Water Research 89 (2016) 282–292

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Respirometric characterization of aerobic sulfide, thiosulfate and elemental sulfur oxidation by S-oxidizing biomass

Mabel Mora [a], Luis R. López [a], Javier Lafuente [a], Julio Pérez [b], Robbert Kleerebezem [b], Mark C.M. van Loosdrecht [b], Xavier Gamisans [c], David Gabriel [a, *]

[a] GENOCOV Research Group, Department of Chemical, Biological and Environmental Engineering, Escola d'Enginyeria, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain
[b] Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands
[c] Department of Mining Engineering and Natural Resources, Universitat Politècnica de Catalunya, Bases de Manresa 61-73, 08240 Manresa, Spain

ARTICLE INFO

Article history:
Received 9 September 2015
Received in revised form
25 November 2015
Accepted 26 November 2015
Available online 2 December 2015

Keywords:
Sulfide
Elemental sulfur
Sulfide oxidizing bacteria
Thiothrix sp.
Respirometry
Kinetic and mechanistic analysis

ABSTRACT

Respirometry was used to reveal the mechanisms involved in aerobic biological sulfide oxidation and to characterize the kinetics and stoichiometry of a microbial culture obtained from a desulfurizing biotrickling filter. Physical–chemical processes such as stripping and chemical oxidation of hydrogen sulfide were characterized since they contributed significantly to the conversions observed in respirometric tests. Mass transfer coefficient for hydrogen sulfide and the kinetic parameters for chemical oxidation of sulfide with oxygen were estimated. The stoichiometry of the process was determined and the different steps in the sulfide oxidation process were identified. The conversion scheme proposed includes intermediate production of elemental sulfur and thiosulfate and the subsequent oxidation of both compounds to sulfate. A kinetic model describing each of the reactions observed during sulfide oxidation was calibrated and validated. The product selectivity was found to be independent of the dissolved oxygen to hydrogen sulfide concentration ratio in the medium at sulfide concentrations ranging from 3 to 30 mg S L^{-1}. Sulfide was preferentially consumed ($SOUR_{max}$ = 49.2 mg DO g^{-1} VSS min^{-1}) and oxidized to elemental sulfur at dissolved oxygen concentrations above 0.8 mg DO L^{-1}. Substrate inhibition of sulfide oxidation was observed ($K_{i,S^{2-}}$ = 42.4 mg S L^{-1}). Intracellular sulfur accumulation also affected negatively the sulfide oxidation rate. The maximum fraction of elemental sulfur accumulated inside cells was estimated (25.6% w/w) and a shrinking particle equation was included in the kinetic model to describe elemental sulfur oxidation. The microbial diversity obtained through pyrosequencing analysis revealed that *Thiothrix* sp. was the main species present in the culture (>95%).

1. Introduction

Desulfurization of biogas under aerobic conditions using biotrickling filters (BTFs) has been demonstrated as an efficient and environmentally friendly process to upgrade biogas (Fortuny et al., 2008). In aerobic BTFs, air blown directly to the liquid phase is the most efficient way to supply oxygen as electron acceptor to oxidize sulfide to sulfate. However, excess air is passed through the packed bed to avoid products from partial sulfide oxidation, as elemental sulfur. Thus, air supply is limited since it lowers the methane content of biogas with the additional risk of reaching flammability limits when too much air is supplied. At high sulfide loading rates, elemental sulfur is produced instead of sulfate and accumulates inside the BTF packed bed as a consequence of oxygen transfer limitation (Fortuny et al., 2008; Rodriguez et al., 2014) (Eqs. (1)–(3)). Elemental sulfur accumulation imposes operational problems such as a pressure drop increase and it may eventually result in bioreactor clogging.

$$H_2S + 0.5\ O_2 \rightarrow S^0 + H_2O \quad (1)$$

$$S^0 + 1.5\ O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \quad (2)$$

$$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (3)$$

In order to gain insight in the factors that determine which products are formed during sulfide oxidation enabling process

* Corresponding author.
E-mail address: david.gabriel@uab.cat (D. Gabriel).

http://dx.doi.org/10.1016/j.watres.2015.11.061
0043-1354/

Nomenclature

a	chemical reaction order (dimensionless)
b	chemical reaction order (dimensionless)
β	chemical reaction order (dimensionless)
DO	dissolved oxygen concentration (mg O_2 L^{-1})
DO*	equilibrium oxygen concentration (mg O_2 L^{-1})
f_{max}	maximum ratio of intracellular sulfur stored to biomass (mg S mg^{-1} VSS)
f_{S0}	ratio of intracellular sulfur stored to biomass (mg S mg^{-1} VSS)
$K_{i,S^{2-}}$	sulfide inhibition constant (mg S L^{-1})
k_La_{O2}	mass transfer coefficient for oxygen (min^{-1})
K_O	affinity constant for oxygen (mg O_2 L^{-1})
$K_{S^{2-}}$	affinity constant for sulfide (mg S L^{-1})
k_{S^0}	shrinking kinetic constant for elemental sulfur oxidation ($mg^{2/3}$ VSS $mg^{-2/3}$ S)
K_{switch}	substrate switch constant (mg S L^{-1})
K_{TS}	affinity constant for thiosulfate (mg S L^{-1})
k_{TS_ab}	rate constant for thiosulfate production under abiotic conditions
k_{TS_p}	kinetic constant for thiosulfate production under biotic conditions
μ_{max}	maximum specific growth rate (h^{-1} or min^{-1})
η_{TS}	energetic correction factor to describe growth on thiosulfate
OUR_{end}	endogenous oxygen uptake rate (mg O_2 L^{-1} min^{-1})
OUR_{ex}	exogenous oxygen uptake rate (mg O_2 L^{-1} min^{-1})
PHB	Polyhydroxy-butyrate
r_{S2-}	biological sulfide oxidation rate (mg S L^{-1} min^{-1})
r_{S0}	biological sulfur oxidation rate (mg S L^{-1} min^{-1})
r_{TS}	biological thiosulfate oxidation rate ($mgS_2O_3^{2-}$–$SL^{-1}min^{-1}$)
r_{TS_ab}	thiosulfate production rate under abiotic conditions ($mgS_2O_3^{2-}$–$SL^{-1}min^{-1}$)
r_{TS_p}	thiosulfate production rate under biotic conditions ($mgS_2O_3^{2-}$–$SL^{-1}min^{-1}$)
S^{2-}	sulfide concentration (mg S L^{-1})
SO	sulfate concentration (mg S L^{-1})
SO_{in}	sulfate concentration in the outlet flow of the CSTR (mg S L^{-1})
SO_{out}	sulfate concentration in the inlet flow of the CSTR (mg S L^{-1})
θ	model parameter to be estimated through minimization
TDS	Total dissolved sulfide concentration (mg S^{2-} L^{-1})
TS	thiosulfate concentration (mg S L^{-1})
VSS_{out}	biomass concentration in the outlet flow of the CSTR (mg VSS L^{-1})
VSS_{in}	biomass concentration in the inlet flow of the CSTR (mg VSS L^{-1})
w_i	weighting coefficient
X	biomass concentration (mg VSS L^{-1})
$Y_{X/S^{2-}}$	biomass growth yield using sulfide as substrate (mg VSS mg^{-1} S)
Y_{X/S^0}	biomass growth yield using elemental sulfur as substrate (mg VSS mg^{-1} S)
$Y_{X/TS}$	biomass growth yield using thiosulfate as substrate (mg VSS mg^{-1} S)

optimization, a rigorous mathematical model describing desulfurization in a BTF is needed. The model can be applied to optimize the operational conditions of a desulfurizing BTF by avoiding elemental sulfur accumulation (Almenglo et al., 2013; López et al., 2015). To develop such process models, detailed kinetic characterization of sulfide-oxidizing biomass (SOB) is required. A validated and calibrated mathematical model can be used for proper prediction of bioreactor performance as a function of the operational conditions imposed.

