Appl Microbiol Biotechnol
DOI 10.1007/s00253-014-5688-5

ENVIRONMENTAL BIOTECHNOLOGY

Kinetic and stoichiometric characterization of anoxic sulfide oxidation by SO-NR mixed cultures from anoxic biotrickling filters

Mabel Mora · Maikel Fernández · José Manuel Gómez · Domingo Cantero · Javier Lafuente · Xavier Gamisans · David Gabriel

Received: 28 January 2014 / Revised: 10 March 2014 / Accepted: 11 March 2014

Abstract Monitoring the biological activity in biotrickling filters is difficult since it implies estimating biomass concentration and its growth yield, which can hardly be measured in immobilized biomass systems. In this study, the characterization of a sulfide-oxidizing nitrate-reducing biomass obtained from an anoxic biotrickling filter was performed through the application of respirometric and titrimetric techniques. Previously, the biomass was maintained in a continuous stirred tank reactor under steady-state conditions resulting in a growth yield of 0.328±0.045 g VSS/g S. To properly assess biological activity in respirometric tests, abiotic assays were conducted to characterize the stripping of CO_2 and sulfide. The global mass transfer coefficient for both processes was estimated. Subsequently, different respirometric tests were performed: (1) to solve the stoichiometry related to the autotrophic denitrification of sulfide using either nitrate or nitrite as electron acceptors, (2) to evaluate the inhibition caused by nitrite and sulfide on sulfide oxidation, and (3) to propose, calibrate, and validate a kinetic model considering both electron acceptors in the overall anoxic biodesulfurization process. The kinetic model considered a Haldane-type equation to describe sulfide and nitrite inhibitions, a non-competitive inhibition to reflect the effect of sulfide on the elemental sulfur oxidation besides single-step denitrification since no nitrite was produced during the biological assays.

Keywords Hydrogen sulfide · Biotrickling filter · SO-NR culture · Kinetics and stoichiometry · Respirometry · Titrimetry

Introduction

Purification of biogas produced in anaerobic digesters is necessary since biogas often contains hydrogen sulfide (H_2S) which potentially causes corrosive damage to combustion engines besides producing adverse environmental effects due to sulfur oxide emissions. Physical and chemical processes traditionally used to remove H_2S from biogas are effective but costly. For this reason, emerging technologies are increasingly focused on the biological treatment of biogas since they are attractive from an economical and technological point of view (Soreanu et al. 2009). In this sense, biogas desulfurization has already been efficiently performed through biofiltration in biotrickling filters (BTF) (Fernandez et al. 2013; Fortuny et al. 2008). In a BTF, biomass immobilized over the surface of an inert packing material degrades the H_2S previously absorbed in a continuously circulating liquid phase (Cox and Deshusses 1998; Syed et al. 2006). The dissolved sulfide is biologically oxidized to elemental sulfur or sulfate using either oxygen (aerobic conditions) or nitrate (anoxic conditions) as final electron acceptors.

Development of mathematical models is necessary to design and optimize the biological H_2S removal process.

Electronic supplementary material The online version of this article (doi:10.1007/s00253-014-5688-5) contains supplementary material, which is available to authorized users.

M. Mora · J. Lafuente · D. Gabriel (✉)
Department of Chemical Engineering, School of Engineering, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
e-mail: david.gabriel@uab.es

M. Fernández · J. M. Gómez · D. Cantero
Department of Chemical Engineering and Food Technologies, Faculty of Sciences, University of Cádiz, 11510 Puerto Real, Cádiz, Spain

X. Gamisans
Department of Mining Engineering and Natural Resources, Universitat Politècnica de Catalunya, Bases de Manresa 61-73, 08240 Manresa, Spain

Published online: 06 April 2014

Particularly, accurate microbial kinetic data is needed in these general models for a proper prediction of the biological activity and, consequently, to properly assess the local and overall performance of such heterogeneous, plug flow-type reactors (Martin et al. 2002). However, characterization of autotrophic sulfide-oxidizing nitrate-reducing (SO-NR) biomass in a BTF implies estimating the biomass concentration and the growth yield coefficient ($Y_{x/s}$), parameters that can hardly be measured in situ when using immobilized biomass. In addition, several physical and chemical processes related with mass transfer phenomena and ionic equilibriums take place simultaneously in the biotrickling filter (López et al. 2013), which increases the uncertainty of model parameter estimation.

