l h⁻¹ and with three different volumes in the aerator, expressed as the aerated fraction volume (V_B^*) referred to the principal reactor. Various air flows (6–240 l h⁻¹) were tested for each aerator volume (1.6, 1.2, 0.8 l equivalent to V_B^* of 0.16, 0.12 and 0.08 respectively). The operation conditions in the RRS at steady state were: $R = 35$ l h⁻¹, $D = 0.5$ day⁻¹ and $[S_2O_3^{2-}]_{in} = 80$ mmol l⁻¹.

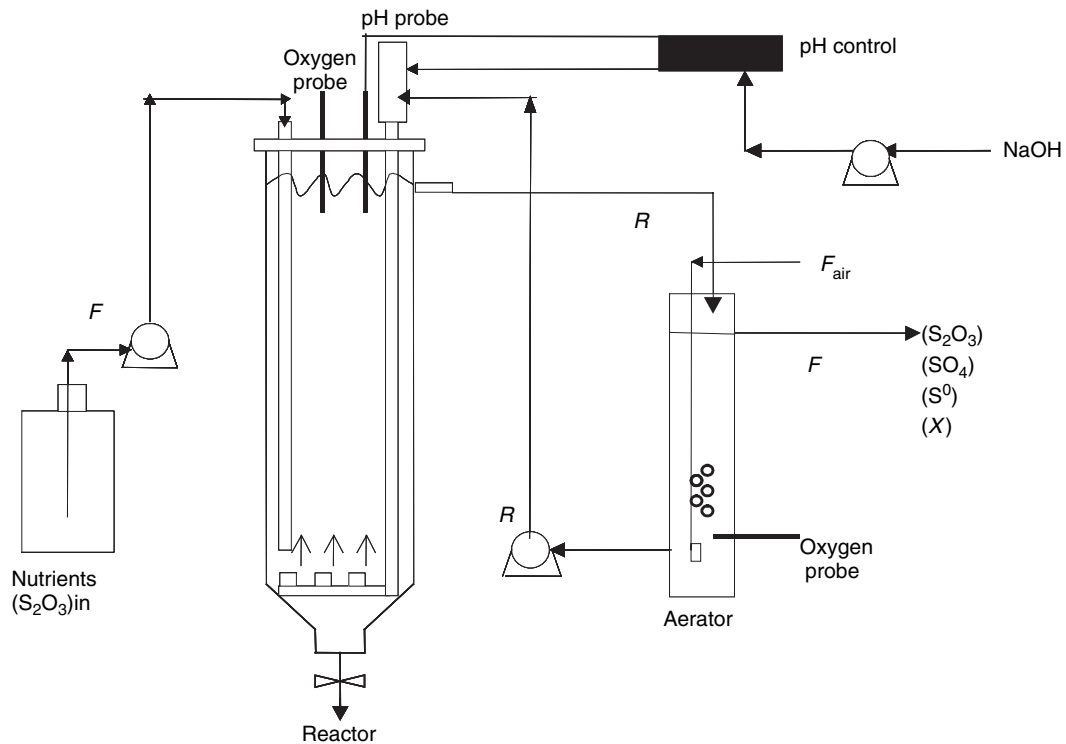


Fig. 1 Sulfoxidizing system

The dissolved oxygen concentration in the aerator and the reactor were measured for each of these conditions. The oxygen consumption rate, $q_{O_2}X$, was concurrently determined by respirometry in a bottle with a 0.25 l sample from the aerator. The $q_{O_2}X$ value was obtained from the steepest slope.

Oxygen balance

From the oxygen balance in the gas and liquid phases in the aerator and assuming complete mixing, constant $q_{O_2}X$, and pseudo-steady state in both phases, the k_La for continuous systems and steady state was calculated from the following equations.

From eqn 8, k_La is a function of both D_G and D_R^B . These variables determine the inlet and outlet dissolved oxygen concentrations. Thus, substituting the dissolved oxygen concentrations in the aerator $[O_2]_B$, in the reactor $[O_2]_A$ and the value of $q_{O_2}X$ in eqns 7 and 8 for each operation condition, the equations were simultaneously solved for $[O_2]_G$ and k_La . The measured $[O_2]_A$ was lower than the detection limit of the probe ($<0.05 \text{ mg l}^{-1}$) and was considered as 0 mg l^{-1} in the mass balances.