Kinetic and stoichiometric characterization of SOB have been successfully conducted by Flowing gas-Static liquid respirometry (LFS), which is based on monitoring the dissolved oxygen (DO) concentration when a pulse of substrate is added to the respirometric vessel while air is continuously supplied to the liquid phase. At the end of the test, the initial dissolved oxygen concentration is recovered, once the substrate is completely depleted. The successful application of the LFS method is largely due to the high sensitivity associated with DO monitoring (Spanjers et al., 1996; Jubany et al., 2005 among many others). In an LFS respirometer, DO is measured in an aerated, suspended microbial culture. In desulfurizing BTFs, SOB are not suspended in the liquid phase but immobilized over the packing material surface. Immobilized biomass makes identification of kinetic and stoichiometric parameters more complicate due to steep substrate and product gradients. To overcome these limitations, Gonzalez-Sanchez et al. (2009) used the LFS respirometry to characterize SOB obtained from a BTF biofilm although the stoichiometry and oxidation mechanisms were not studied. Delhomenie et al. (2008) proposed a respirometric technique using directly the packing material with immobilized biomass. The problem with this method was that the conditions were not perfectly controlled (pH, homogeneity, active fraction of biomass and nutrients recirculation among others). Bonilla-Blancas et al. (2015) also proposed a novel respirometric methodology for SOB characterization using direct measurements in biotrickling beds, namely heterogeneous respirometry. However, the configuration of the respirometer did not allow sampling the liquid phase of the system, which hindered the validation of any kinetic model proposed to characterize SOB. LFS respirometry overcomes such limitations associated with direct measurement on a biofilm system. The main uncertainty to be considered when applying the kinetic data obtained using LFS in a biofiltration model is the correction needed for the fraction of active biomass and the actual biomass concentration in the biofilm.

Many authors have characterized suspended SOB but there is no clear agreement about the processes occurring or the kinetic equation to describe sulfide oxidation and elemental sulfur production and consumption rates. Most authors consider simple Monod or Haldane equations to describe limitation or substrate inhibition by sulfide, respectively (Roosta et al., 2011; Mora et al., 2014a). More complex kinetic models related to the physiology of SOB have also been reported (Klok et al., 2013). With regards to biological oxidation of elemental sulfur, kinetic models considering Monod equation or half or zero-order equations have also been reported (Koenig and Liu, 2001; Munz et al., 2009; Roosta et al., 2011; Mora et al., 2014a). The main objective of this work was to develop, calibrate and validate a complete kinetic model to characterize the aerobic biological sulfide oxidation process since no widely accepted mathematical framework to describe this process has been established so far. The model was developed taking into account the most relevant mechanisms involved in the process in order to be subsequently used in

general models describing aerobic desulfurization in BTFs. The Fisher Information Matrix (FIM) method was also used to evaluate the parameter sensitivities and the quality of the estimated parameter values (confidence intervals).

2. Materials and methods

2.1. SOB cultivation and biomass growth yield calculation

A sample of biomass was extracted from a lab-scale aerobic desulfurizing BTF (Lopez et al., 2015). The bioreactor had been treating 2000 ppm_v of H_2S during 1 year at room temperature and pH 7.0. The BTF was packed with plastic pall rings (3 L of packing material) and was divided into three sequential sections. From each Section 3 pieces of packing material with biofilm attached were collected and washed in 500 mL of mineral medium with the following composition (g L^{-1}): $NaHCO_3$ (3.5); NH_4Cl (1); KH_2PO_4 (0.12); K_2HPO_4 (0.15); $CaCl_2$ (0.02); $MgSO_4{\cdot}7H_2O$ (0.2); and trace elements, 1 mL L^{-1} (Fortuny et al., 2008). Afterwards, the mineral medium with the suspended biomass was used to inoculate a sterilized reactor (2.8 L), which was operated as a continuous stirred tank reactor (CSTR) without biomass retention.

Mineral medium and dissolved sodium sulfide were fed separately to avoid reactions between sulfide and metallic ions in the mineral medium (trace elements). A flow of 7.9 mL h^{-1} of sulfide solution (10 g $Na_2S{\cdot}9H_2O$-S L^{-1}) was supplied during the whole operation, which corresponded to a loading rate of 27.5 g S m^{-3} reactor h^{-1}. A total flow of 42.5 mL h^{-1} was supplied thus obtaining a dilution rate of 0.015 h^{-1}. Air was supplied at a flow rate of 20 L h^{-1}. The biomass was cultivated during almost 4 weeks. After steady-state conditions were reached, aliquots of the suspended culture were withdrawn from the CSTR to perform the corresponding respirometric tests.

The liquid phase of the CSTR was daily sampled to analyze sulfate, thiosulfate, sulfide and volatile suspended solids (VSS). Moreover, biomass samples from the inoculum as well as at the steady-state were taken from the reactor to verify microbial diversity preservation by pyrosequencing analysis. The $Y_{x/s}$ was also calculated at steady-state according to Eq. (4).

$$Y_{x/s} = (VSS_{out} - VSS_{in})/(SO_{out} - SO_{in}) \quad (4)$$

where $Y_{x/s}$ is the biomass growth yield (mg VSS mg^{-1} S), VSS_{in} and VSS_{out} are the inlet and outlet biomass concentrations (mg VSS L^{-1}) in the CSTR, respectively. SO_{in} and SO_{out} are the inlet and outlet sulfate concentrations in the CSTR (mgS $- SO_4^{2-} L^{-1}$), respectively.

2.2. Characterization of H_2S stripping and chemical oxidation

H_2S stripping and chemical oxidation processes were characterized through several abiotic tests conducted prior to biotic tests. A batch-type, magnetically stirred respirometer with a capacity of 350 mL was used. The respirometer was equipped with a gas diffuser, a pH control system and a water jacket connected to a thermostatic bath (Lauda Ecoline StarEdition RE107 E100 Circulator, Lauda Dr. R. Wobser) (Mora, 2014). Total Dissolved Sulfide (TDS) concentration was continuously monitored.

The stripping of sulfide was characterized using nitrogen gas to avoid any chemical oxidation of sulfide that could contribute to sulfide removal from the liquid phase. The conditions and the mineral medium set to perform the abiotic tests were those used in biotic tests. A buffered mineral medium (50 mM P-Phosphate at pH = 7) with NH_4Cl (100 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (20 mg L^{-1}), $CaCl_2$ (2 mg L^{-1}) without trace elements solution was used for stripping tests. It must be noted that the presence of metallic compounds that catalyze chemical sulfide oxidation (Obrien and Birkner, 1977; Buisman et al., 1990) were avoided during respirometric tests. Two different initial sulfide concentrations (15 and 30 mg $S^{2-} L^{-1}$) at different gas flows (15, 50 and 100 mL min^{-1}) were tested to characterize H_2S stripping. Mass balances and chemical equilibria of H_2S reported by Gonzalez-Sanchez et al. (2009) were used to estimate the k_La by curve fitting of time dependent dissolved sulfide concentration profiles.

Chemical sulfide oxidation tests were performed by using an air flow of 50 mL min^{-1} and a sulfide concentration of 30 mg S L^{-1}. Thiosulfate and sulfate were monitored during the test in order to find out which product was being produced from the chemical reaction.