Respirometry and titrimetry are two techniques that have been successfully applied to characterize autotrophic and heterotrophic suspended biomass cultures under both anoxic and aerobic conditions, especially in activated sludge from wastewater treatment processes (Spanjers and Vanrolleghem 1995). On the one hand, respirometry is the measurement and interpretation of the biological oxygen consumption rate under well-defined experimental conditions according to Young and Cowan (2004). The exogenous oxygen uptake rate (OUR) curves obtained after the injection of substrate pulses are related with the aerobic biodegradation process, which allow the estimation of kinetic and stoichiometric parameters. Moreover, respirometry can be analogously applied under anoxic conditions by measuring the nitrate uptake rate (NUR) (Kristensen et al. 1992). On the other hand, titrimetry is a volumetric analysis that has been commonly used to measure the addition of diluted acidic or alkaline solutions to maintain, under perfectly controlled conditions, a constant pH in systems where pH-affecting reactions are taking place (Marcelino et al. 2009; Spanjers et al. 1996). This technique can be also applied to indirectly measure oxygen uptake rate when adding hydrogen peroxide or sulfide solutions to the system. Although these techniques have been successfully applied to characterize several biological processes and microbial cultures (Decostere et al. 2013; Guisasola et al. 2007; Munz et al. 2009; Sin and Vanrolleghem 2007), respirometry and tritrimetry have not been extensively used to study SO-NR biomass obtained from BTFs (Gonzalez-Sanchez et al. 2009). In this study, the characterization of a SO-NR mixed culture, obtained from a BTF operated under anoxic conditions, was performed by using both techniques and the methodology proposed by Mora et al. (2014). Sulfide oxidation mechanisms associated to denitrification are proposed, and, in addition, $Y_{x/s}$ and kinetic parameters are defined and estimated for the calibration and validation of a microbial kinetic model. Inhibition of nitrite was also studied as an intermediate of the denitrification process that could have detrimental effects on the overall reaction when denitritation is the limiting step (McMurray et al. 2004).

Materials and methods

Sulfide-oxidizing biomass

The SO-NR mixed culture used in this study was collected from a pilot-scale anoxic BTF (0.17 m^3 working volume) treating biogas under anoxic conditions containing an average inlet H_2S concentration of about 4,500 ppm_v (Almenglo et al. 2013). The BTF was packed with open-pore polyurethane foam (OPUF) cubes and operated during several months at pH 7.4. The SO-NR biomass was withdrawn from the packing material and suspended in 500 mL of nutrient solution to subsequently inoculate a 5-L sterilized fermenter (Applikon Biotechnology). A modified ATCC-1255 *Thiomicrospira denitrificans* nutrient solution was used for the biomass growth without thiosulfate and with a higher concentration of bicarbonate and nitrate: $NaHCO_3$ (2 g L^{-1}) and KNO_3 (3 g L^{-1}). Previously to the inoculation, the fermenter was filled with nutrient solution (4.2 L) and gassed with argon to remove oxygen. Afterwards, the suspended biomass was transferred to the fermenter, and the operation was started up in fed-batch mode by continuously supplying 5.37 mL h^{-1} of a dissolved sulfide solution (8 g S-$Na_2S{\cdot}9H_2O$ L^{-1}), which corresponded to a loading rate of 8.6 g S m^{-3} reactor h^{-1}, with a temporized peristaltic pump (Masterflex, Cole Parmer). During this stage, substrate inhibition did not occur, and the biomass was acclimated to operate as a suspended culture. Once nitrate was almost depleted, an operation with continuous stirred tank reactor (CSTR) without biomass recirculation was set during 3 weeks to maintain the SO-NR biomass under steady-state conditions. During this phase, the reactor was fed with 46.2 mL h^{-1} of nutrient solution, thus obtaining a dilution rate of 0.01 h^{-1}, which was low enough to avoid the washout of the biomass. Aliquots were withdrawn for respirometric tests as described in the next section. The nutrient solution was pumped also with a temporized peristaltic pump (Masterflex, Cole Parmer). During CSTR operation, temperature was controlled at 30 °C with a thermostatic water bath (MA Heating Immersion Circulator, Julabo GmbH). The pH was also measured and controlled at 7.5 (pH analyzer AX400, ABB Group) through the automatic addition of HCl (1 M) or NaOH (1 M). The headspace of the CSTR was continuously gassed with argon (100 mL min^{-1}) to operate under anoxic conditions. The liquid phase was daily sampled to analyze nitrite, nitrate, sulfate, sulfide, and volatile suspended solids (VSS). Moreover, biomass samples were weekly taken from the reactor to verify the microbial diversity preservation by denaturing gradient gel electrophoresis (DGGE). The biomass growth yield ($Y_{x/s}$) was also calculated at steady-state conditions according to Eq. 1:

$$Y_{x/s} = \frac{[\mathrm{VSS}]_{out} - [\mathrm{VSS}]_{in}}{\left[\mathrm{S{-}SO_4^{2-}}\right]_{out} - \left[\mathrm{S{-}SO_4^{2-}}\right]_{in}} \quad (1)$$

where VSS_{out} and VSS_{in} are the biomass concentrations (mg VSS L^{-1}) at the outlet and inlet flows of the reactor, respectively, and $S\text{-}SO_4^{2-}{}_{in}$ and $S\text{-}SO_4^{2-}{}_{out}$ are the inlet and outlet sulfate concentrations (mg $S\text{-}SO_4^{2-}L^{-1}$), respectively.

Respirometric tests

Respirometric tests were conducted in a batch-type magnetically stirred respirometer with a capacity of 600 mL (Fig. S1, Supplementary Material). A pulse of bicarbonate (50 mg C L^{-1}) was added before each respirometric test to avoid slower specific sulfide uptake rates due to carbon source limitation (Tora et al. 2010). An argon flow of 50 mL min^{-1} was sparged to the headspace of the respirometer and controlled with a mass flow meter (EL-FLOW, Bronckhorst High-Tech) in order to minimize the carbon source and sulfide stripping while maintaining anoxic conditions.

A set of biotic tests (Table 1) were performed at pH 7.5 and 30 °C with SO-NR biomass obtained from the 5-L reactor. The methodology proposed by Mora et al. (2014) regarding the preparation of the biomass (centrifugation and resuspension in free substrate diluted in mineral medium), as well as the performance of the endogenous phase, the wake-up pulses and the sampling frequency of the respirometer, was followed. Tests I-1, I-2, S-1, and S-2 were used to calibrate the kinetics of the process, while test S-3 was used in order to validate the kinetic model (Table 1). Nitrite and sulfide inhibitions were firstly studied in tests I-1 and I-2 by adding sequential pulses of the species to the respirometer and estimating the specific uptake rate in each pulse. Afterwards, tests S-1 and S-2 were conducted to study separately the two-step sulfide oxidation with each electron acceptor. Finally, model validation in experiment S-3 was performed with the single addition of nitrate as the electron acceptor. Further details of the respirometer setup and the methodology used for the tests are provided in the Supplementary Material. It is worth mentioning that replicates of respirometric tests were not performed since a respirometer can be considered to be a sensor (Spanjers et al. 1996). This device is constructed in such a compact way that can be easily considered as one unit. In the present work, errors associated to the analysis of the samples were 5, 10, and 2 % for ionic chromatography analysis, volatile suspended solids analysis, and the dilution of each sample, respectively.

Characterization of sulfide and CO_2 stripping

To study the biological mechanisms and kinetics of a SO-NR microbial population in a respirometer is essential to firstly characterize the physical and chemical processes that take place simultaneously to the biological processes. In this particular case, anoxic respirometric assays must be performed using a continuous inert gas flow, thus implying the stripping of both the volatile substrate and the carbon source. The characterization of CO_2 and H_2S stripping processes was made through several abiotic tests conducted previously to the biological studies. Experimental conditions and the mineral medium used in abiotic tests were the same than those used in biotic tests. On the one hand, H_2S stripping was characterized with three different initial sulfide concentrations (3.2, 12, and 40 mg S^{2-} L^{-1}). Equations 2 and 3, corresponding to the mass balance and chemical equilibriums corresponding to H_2S (Gonzalez-Sanchez et al. 2009), were used to estimate the global mass transfer coefficient K_La by curve fitting of experimental profiles of dissolved sulfide to Eq. 2 by means of MATLAB 7.7 (Mathworks, Natik, MA).