Gas phase

$$[O_2]_G = \frac{D_G[O_2]_G^{\text{IN}} + k_La[O_2]_B m}{k_La + D_G} \quad (7)$$

Liquid phase

$$k_La = \frac{Q_{O_2 \text{ reactor}}^B + q_{O_2}X}{([O_2]_G/m - [O_2]_B)} \quad (8)$$

RESULTS

The oxygen concentrations in the aerator and reactor were measured at steady state at different liquid residence times and air flows in the aerator. To promote elemental sulfur production with $R_{\text{me}} \approx 0.5$ (eqn 1), R was maintained at 35 l h^{-1} and consequently F_{air} and V_B^* became the main operational parameters to control the oxygen transfer rates. Figure 2(a) shows the $[O_2]_B$ and oxygen loads to the reactor $Q_{O_2 \text{ reactor}}^A$ (eqn 5) at different V_B^* and F_{air} . The concentration profiles were typical saturation curves and it was observed that flows from 1.0 to 1.5 vvm were sufficient to attain the maximum oxygen concentration in the aerator for V_B^* of 0.16 and 0.12, while 2.5–3.0 vvm were required for 0.084. These conditions, with a R of 35 l h^{-1} , resulted in the maximum $Q_{O_2 \text{ reactor}}^A$ for each aerator volume. Under these conditions, a $q_{O_2}X$ of $204 \pm 6 \text{ mg O}_2 \text{ l}^{-1} \text{ h}^{-1}$ (with a biomass content of $110 \text{ mg}_{\text{protein}} \text{ l}^{-1}$) was experimentally obtained and used to solve eqns 7 and 8 for $[O_2]_G$ and k_La . The k_La profiles are shown in Fig. 2(b).

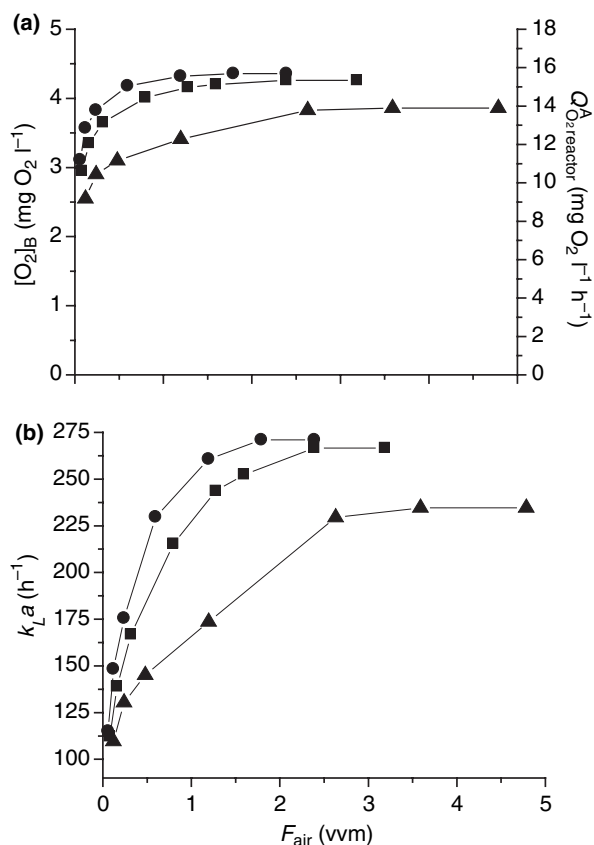


Fig. 2 Variation of (a) $[O_2]_B$ and $Q_{O_2,reactor}^A$, (b) k_{La} with F_{air} for V_B^* of (●) 0.16, (■) 0.12 and (▲) 0.08

The contribution of the aerator to the total thiosulfate oxidation was demonstrated from the oxygen balance (eqn 8). The comparison of oxygen feed rate to the reactor ($Q_{O_2,reactor}^B$) and the oxygen consumption rate in the aerator ($q_{O_2}X$) in Fig. 3 indicate that for the higher V_B^* , the biomass in the aerator consumed >50% of the oxygen fed to the system but was *c.* 40% for the smaller $V_B^* = 0.08$.