2.3. LFS respirometric tests

LFS respirometry was used in order to study the mechanisms of sulfide oxidation under aerobic conditions and to identify the kinetic parameters. Two biotic tests (Table 1) were performed at 25 °C and at pH 7.0 with SOB obtained from the CSTR following the methodology proposed in Mora et al. (2014b). The difference in this case was that the liquid phase with the suspended biomass was continuously sparged with 50 mL min^{-1} of air with 2% (v/v) of CO_2. In this way non-limiting inorganic carbon concentrations were obtained as verified through total inorganic carbon analysis. Tests C-1 to C-4 corresponded to 4 consecutive substrate pulses performed in the same single respirometric test and were used to calibrate the kinetics of the process. Since tests were performed one after another, elemental sulfur not degraded in previous test was accumulated inside the cells, thus reaching a higher initial elemental sulfur concentration in the following test. Test V-1 corresponded to a substrate pulse performed in a different respirometric test and used in order to validate the kinetic model. In each test the pulse of substrate was added once the oxygen concentration was almost recovered from the previous test. At the beginning of each respirometric test, when the biomass was under endogenous conditions, the endogenous OUR and the k_La for oxygen were calculated following the methodology described in Guisasola (2005).

2.4. Analytical methods

Sulfate and thiosulfate concentrations were analyzed by ion chromatography with conductivity detection using a Dionex ICS-2000 equipment. Biomass concentration was determined according to Standard Methods (APHA, 2005) to obtain VSS concentration. Inorganic carbon concentration was measured with an OI Analytical TIC/TOC Analyzer (Model 1020A) equipped with a non-dispersive infrared detector and a furnace maintained at 680 °C. TDS concentration was analyzed off-line with a sulfide selective electrode (VWR International Eurolab, S.L). DO concentration and pH in the respirometer were monitored with a CellOx® 325 (WTW) and a SenTix® 82 (WTW) sensors both connected to a bench-top multimeter (Inolab® Multi 740 – WTW). Pyrosequencing analyses of SOB were performed by following the methodology reported in Montebello et al. (2013).

3. Kinetic model development

A kinetic model was developed based on the data obtained in respirometric tests and using mathematical terms typically used in activated sludge models as Monod-type or competitive inhibition-type equations (Dochain et al., 2001). Full oxidation of sulfide was assumed to proceed via thiosulfate and elemental sulfur as intermediate products. In the following sections all processes

Table 1
Biotic tests conducted to calibrate and validate the kinetics of sulfide oxidation under anoxic conditions.

Test	Process	Sulfide (mg S L^{-1})	Air flow (mL min^{-1})	Biomass (mg VSS L^{-1})
C-1	Calibration	3.5	50	155
C-2		10.5	50	155
C-3		23	50	155
C-4		36	50	155
V-1	Validation	25	15	260

considered are described in detail.

3.1. Sulfide biological oxidation

The volumetric rate of sulfide oxidation to elemental sulfur and thiosulfate was described through Eq. (5). A Haldane equation was considered to describe sulfide inhibition (Mora et al., 2015). A term describing the decrease of the sulfide oxidation rate due to the accumulation of intracellular elemental sulfur was also included in the kinetic model (Tamis et al., 2014). The limitation of oxygen was described with Monod-type kinetics.

$$r_{S^{2-}} = \frac{1}{Y_{X/S^{2-}}} \cdot \mu_{max} \cdot \frac{S^{2-}}{K_{S^{2-}} + S^{2-} + \frac{(S^{2-})^2}{K_{i,S^{2-}}}} \cdot \left[1 - \left(\frac{f_{S^0}}{f_{max}}\right)^{\alpha}\right] \cdot \frac{DO}{K_O + DO} \cdot X \quad (5)$$

where μ_{max} is the maximum specific growth rate (h^{-1}), $K_{S^{2-}}$ and K_O are the affinity constants for sulfide and oxygen, respectively (mg L^{-1}), $K_{i,S^{2-}}$ is the sulfide inhibition constant (mg S L^{-1}), f_{S0} is the ratio of intracellular elemental sulfur stored to biomass (mg S mg^{-1} VSS), f_{max} is the maximum ratio of intracellular elemental sulfur stored to biomass (mg S mg^{-1} VSS), X is the biomass concentration (mg VSS L^{-1}), $Y_{X/S^{2-}}$ is the biomass growth yield using sulfide as substrate (mg VSS mg^{-1} S) and S^{2-} and DO are sulfide and DO concentrations, respectively (mg S L^{-1} or mg O_2 L^{-1}).

3.2. Thiosulfate production and biodegradation

Thiosulfate is chemically produced as an intermediate compound during sulfide oxidation under abiotic conditions (r_{TS_p}) according to Eq. (6), while Eq. (7) describes the biological oxidation of thiosulfate (r_{TS}) through a Monod-type kinetics to consider thiosulfate limitation.

$$r_{TS\_p} = k_{TS\_p} \cdot \left(S^{2-}\right)^{\beta} \quad (6)$$

$$r_{TS} = \frac{1}{Y_{X/TS}} \cdot \mu_{max} \cdot \eta_{TS} \cdot \frac{TS}{K_{TS} + TS} \cdot \frac{K_{switch}}{K_{switch} + S^{2-}} \cdot \frac{DO}{K_O + DO} \cdot X \quad (7)$$

Where k_{TS_p} is the kinetic constant for thiosulfate production under biotic conditions, β is an order constant (dimensionless), η_{TS} is an energetic correction factor to describe growth on thiosulfate, TS is the thiosulfate concentration in the liquid (mg S L^{-1}), K_{TS} is the affinity constant for thiosulfate consumption (mg S L^{-1}), K_{switch} is a substrate switch constant (mg S L^{-1}) and $Y_{X/TS}$ is the biomass growth yield using thiosulfate as substrate (g VSS g^{-1} S).

3.3. Elemental sulfur biological oxidation

Elemental sulfur is the most important intermediate during biological sulfide oxidation. In Eq. (8) the reaction rate equation that describes the biodegradation of this compound is presented. As found in previous studies (Mora et al., 2015), elemental sulfur was degraded once the sulfide was almost depleted. For this reason a non-competitive inhibition term was used in order to describe the substrate switch. Moreover, elemental sulfur biodegradation rate included a shrinking particle term analogous to that used for polyhydroxy-butyrate (PHB) biodegradation modeling (Murnleitner et al., 1977; Tamis et al., 2014).

$$r_{S^0} = \frac{1}{Y_{X/S^0}} \cdot \mu_{max} \cdot k_{S^0} \cdot (f_{S^0})^{2/3} \cdot \frac{K_{switch}}{K_{switch} + S^{2-}} \cdot \frac{DO}{K_O + DO} \cdot X \quad (8)$$

Where k_{S^0} is the shrinking kinetic constant for elemental sulfur ($mg^{2/3}$ VSS $mg^{-2/3}$ S) and Y_{X/S^0} is the biomass growth yield using elemental sulfur as substrate (mg VSS mg^{-1} S). It must be mentioned that k_{S0} takes into account the energetic correction factor to describe growth on elemental sulfur, which would be equivalent to η_{TS}.

3.4. Oxygen uptake rate

DO was modeled taking into account sulfide, thiosulfate and elemental sulfur biodegradation and the corresponding stoichiometric coefficients from the biological reactions. Eq. (9) is the general equation used to describe the biomass specific oxygen uptake rate (SOUR) in an LFS respirometer.

$$SOUR = \frac{1}{X} \cdot \left[k_L a_{O_2} \cdot (DO^* - DO) - OUR_{ex} - OUR_{end}\right] \quad (9)$$

Where k_La_{O2} is the mass transfer coefficient for oxygen (min^{-1}), DO* and DO are the equilibrium and actual DO concentration (mg O_2 L^{-1}), respectively, OUR_{ex} and OUR_{end} are the exogenous and endogenous OUR (mg O_2 L^{-1} min^{-1}), respectively, which were calculated from the derivative of the experimental DO profile.