$$\frac{dH_2S}{dt} = \frac{V_L}{V_G} \cdot K_L a_{H_2S} \cdot \left[\left(\frac{S_{H_2S}}{1 + 10^{[pH-pk1]} + 10^{[2 \cdot pH-(pk_1+pk_2)]}} \right) - \frac{H_2S_{(g)}}{He} \right] - \left(\frac{F_{ar}}{V_G} \cdot H_2S_{(g)} \right) \quad (2)$$

$$\frac{dS_{H_2S}}{dt} = -\left[1 + 10^{[pH-pk1]} + 10^{[2 \cdot pH-(pk_1+pk_2)]} \right] \cdot K_L a_{H_2S} \cdot \left[\left(\frac{S_{H_2S}}{1 + 10^{[pH-pk1]} + 10^{[2 \cdot pH-(pk_1+pk_2)]}} \right) - \frac{H_2S_{(g)}}{He} \right] \quad (3)$$

where V_L and V_G are the liquid and gas volumes in the respirometer (L), S_{H_2S} is the dissolved sulfide concentration (mM), He is the dimensionless Henry's law constant, pka_1 and pka_2 are the logarithmic values of the acid dissociation constants, and F_{ar} is the argon flow (L h^{-1}). On the other hand, a titrimetric test was conducted following the methodology proposed by López et al. (2013) to characterize CO_2 stripping. By this way, an initial pulse of 50 mg C L^{-1} was added to investigate whether such carbon concentration would limit the reaction rate during respirometric tests. Mass balances and equations for CO_2 stripping characterization are provided in the Supplementary Material.

Kinetic model

Two different kinetic equations were proposed to describe the experimental respirometric profiles obtained using nitrate and nitrite as the electron acceptors. The kinetic model proposed

Table 1 Biotic tests conducted to calibrate the kinetics of sulfide oxidation in anoxic conditions

Test	Process	Nitrite (mg N L^{-1})	Nitrate (mg N L^{-1})	Sulfide (mg S L^{-1})
I-1	Nitrite inhibition	1.5–95	0	3.5–15
I-2	Sulfide inhibition	3.5–15	0	1.5–110
S-1	Sulfide oxidation	0	35	30
S-2	Sulfide oxidation	20	0	13
S-3	Sulfide oxidation	0	40	10

for H_2S and elemental sulfur oxidation associated to nitrate reduction is presented through Eqs. 4 and 5. For sulfide oxidation, a Haldane-type term was proposed to describe substrate inhibition by sulfide, while nitrate reduction followed a Monod-type kinetic equation. For elemental sulfur oxidation, a Monod-type term was considered for both elemental sulfur and nitrate. However, a noncompetitive inhibition term was included to investigate the effect of sulfide on elemental sulfur oxidation.

$$r_{N,1} = \frac{1}{Y_{\left(x/S^{2-}\right)_N}} \cdot \frac{\mu_{\max_1,N} \cdot S^{2-}}{K_{S^{2-}} + S^{2-} + \frac{\left(S^{2-}\right)^2}{K_{\text{is}}}} \cdot \frac{N}{K_N + N} \cdot X \quad (4)$$

$$r_{N,2} = \frac{1}{Y_{\left(X/S^0\right)_N}} \cdot \frac{\mu_{\max_2,N} \cdot S^0}{K_{S^0} + S^0} \cdot \frac{N}{K_N + N} \cdot \frac{K}{K + S^{2-}} \cdot X \quad (5)$$

where $\mu_{\max 1,N}$ and $\mu_{\max 2,N}$ are the maximum specific uptake rates for sulfide and sulfur oxidation (h^{-1}), respectively; $K_{S^{2-}}$, K_{S^0}, and K_N are the affinity constants for the substrates (sulfide and sulfur) and nitrate (mg L^{-1}); K_{is} is the sulfide inhibition constant (mg S L^{-1}); K is the inhibition constant for elemental sulfur oxidation (mg S L^{-1}); X is the biomass concentration (mg VSS L^{-1}); $Y_{(x/s)N}$ is the biomass growth yield using nitrate as the electron acceptor (g VSS g substrate^{-1}); and S^{2-}, S^0, and N are sulfide, elemental sulfur, and nitrate concentrations, respectively (mg S L^{-1} or mg N-NO_3^- L^{-1}).