The effect of oxygen transfer rate on elemental sulfur production was evaluated with the methodology proposed in this work and using the data reported by Velasco *et al.* (2004) for different steady state conditions in RRS. Each steady state was established by varying operational parameters that were adjusted to the tested R_{mt} . Figure 4 shows the effect of k_{La} on the percentage of elemental sulfur and sulfate yield for the steady states reported. Here the elemental sulfur yield Y_S^0 is defined considering that 1 mol of elemental sulfur can be formed from 1 mol of thiosulfate that reacts according to eqn (1).

At very low k_{La} , (<50 h $^{-1}$), limited bacterial viability and elemental sulfur and sulfate production stopped. Beyond certain values of k_{La} , elemental sulfur production diminishes provoking increased sulfate concentration. For the

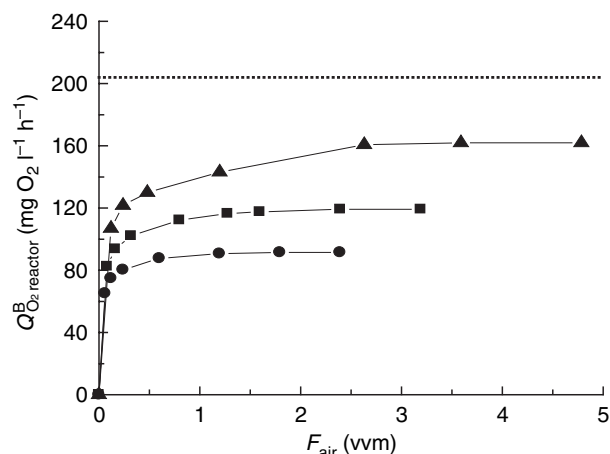


Fig. 3 Variation of $Q_{O_2,reactor}^B$ with F_{air} for V_B^* of (●) 0.16, (■) 0.12, (▲) 0.08. (---) $q_{O_2}X$. R was 35 l h $^{-1}$

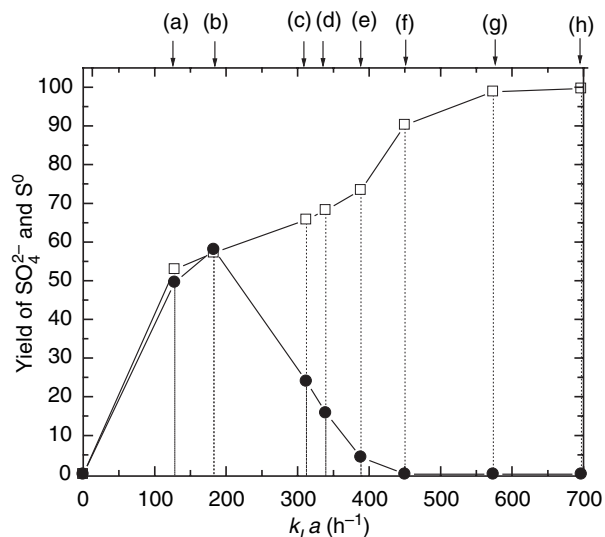


Fig. 4 Variation of (□) SO_4^{2-} and (●) S^0 yield with k_{La} for R_{mt} of (a) 0.13, (b) 0.20, (c) 0.44, (d) 0.55, (e) 0.75, (f) 1.00, (g) 1.50, (h) 2.00. $Q_{S_2O_3} = 2$ mmol l $^{-1}$ $S_2O_3^{2-}$ h $^{-1}$

configuration used, k_{La} values around 200 h $^{-1}$ were found to be appropriate to increase sulfur production.