3.5. Parameters estimation

Kinetic parameters were estimated by fitting the experimental data to model predictions. The fitting method was based on seeking the minimum value of the objective function (Eq. (10)). This function is usually defined as the norm of the differences between the predicted values of the mathematical model and the experimental data. However, in this work a weighted least squares objective function (G) was used (Eq. (10)) since the number of experimental points and the magnitude of the variables considered for the optimization were very different.

$$\begin{aligned} G(\theta) = {} & w_1 \cdot \text{norm}([S^{2-}]_{exp} - [S^{2-}]_{model} \\ & (\theta)) + w_2 \cdot \text{norm}([DO]_{exp} - [DO]_{model} \\ & (\theta)) + w_3 \cdot \text{norm}([SO]_{exp} - [SO]_{model} \\ & (\theta)) + w_4 \cdot \text{norm}([TS]_{exp} - [TS]_{model}(\theta)) \end{aligned} \quad (10)$$

Where θ is the model parameter to be estimated through minimization, w_i are the weighting coefficients used for each one of the data sets included in the objective function and norm is the

Euclidean norm of a vector. As an example, the calculation of the weighting coefficient w_1 is shown in Eq. (11).

$$w_1 = \frac{\sum_1^i (SO)_{i,1}}{i} \cdot \left[\frac{\sum_1^j (S^{2-})_{j,1}}{j}\right]^{-1} \cdot j^{-1} \quad (11)$$

Where $(SO)_{i,1}$ is the sulfate concentration column vector with *i* elements and $(S^{2-})_{j,1}$ is the sulfide concentration column vector with *j* elements. Sulfate was used to calculate each weighting coefficient since was the species with highest mean concentration along each test.

All calculations were implemented in MATLAB 7.7 (Mathworks, Natik, MA) using the function *ode45* (unconstrained nonlinear optimization method based on an explicit Runge-Kutta (4,5) formula) to solve the numerical differentiation formulas. The MATLAB function *fminsearch* was used to minimize the fitting error between the experimental and model data. A cubic spline polynomial interpolation algorithm (*interp1*) was used to interpolate the modeling results for sulfide, sulfate, DO and OUR at different experimental sampling times.

3.6. Fisher Information Matrix

The confidence intervals of the estimated parameters were assessed through the Fisher Information Matrix (FIM) method. This method is a proven tool that accurately provides confidence intervals for kinetic parameters and allows evaluating the sensitivities of parameters and the quality of estimations (Dochain and Vanrolleghem, 2001). The FIM method is based on the calculation of the covariance matrix inverse, which is directly associated to the uncertainty of model parameters estimated and the quantity and quality of the experimental data since it considers the output sensitivity functions and the measurement errors of experimental data (i.e. accuracy of an experiment). Many authors have successfully used this mathematical method to evaluate the reliability of parameters estimated both in wastewater and waste gas treatment modeling (Guisasola et al., 2006; Dorado et al., 2008).

4. Results and discussion

4.1. Assessment of SOB cultivation in a CSTR

The operational performance of the CSTR in which the SOB culture was cultivated is shown in Fig. 1. It was observed that the steady-state was reached after 12 d of operation. A biomass growth yield of 0.258 ± 0.025 mg VSS mg^{-1} S (0.073 mmol VSS $mmol^{-1}$ S) was estimated and complete sulfide oxidation to sulfate was obtained during the steady-state period (15–18 d). This biomass yield was similar to the value obtained by Nelson et al. (1986) (0.247 g VSS g^{-1} S) and Kelly et al. (1982) (0.213 g VSS g^{-1} S) for sulfide oxidizing bacteria as *Beggiatoa* sp. and *Thiobacillus* sp., respectively.

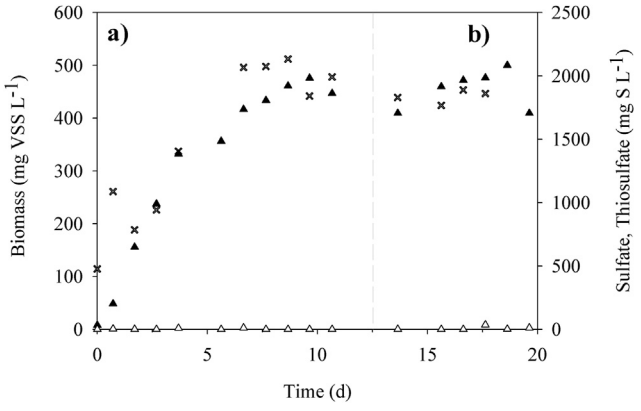

Fig. 1. SOB cultivation in a CSTR. (a) Unsteady-state operation (b) Steady-state operation. Sulfate (▲), thiosulfate (△) and biomass (X).

Considering the biomass growth yield calculated, the stoichiometry of the overall oxidation reaction was obtained (Eq. (12)) by solving the mass and charge balances according to Roels (1983), assuming $C_5H_7O_2N$ as typical biomass composition (Hoover et al., 1951) and NH_4^+ as the nitrogen source.

$$0.5\ H_2S + 0.5\ HS^- + 1.64\ O_2 + 0.342\ CO_2 + 0.023\ HCO_3^- + 0.073\ NH_4^+ + 0.123\ H_2O \rightarrow 0.073\ C_5H_7NO_2 + SO_4^{2-} + 1.55\ H^+ \quad (12)$$

In Fig. 2 the pyrosequencing analysis performed from the inoculum and steady-state biomass samples collected from the CSTR are presented. As can be observed, the inoculum diversity (Fig. 2a) was very low and the main species found was a member of the *Thiothrix* genus (95%) which was successfully preserved until the steady-state, where its predominance was even higher (99%) (Fig. 2b). *Thiotrix* sp. are filamentous γ-proteobacteria that grow well under heterotrophic, mixotrophic or autotrophic conditions and form intracellular deposits of elemental sulfur as intermediary product during sulfide oxidation. *Thiothrix* sp. are also capable of oxidizing thiosulfate and other reduced sulfur compounds (Nielsen et al., 2000). Thus, the CSTR operational conditions allowed preserving the dominant strains found of the inoculum, which had been growing on the packing material as a biofilm and had different structure and growth conditions than those set in the CSTR. This result indicates that the immobilized SOB mixed culture from the BTF acclimated well to suspended conditions. From Eq. (12), a stoichiometric O_2/S ratio of 1.64 was obtained. This ratio is lower than that associated to the corresponding chemical reaction (O_2/S = 2) (Eq. (3)). This was expected since a fraction of the reducing power of H_2S is used to reduce CO_2 for cell synthesis instead of oxygen reduction for energy generation (Eq. (3)).