Since some authors have found that equivalent reactions occur with nitrite as an intermediate of the denitrification process with sulfide and elemental sulfur (An et al. 2010; Dogan et al. 2012), Eqs. 6 and 7 have been defined to describe denitritation with sulfide and elemental sulfur, respectively. Also, a Haldane-type inhibition term for nitrite has been considered for substrate inhibition.

$$r_{\text{Nit},1} = \frac{1}{Y_{\left(X/S^{2-}\right)_{\text{Nit}}}} \cdot \frac{\mu_{\max_1,\text{Nit}} \cdot S^{2-}}{K_{S^{2-}} + S^{2-} + \frac{\left(S^{2-}\right)^2}{K_{\text{is}}}} \cdot \frac{\text{Nit}}{K_{\text{Nit}} + \text{Nit} + \frac{(\text{Nit})^2}{K_{i\text{Nit}}}} \cdot X \quad (6)$$

$$r_{\text{Nit},2} = \frac{1}{Y_{\left(X/S^0\right)_{\text{Nit}}}} \cdot \frac{\mu_{\max,\text{Nit}}}{2} \cdot \frac{S^0}{K_{S^0} + S^0} \cdot \frac{\text{Nit}}{K_{\text{Nit}} + \text{Nit} + \frac{(\text{Nit})^2}{K_{i\text{Nit}}}} \cdot \frac{K}{K + S^{2-}} \cdot X \quad (7)$$

where $\mu_{\max 1,\text{Nit}}$ and $\mu_{\max 2,\text{Nit}}$ are the maximum specific uptake rates for sulfide and elemental sulfur oxidation (h^{-1}), respectively; K_{Nit} and $K_{i\text{Nit}}$ are the affinity and the inhibition constant for nitrite (mg N L^{-1}), respectively; $Y_{(x/s)\text{Nit}}$ is the biomass growth yield using nitrite as the electron acceptor (g VSS g substrate^{-1}); and Nit is the nitrite concentration (mg N L^{-1}).

Maximum specific growth rates, half-saturation constants, and inhibition constants were determined by curve fitting to respirometric profiles by means of MATLAB 7.7 (Mathworks, Natik, MA).

Analytical methods

Nitrite (NO_2^-), sulfate (SO_4^{2-}), thiosulfate ($S_2O_3^{2-}$), and nitrate (NO_3^-) concentrations were analyzed by ion chromatography with conductivity detection using Dionex ICS-2000 equipment. The biomass concentration was determined according to Standard Methods (APHA-AWWA-WPCF 2005) to obtain VSS concentration. The inorganic carbon concentration was measured with an OI Analytical TIC/TOC Analyzer (Model 1020A) equipped with a nondispersive infrared

detector and a furnace maintained at 680 °C. The sulfide concentration was analyzed off-line with a sulfide selective electrode (VWR International Eurolab, S.L). Since no other S species were produced, the elemental sulfur concentration was calculated from the sulfur mass balance, i.e., from the difference between the sulfide consumed and the sulfate produced. The DGGE analysis was performed by following the methodology reported by Fernandez et al. (2013).