DISCUSSION

The proposed method for the k_{La} determination is based on the oxygen balance, which incorporates the oxygen concentrations measured in the liquid in steady state at the inlet and outlet of the aerator under different operation conditions. The maximum values of k_{La} under the experimental conditions were in the same order of magnitude with those estimated by the correlation reported by Van't Riet (1983)

for bubble columns with coalescence. With this correlation, $k_L a$ values of 386, 224 and 213 h^{-1} were calculated for the V_B^* 0.16, 0.12 and 0.08 respectively.

Under the conditions of operation described, the RRS showed two opposite regions with low performance. In the first, where the dissolved oxygen was fed in excess, an increase in the specific growth rate of the microbial consortium was reached, but the end product was the less desirable sulfate. On the other side, when oxygen transfer was limited, a slow specific growth rate was reached and both the production of SO_4^{2-} and S^0 were strongly reduced and thiosulfate accumulated. In a previous work, González-Sánchez (2002), reported a μ_{\max} of 0.36 h^{-1} for this microbial consortium under aerobic conditions. This rate was reduced by 80% under the microaerophilic conditions ($<0.3 \text{ mg O}_2 \text{ l}^{-1}$) that favour elemental sulfur production.

The analyses showed that the performance of the RRS was reduced by relevant sulfate production under the oxygen rich conditions prevailing in the aerator. Sulfate production can be lowered by two ways. First, by reducing the hydraulic retention time in the aerator (although there is a practical limit when V_B is too low that insufficient contact area and time are found and thus $[\text{O}_2]_B \approx [\text{O}_2]_A$) and by maintaining a $k_L a$ around 200 h^{-1} as a scale-up criterion. Secondly, by increasing the cell retention in the reactor with a suitable support, such as polyurethane foam, the suspended bacterial concentration in the recirculating liquid is reduced and concomitantly the volumetric sulfate production in the aerator.

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NOMENCLATURE

D	Dilution rate (h^{-1})
D_F^A	Dilution rate, referred to reactor volume (h^{-1})
D_R^A	Recycling dilution rate, referred to reactor volume (h^{-1}) $D_R^A = \frac{R}{V_A}$
D_F^B	Dilution rate, referred to aerator volume (h^{-1})
D_R^B	Recycling dilution rate, referred to aerator volume (h^{-1}) $D_R^B = \frac{R}{V_B}$
D_G	Air dilution rate (h^{-1}) $D_G = \frac{F_{\text{air}}}{V_{\text{air}}}$
F	Volumetric flow rate (l h^{-1})
F_{air}	Volumetric air flow rate (l h^{-1})
$k_L a$	Volumetric oxygen transfer coefficient (h^{-1})