4.2. Mass transfer and sulfide stripping characterization

Mass transfer coefficient (k_La) for stripping of sulfide was obtained by fitting measured sulfide concentrations to the model proposed by Gonzalez-Sanchez et al. (2009). In Fig. 3a the experimental and model data from the stripping test performed at 30 mg S L^{-1} and at 50 mL min^{-1} are presented. A k_La value of 0.075 ± 0.010 min^{-1} (4.5 ± 0.6 h^{-1}) was estimated. Since air was sparged through the liquid the k_La was higher than the value obtained for a similar system just with a gassed headspace (Mora et al., 2015). The air flow used was 50 mL min^{-1} to minimize the H_2S stripping without limiting the DO concentration during respirometric tests (DO > 0.8 mg O_2 L^{-1}). In Fig. 3b, k_La values obtained for different air flows are presented. As can be observed, a linear correlation was found to describe the relation between both variables. At 15 mL N_2 min^{-1} the k_La obtained allowed minimizing the stripping of H_2S. However, in biotic experiments the time needed to recover the initial DO concentration after a substrate pulse was extremely long using this flow. This fact did not allow spiking several times the biomass suspension with substrate during the same experiment. On the other hand, a gas flow of 100 mL min^{-1} lead to a k_La of 0.143 ± 0.008 min^{-1} (8.6 ± 0.5 h^{-1}),

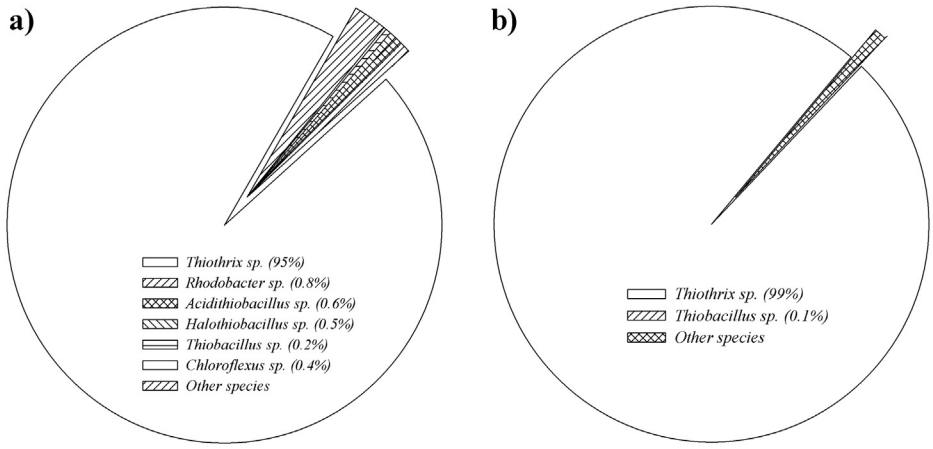

Fig. 2. Pyrosequencing microbial diversity results of CTSR biomass samples. (a) Inoculum (b) Steady-state.

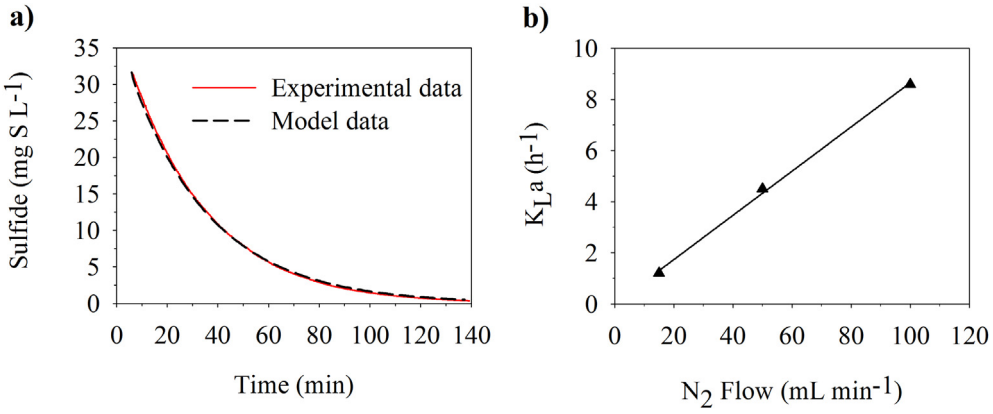

Fig. 3. Experimental and modeled data obtained from H_2S stripping tests performed in the respirometer. (a) Stripping test performed at 30 mg S L^{-1} and a gas flow of 50 mL min^{-1} (b) Linear correlation between gas flow and k_La values. Experimental k_La (symbol), linear correlation between k_La and gas flow (black solid line).

which was too high to obtain a sensitive DO profile associated to a substrate pulse. Consequently, the gas flow was finally set at 50 mL min^{-1}.

4.3. Chemical oxidation of sulfide

Chemical oxidation of sulfide has been studied by many authors (Chen and Morris, 1972; Kuhn et al., 1983; Alper and Ozturk, 1985; Nielsen et al., 2003). Sulfide is a reactive species that may be oxidized even in absence of a catalyst (Buisman et al., 1990). Since respirometric tests were conducted under aerobic conditions, an abiotic test was performed to identify the contribution of chemical sulfide oxidation. In Fig. 4 the experimental data obtained from the test is presented. A minimum amount of thiosulfate was produced due to chemical oxidation in the control experiment. No significant sulfate production was observed. Chemical thiosulfate production (Eq. (13)) has been previously described with Eq. (14) by other authors (Buisman et al., 1990 among others). We therefore used this equation to fit the thiosulfate experimental data (Fig. 4) and to estimate the kinetic parameters (k_{TS_ab}, a and b).

$$2HS^- + 2O_2 \rightarrow {S_2O_3}^{2-} + 2H_2O \quad (13)$$

$$r_{TS\_ab} = k_{TS\_ab} \cdot \left(S^{2-}\right)^a \cdot (O_2)^b \quad (14)$$

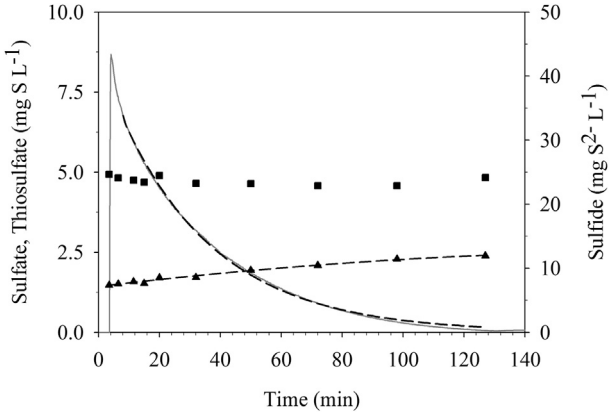

Fig. 4. Experimental and model data obtained from sulfide chemical oxidation test performed in the respirometer without trace elements solution, an air flow of 50 mL min^{-1} and a sulfide concentration of 30 mg S L^{-1}. Sulfate (■), thiosulfate (▲), experimental dissolved sulfide (grey solid line) and model predictions (black dashed line).

Where r_{TS_ab} is the oxidation rate (mg S – ${S_2O_3}^{2-}$ L^{-1} min^{-1}), k_{TS_ab} is the rate constant for thiosulfate production (k_{TS_ab} = 0.0030 $min \cdot (mg\ S\ L^{-1})^{-0.75} \cdot (mg\ O_2\ L^{-1})^{-0.77}$) in abiotic conditions, a is the reaction order with respect to sulfide (a = 0.25) and b is the reaction order with respect to oxygen (b = 0.23).

From the kinetic values estimated it was concluded that, given the biological sulfide oxidation rates typically found, the contribution of chemical sulfide oxidation could be neglected.

4.4. Biological sulfide oxidation mechanisms

Respirometric profiles obtained from experimental tests were previously studied in order to elucidate which the mechanisms of the process were. In this way, from a single test (Test C-4, Fig. 5), the mechanisms depicted in Fig. 6 was proposed. The first step of the sulfide oxidation process corresponds not only to the partial oxidation of sulfide to elemental sulfur but also to its oxidation to sulfite, which further reacts with elemental sulfur in the presence of sulfide to form polysulfides (Kleinjan et al., 2005) in the periplasmatic space of the cell. Then, polysulfide is subsequently oxidized to thiosulfate. This mechanism has been previously described by Chen and Morris (1972) and is in line with the results obtained from the sulfide chemical oxidation experiments described previously. In the model described herein sulfide conversion to sulfite, polysulfide, and thiosulfate is lumped into one chemical equation describing chemical sulfide oxidation to thiosulfate. Finally, after sulfide depletion, both elemental sulfur and thiosulfate are further oxidized to sulfate, which is the end product of the reaction.

Since the abiotic test showed that a much lower amount of thiosulfate was chemically produced compared to that produced in test C-4 (Fig. 4), it is logical that another species not present under abiotic conditions was responsible for thiosulfate formation. This could be the case of sulfite, which was only present during the biotic test and probably the key compound to obtain thiosulfate as an intermediate during biological sulfide oxidation. However, this hypothesis must be further investigated since, in this preliminary study, sulfite and polysulfides were not analyzed during respirometric tests. Moreover, biological thiosulfate formation has not been previously reported by other authors at the conditions set in this study, probably because of the particular characteristics of the microbial culture studied herein. Therefore, there is no data available to be compared with that obtained in this study.

4.5. Stoichiometry of the process

The stoichiometry of the process was identified using the profile shown in Fig. 5 and Eq. (12). As can be observed in Fig. 5, the sulfate concentration was almost constant during sulfide uptake, suggesting that intermediate elemental sulfur was initially produced. From this stage of the respirometric test (with the presence of sulfide) the total oxygen used for sulfide oxidation to elemental

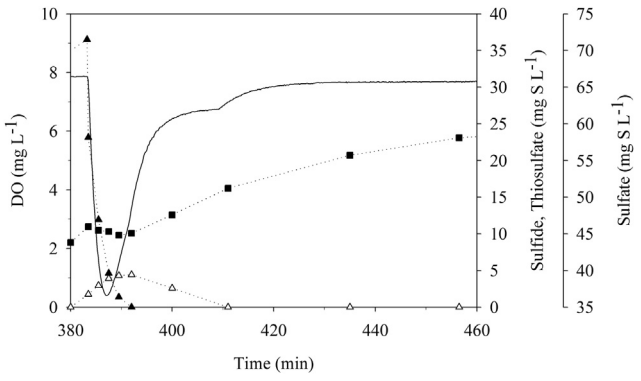

Fig. 5. Respirometric profile obtained from Test C-4. Sulfide (▲), thiosulfate (△), sulfate (■), DO (solid line).

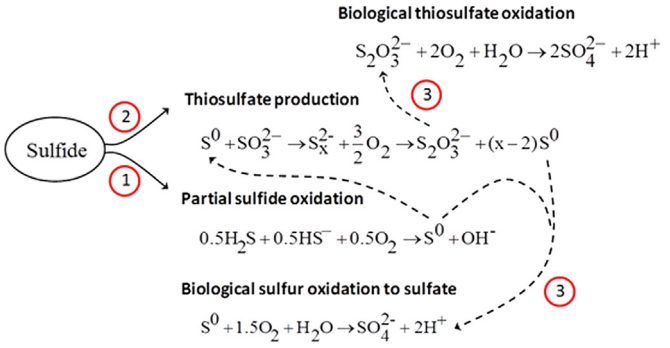

Fig. 6. Mechanism proposed for biological sulfide oxidation. (1) Partial sulfide oxidation to elemental sulfur (2) Thiosulfate production from polysulfide pathway (3) Biological oxidation of thiosulfate and intracellular elemental sulfur

sulfur was calculated by integrating Eq. (9). The ratio between the oxygen consumed and the sulfide consumed was 0.42 mol O_2 mol^{-1} S^{2-}. The remaining stoichiometric coefficients corresponding to the considered reaction (Eq. (15)), including the biomass growth yield, were calculated as described above (section 4.1).

$$0.5\ H_2S + 0.5\ HS^- + 0.42\ O_2 + 0.075\ CO_2 + 0.005\ HCO_3^- + 0.016\ NH_4^+ + 0.489\ H^+ \rightarrow 0.016\ C_5H_7NO_2 + S^0 + 0.973\ H_2O \quad (15)$$

A biomass growth yield of 0.016 mol VSS mol^{-1} S was calculated, which represents a 22% of the experimental $Y_{x/s}$ obtained for the complete reaction (0.073 mol VSS mol^{-1} S). This result is coherent since 25% of the electrons required for the complete oxidation (2 of 8 mol e$^-$ mol^{-1} S^{2-}) were involved in this reaction. From Eq. (12) and Eq. (15) the stoichiometry corresponding to the elemental sulfur oxidation reaction was solved (Eq. (16)).

$$S^0 + 1.22O_2 + 0.267CO_2 + 0.018HCO_3^- + 0.057NH_4^+ + 1.10H_2O \rightarrow 0.057C_5H_7NO_2 + SO_4^{2-} + 2.04H^+ \quad (16)$$

A higher biomass growth yield was obtained from elemental sulfur oxidation to sulfate (0.057 mol VSS mol^{-1} S) since 6 mol e$^-$ mol^{-1} S are gained in this reaction. For thiosulfate oxidation, a biomass growth yield of 0.071 mol VSS mol^{-1} $S_2O_3^{2-}$ – S reported by Odintsova et al. (1993) for *Thiothrix ramosa* was used to solve the thiosulfate oxidation stoichiometry (Eq. (17)).

$$S_2O_3^{2-} + 1.65O_2 + 0.333CO_2 + 0.022HCO_3^- + 0.071NH_4^+ + 1.12H_2O \rightarrow 0.071C_5H_7NO_2 + SO_4^{2-} + 2.05H^+ \quad (17)$$

4.6. Kinetic model calibration

The kinetic model was calibrated using tests C-1 to C-4 which, as mentioned in Section 2.3, corresponded to 4 different substrate pulses performed in the same single respirometric test. In Fig. 7 two of the modeled respirometric profiles are presented (C-2 and C-4). In Table 2 the kinetic parameters obtained are listed. In each one of the tests different parameters were estimated since the sensitivity of the kinetic model for each parameter estimated depends on the conditions tested (substrate and product concentrations, presence of inhibiting compounds, etc.). Previous to model calibration, the