$m = 38$	Gas liquid partition coefficient (corrected by salinity)
$[\text{O}_2]_G^{\text{IN}}$	Oxygen concentration in air ($300 \text{ mg O}_2 \text{ l}^{-1} \text{ air}$)
$q_{\text{O}_2} X$	Oxygen consumption rate in aerator ($204 \pm 6 \text{ mg O}_2 \text{ l}^{-1}$)
$Q_{\text{O}_2, \text{reactor}}^A$	Oxygen load rate, referred to reactor volume ($\text{mg O}_2 \text{ l}^{-1} \text{ h}^{-1}$), $Q_{\text{O}_2, \text{reactor}}^A = D_R^A * ([\text{O}_2]_B - [\text{O}_2]_A)$
$Q_{\text{O}_2, \text{reactor}}^B$	Oxygen load rate, referred to aerator volume ($\text{mg O}_2 \text{ l}^{-1} \text{ h}^{-1}$), $Q_{\text{O}_2, \text{reactor}}^B = D_R^B * ([\text{O}_2]_B - [\text{O}_2]_A)$
$Q_{\text{S}_2\text{O}_3}^{2-}$	Thiosulfate consumption rate ($\text{mmol l}^{-1} \text{ S}_2\text{O}_3^{2-} \text{ l}^{-1} \text{ h}^{-1}$); $Q_{\text{S}_2\text{O}_3}^{2-} = \frac{F * ([\text{S}_2\text{O}_3^{2-}]_{\text{in}} - [\text{S}_2\text{O}_3^{2-}]_{\text{B}})}{V_{\text{total}}}$
Q_{O_2}	Oxygen consumption rate ($\text{mmol l}^{-1} \text{ O}_2 \text{ l}^{-1} \text{ h}^{-1}$), $Q_{\text{O}_2} = \frac{R * ([\text{O}_2]_B - [\text{O}_2]_A)}{V_{\text{total}}}$
R	Volumetric recycling flow rate (l h^{-1})
R_{mc}	Stoichiometric $\text{O}_2/\text{S}_2\text{O}_3^{2-}$ molar ratio (dimensionless)
R_{mt}	Theoretical $\text{O}_2/\text{S}_2\text{O}_3^{2-}$ molar ratio (dimensionless); $R_{\text{mt}} = \left(\frac{Q_{\text{O}_2}}{Q_{\text{S}_2\text{O}_3}^{2-}} \right)$
t_r^B	Hydraulic retention time in aerator (s)
V_A	Reactor volume (l)
V_{air}	Air volume inside of aerator (l)
V_B	Aerator volume (l)
V_B^*	Fraction volume of aerator; $V_B^* = V_B/V_A$
vvm	Air volume by liquid volume by minute (min^{-1})
$[\text{O}_2]_A$	Dissolved oxygen concentration in reactor ($\text{mg O}_2 \text{ l}^{-1}$)
$[\text{O}_2]_B$	Dissolved oxygen concentration in aerator ($\text{mg O}_2 \text{ l}^{-1}$)
$[\text{S}_2\text{O}_3^{2-}]_{\text{in}}$	Thiosulfate concentration on feed ($\text{mol S}_2\text{O}_3^{2-} \text{ l}^{-1}$)
$[\text{S}_2\text{O}_3^{2-}]_B$	Thiosulfate concentration on exit of the system ($\text{mol S}_2\text{O}_3^{2-} \text{ l}^{-1}$)
V_{total}	Total volume (l)
Subscripts	
A	Reactor
B	Aerator
G	Gas phase

REFERENCES

- Alcántara, S., Velasco, A., Muñoz, A., Cid, J., Revah, S. and Razo-Flores, E. (2004) Sulfide oxidation by a microbial consortium in a recirculation reactor system: sulfur formation under oxygen limitation and removal of phenols. *Environ Sci Technol* **38**, 918–923.
- Bailey, J.E. and Ollis, D.F. (1986) *Biochemical Engineering Fundamentals*, 2nd edn. New York, NY: McGraw-Hill.
- Bartlett, J. and Skoog, D. (1954) Colorimetric determination of elemental sulfur in hydrocarbons. *Anal Chem* **26**, 1008–1012.
- Buisman, C., Post, R., Ijspeert, P., Geraats, G. and Lettinga, G. (1989) Biotechnological process for sulfide removal with sulphur reclamation. *Acta Biotechnol* **9**, 255–267.
- González-Sánchez, A. (2002) Operación, caracterización y modelado de un sistema de oxidación biológica de tiosulfato a azufre elemental.

- MSc thesis, Universidad Autónoma Metropolitana- Iztapalapa, México DF, México.
- Janssen, A., Ma, S., Lens, P. and Lettinga, G. (1997) Performance of a sulfide-oxidizing expanded-bed reactor supplied with dissolved oxygen. *Biotechnol Bioeng* **53**, 32–40.
- Sublette, K. (1987) Oxidation of hydrogen sulfide by *Thiobacillus denitrificans*: desulfurization of natural gas. *Biotechnol Bioeng* **29**, 249–257.
- Van't Riet (1983) Mass transfer in fermentation equipment. *Trends Biotechnol* **1**, 113–121.
- Velasco, A., Alcántara, S., Razo-Flores, E. and Revah, S. (2004) Partial thiosulfate oxidation by steady-state continuous culture in a bioreactor-settler system. *J Chem Technol Biotechnol* **79**, 132–139.
- Visser, J., Robertson, L., Verseveld, H. and Kuenen, J. (1997) Sulfur production by obligately chemolithoautotrophic *Thiobacillus* species. *Appl Environ Microbiol* **63**, 2300–2305.