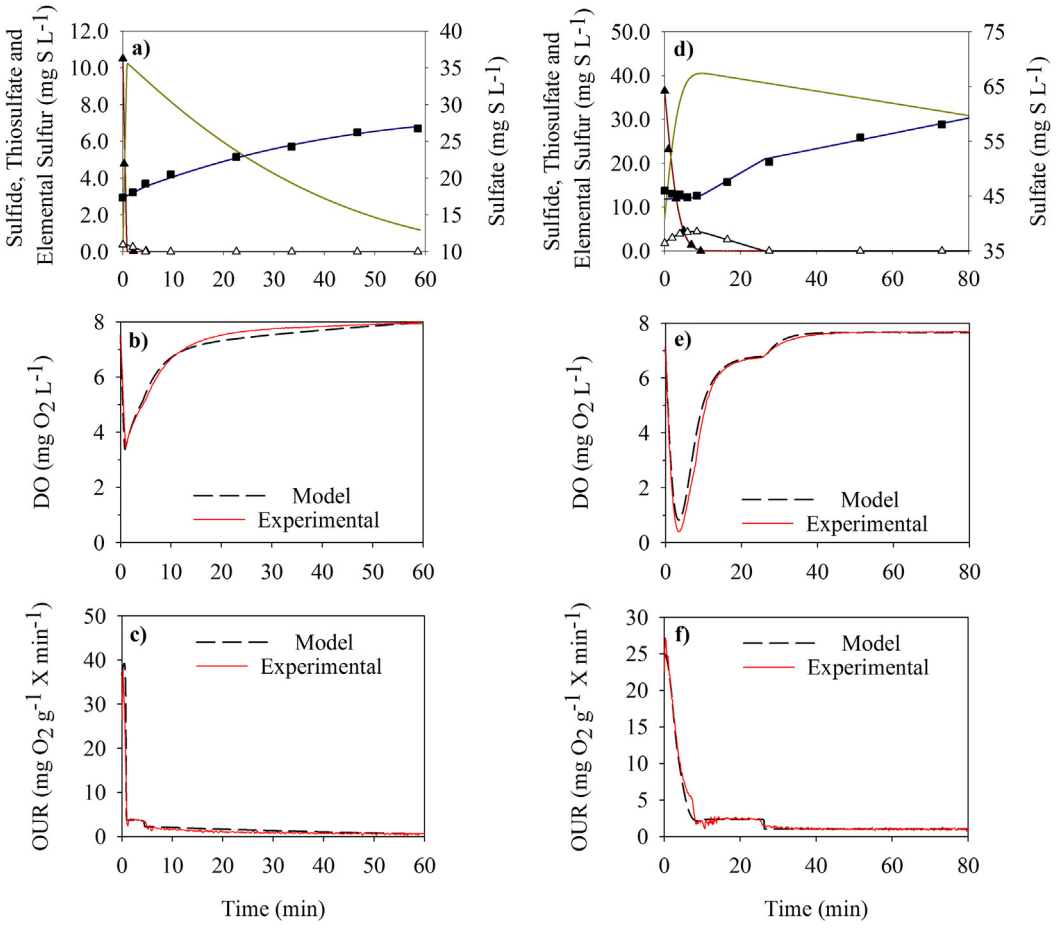

Fig. 7. Calibration of the kinetic model with experimental respirometric profiles obtained from tests C-2 (a, b, c) and C-4 (d, e, f). Experimental and modeled sulfide concentration (▲ and dark red solid line, respectively), modeled elemental sulfur concentration (dark yellow solid line), experimental and modeled thiosulfate concentration (Δ and black solid line, respectively), experimental and modeled sulfate concentration (■ and blue solid line, respectively).

Table 2
Kinetic parameters, error percentage and confidence intervals estimated and calculated from the fitting of the model proposed to the experimental respirometric profiles.

Parameter	Value	% Error	Confidence intervals	Units	Calibration test
μ_{max}	$6.60 \cdot 10^{-3}$	0.89	$[6.66 \cdot 10^{-3} - 6.54 \cdot 10^{-3}]$		C-1
	0.396		[0.400–0.392]	min^{-1} h^{-1}	
$K_{S^{2-}}$	0.317	6.12	[0.336–0.298]	mg S L^{-1}	C-1
$K_{i,S^{2-}}$	42.4	0.29	[42.5–42.3]	mg S L^{-1}	C-3
f_{max}	0.256	0.04	0.256	g S g^{-1} VSS	C-4
α	1.71	0.09	1.71	–	C-4
η_{TS}	0.030	4.30	[0.031–0.029]	–	C-3
K_{TS}	0.0023	28.7	[0.0030–0.0016]	mg S L^{-1}	C-3
k_{TS_p}	0.107	0.28	[0.1073–0.1067]	mg $S^{0.47}$ $L^{-0.47}$ min^{-1}	C-3, C-4
β	0.530	0.21	[0.531–0.529]	–	C-3, C-4
k_{S^0}	[0.833–0.030]	[0.26–0.10]	–	g $S^{1/3}$ $g^{-1/3}$ VSS	C-1 to C-4
K_{switch}	0.455	3.05	[0.469–0.441]	mg S L^{-1}	C-4
$SOUR_{max}$	49.2		–	mg DO g^{-1} VSS min^{-1}	calculated
K_O	0.146	0.21	0.146	mg O_2 L^{-1}	C-4

endogenous OUR (0.132 mg O_2 g^{-1} VSS min^{-1}) and the k_La for oxygen (0.257 min^{-1} or 15.4 h^{-1}) were calculated. As can be observed in Fig. 7, the model described satisfactorily the experimental data although differences were observed for DO concentration when elemental sulfur was the remaining substrate (Test C-2). This difference was also found in tests C-1 and C-3 (see Fig. SM1 in Supplementary Material section). Thus, the kinetic constant corresponding to elemental sulfur oxidation (k_{S0}) was calibrated in each substrate pulse to describe properly the experimental data since sulfur degradation depended directly on this parameter which makes it sensitive to be calibrated for any concentration tested. Differences were attributed to the shrinking particle model used, which was probably not the optimal to describe the respirometric profiles. However, such model provided the best predictions of experimental data compared with other kinetic equations tested such as Monod, Haldane, zero-order and half-order equations. Although the sulfate profile was properly described with most models, the DO profile was not well described

in the whole range of sulfur concentrations (data not shown) except with the shrinking particle model.

Results demonstrate that sulfate data from off-line monitoring are not as sensitive as DO data for proper calibration of kinetic models. Regarding to the variation of kinetic parameters between tests, Koenig and Liu (2001) also observed a large variability when describing elemental sulfur oxidation in a sulfur packed bed reactor with a half-order kinetic equation. Authors eventually associated this variation to the sulfur particle size. Another explanation for kinetic parameters variation could be the fact that part of the intracellular elemental sulfur was stored as orthorhombic α-sulfur which is most likely inaccessible to bacterial enzymes (Berg et al., 2014). Other authors have also observed kinetic parameters variation when describing PHB degradation with the shrinking particle model (Tamis et al., 2014), which was associated to the number and size of the particles related to the biomass concentration. Therefore, the variation of k_{S^0} obtained in this study was also associated to the sulfur particles size and the elemental sulfur speciation (i.e. S_8 rings, polythionates, etc.), which could have been changing during respirometric tests. Further research is required in order to clarify which the specific factors that affect k_{S^0} are.

As presented in Table 2, the maximum growth rate obtained was 0.396 h^{-1} ($6.60 \cdot 10^{-3}$ min^{-1}), which was slightly higher than that found by Munz et al. (2009) (0.308 h^{-1}) for SOB using elemental sulfur as substrate in a respirometer. The difference found is probably because the calibration of this parameter takes into account the maximum specific growth rate that describes only the sulfide oxidation to elemental sulfur, which has associated an extremely high specific consumption rate. This was somehow expected since SOB grow faster with sulfide than with elemental sulfur.

The SOB obtained from the BTF presented high affinity for oxygen, sulfide and thiosulfate according to K_O, $k_{S^{2-}}$ and K_{TS} values, respectively (Table 2). The estimated inhibition constant for sulfide was also high (42.4 mg S L^{-1}) compared with values reported in previous studies (Mora et al., 2015), indicating that more than 80% of the maximum oxidation rate was reached for concentrations below 10 mg S L^{-1} of dissolved sulfide. It must be mentioned that during the operation of the BTF the dissolved sulfide concentration remained below 1 mg S L^{-1} (data not shown), which indicated that the biomass was not inhibited by sulfide. As mentioned in previous sections, sulfide oxidation was also affected by the accumulation of intracellular sulfur. From model calibration, the maximum capacity of elemental sulfur storage inside the cells was calculated as 25.6%. No data has been reported regarding sulfur storage capacity for SOB under the same conditions but compared to PHB degraders (89% of PHB related to dry weight reported by Johnson et al. (2009)) the accumulation percentage was not especially high probably because of the *Thiothrix* sp. morphology.

Regarding the substrate preference, no correlation between the O_2/S ratio and the product selectivity was found as concluded in previous studies (Mora et al., 2015). This result is not in agreement with Gonzalez-Sanchez et al. (2009) who considered product selectivity depending on the O_2/S ratio available in the media. In this study the biomass was consuming preferentially sulfide, regardless of the O_2/S ratio, to convert it into elemental sulfur and thiosulfate. All other substrates were consumed after sulfide depletion and presented the same inhibition-affinity constant (K_{switch} = 0.455 mg S L^{-1}), which indicates a simultaneous biodegradation under the absence of sulfur. This result indicates that sulfide is energetically favorable so that it is immediately oxidized to elemental sulfur, even with O_2/S ratio greater than the corresponding for the complete oxidation stoichiometry (Eq. (12)). In fact, Visser et al. (1997) reported that elemental sulfur formation occurred when the maximum oxidative capacity of the culture was approached independently of the O_2/S ratio. This means that, in order to be able to oxidize increasing amounts of sulfide, the organism has to convert part of the sulfide to sulfur instead of sulfate to keep the electron flux constant regardless of the DO concentration.

As can be observed in Table 2, the error associated with the estimation of each kinetic parameter was calculated, as well as the corresponding confidence interval, in order to assess the sensitivities of the parameters and the quality of estimations. In general, the error percentages calculated with the FIM method were low (<6.5%) indicating that the high sensitivity of all kinetic parameters at these concentration ranges guarantees the parameter identifiability during model calibration (i.e. the possibility in obtaining a unique value of each parameter during model calibration). Such low confidence intervals indicate both the use of an accurate measurement technique as well as a correct experimental design that satisfactorily described the experimental behavior with the kinetic model proposed. The maximum error calculated was that associated to the thiosulfate affinity constant (K_{TS} = 28.4%), which indicates a low sensitivity of this parameter. This result was somehow expected since the quantity of experimental data was really low around the concentration in which the parameter was calibrated.

4.7. Validation of the kinetic model

The kinetic model was validated in respirometric test V-1. In Fig. 8 the experimental and respirometric profiles are presented. This test was performed with the same biomass used for the calibration tests (C-1 to C-4) but withdrawn from the CSTR in a different operation date. An air flow of 15 mL min^{-1} and a biomass concentration of 260 mg VSS L^{-1} were used in order to validate the model under different conditions. A k_{S^0} of 0.053 mg $S^{1/3}$ $mg^{-1/3}$ VSS was obtained since, as mentioned in Section 4.6, this constant must be calibrated in each test. As expected, the k_La for oxygen was almost 3.5-fold less than that obtained for an air flow of 50 mL min^{-1} (0.069 min^{-1} or 4.14 h^{-1}).

As can be observed, the DO profile was not perfectly described despite sulfate, thiosulfate and OUR profiles were properly fitted with the calibrated model. Overall, results provided a satisfactory description of the system, indicating that the model can be included in a general model describing aerobic desulfurization in a BTF. However, the kinetic constant related to elemental sulfur oxidation requires to be calibrated in each particular case probably because the elemental sulfur that is being formed inside the cells has different speciation, which affects directly the oxidation rate. Further investigation to find a mathematical equation describing better this biodegradation process is warranted.

5. Conclusions

Respirometry was demonstrated to be a powerful tool for assessing the mechanistic and kinetic properties of biological sulfide oxidation. Here we used respirometry to characterize SOB obtained from a desulfurizing BTF. The proposed mechanisms describing aerobic sulfide oxidation explained the intermediate production and consumption of thiosulfate and elemental sulfur. The kinetic model proposed was properly calibrated with experimental data. Several kinetic parameters were estimated, together with their confidence intervals, from the calibration. These parameters were further used to validate the model with an additional respirometric test under different experimental conditions. Regarding the characterization of elemental sulfur oxidation, it was concluded that more research is required since the intracellular particles size and the variations in elemental sulfur speciation

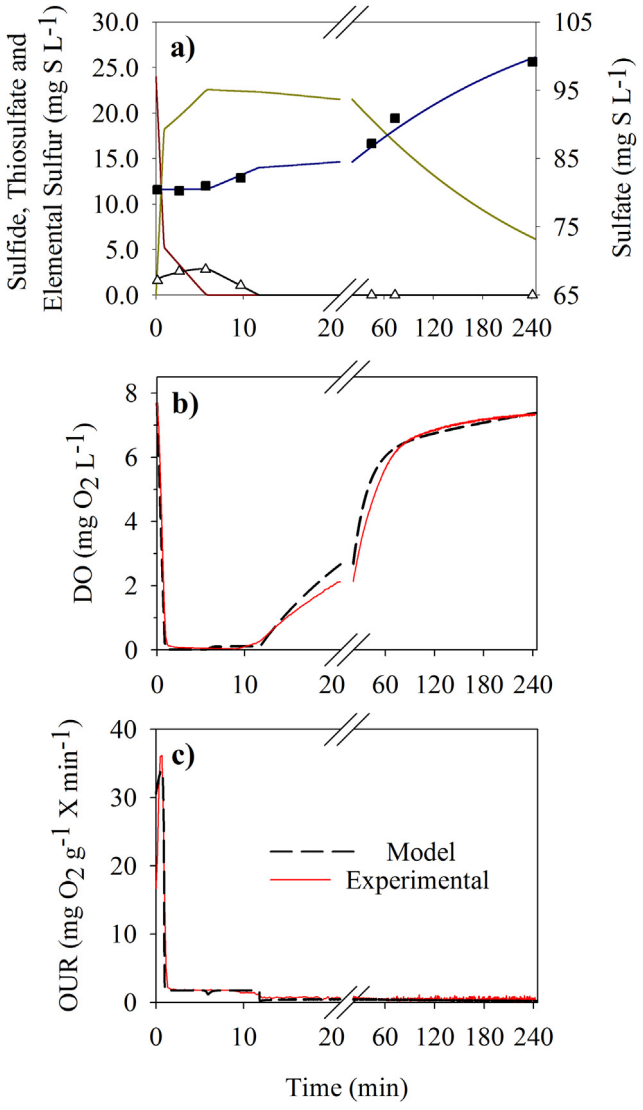

Fig. 8. Validation of the kinetic model with experimental data from respirometric test V-l. Modeled sulfide concentration (dark red solid line), modeled elemental sulfur concentration (dark yellow solid line), experimental and modeled thiosulfate concentration (△ and black solid line, respectively), experimental and modeled sulfate concentration (■ and blue solid line, respectively).

probably affected the kinetics corresponding to its oxidation.

This research provides knowledge relevant for the application of sulfide oxidation processes, which is essential to improve the operation of aerobic desulfurizing BTFs from which the biomass is extracted. In addition, the results obtained in this work also provide fundamental background to aerobic sulfide oxidation processes occurring in the treatment of wastewaters containing sulfide as, for example, these generated from anaerobic treatment of sulfate-containing wastewaters.

Acknowledgments

The Spanish government (MEC) provided financial support through the Project CICYT CTM2009-14338-C03/FEDER and CTM2012-37927-C03/FEDER. The Department of Chemical Engineering at UAB (Universitat Autònoma de Barcelona) is a unit of Biochemical Engineering of the Xarxa de Referència en Biotecnologia de Catalunya (XRB), Generalitat de Catalunya. Authors also acknowledge Dr. Dimitri Sorokin from the department of Biotechnology of TU Delft (Delft University of Technnology) for his valuable inputs during results discussion.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.11.061.

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