Results

SO-NR suspended culture

Biomass withdrawn from the anoxic biotrickling filter was grown in a fermenter as a suspended culture (Fig. 1a). During the fed-batch operation (65 h), biomass gradually consumed nitrate to oxidize sulfide to sulfate while being acclimated to suspended culture conditions. After the fed-batch operation, the continuous operation was started and biomass was progressively washed out from the reactor until reaching the steady state after 350 h of operation. During the nonsteady operation (from 65 to 350 h), nitrite was not accumulated, and a maximum 6 % of elemental sulfur was detected. Nitrate was below 15 mg N L^{-1} during almost the whole continuous operation. The dilution rate set (0.01 h^{-1}) avoided biomass wash out. Diversity was then preserved, which was verified with the DGGE analysis. Results showed that most of the species detected in the inoculum were preserved during the reactor operation as CSTR (Fig. 1b). In addition, a pure culture of *Thiobacillus denitrificans* DSM 12475 was also analyzed since this has been commonly reported as a typical bacteria in anoxic biotrickling filters (Soreanu et al. 2008). However, no coincidence was found between SO-NR mixed culture species lanes and the abovementioned pure culture lane. An $Y_{x/s}$ of 0.328±0.045 g VSS/g S was calculated after 350 h of operation when steady-state conditions were reached (Fig. 1a). Considering the $Y_{x/s}$ calculated, the stoichiometry of the overall oxidation reaction was obtained (Eq. 8) by solving the mass and charge balances according to Roels (1983), assuming $C_5H_7O_2N$ as typical biomass composition (Heijnen 2002) and NH_4^+ as the nitrogen source.

$$HS^- + 1.23\ NO_3^- + 0.573\ H^+ + 0.438\ HCO_3^- + 0.027\ CO_2 + 0.093\ NH_4^+ + \rightarrow 0.093\ C_5H_7O_2N + 0.866\ H_2O + 0.614\ N_2 + SO_4^{2-} \quad (8)$$

Characterization of CO_2 and H_2S stripping

The titrimetric test performed per triplicate to characterize the CO_2 stripping process as well as the modeled profile and the predicted TIC evolution is shown in Fig. 2a. An overall mass transfer coefficient (K_La) of 0.840±0.186 h^{-1} was found to accurately fit the experimental data with the CO_2 stripping model. Model prediction for the sulfide stripping is shown in Fig. 2b corresponding to an initial hydrogen sulfide concentration of 40 mg S^{2-} L^{-1}. An average K_La of 0.739± 0.171 h^{-1} was calculated, taking into account the estimations of the three different concentrations tested.

Sulfide and nitrite inhibition tests

As already known, nitrite is an intermediate in the denitrification process ($NO_3^- \rightarrow NO_2^- \rightarrow N_2$) being accumulated when denitritation is the rate limiting step. In this work, although nitrite

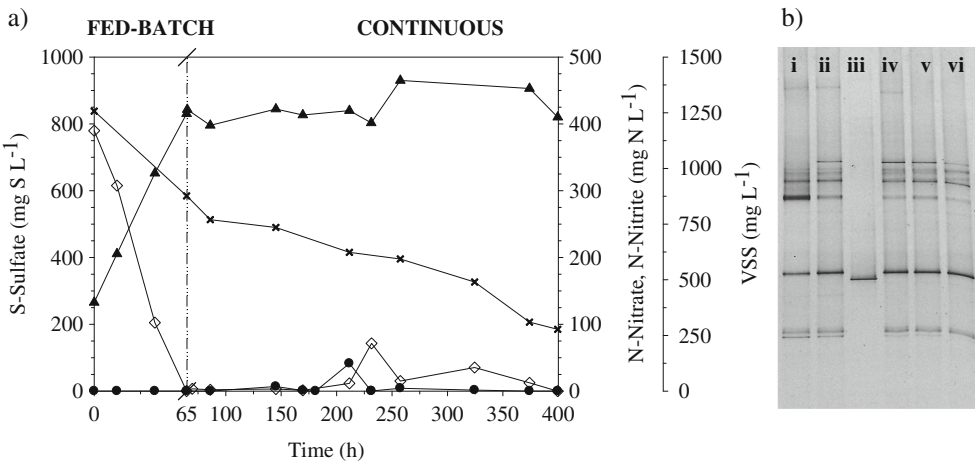

Fig. 1 **a** Operation of the CSTR until steady-state condition and **b** DGGE fingerprinting (*i*) 21 days of operation, (*ii*) 15 days of operation, (*iii*) *Thiobacillus denitrificans* pure culture, (*iv*) three times of the hydraulic retention time (HRT), (*v*) two times of the HRT, and (*vi*) inoculation. Sulfate (*black triangle*), nitrate (*diamond*), biomass (*multiplication symbol*), and nitrite (*black circle*)

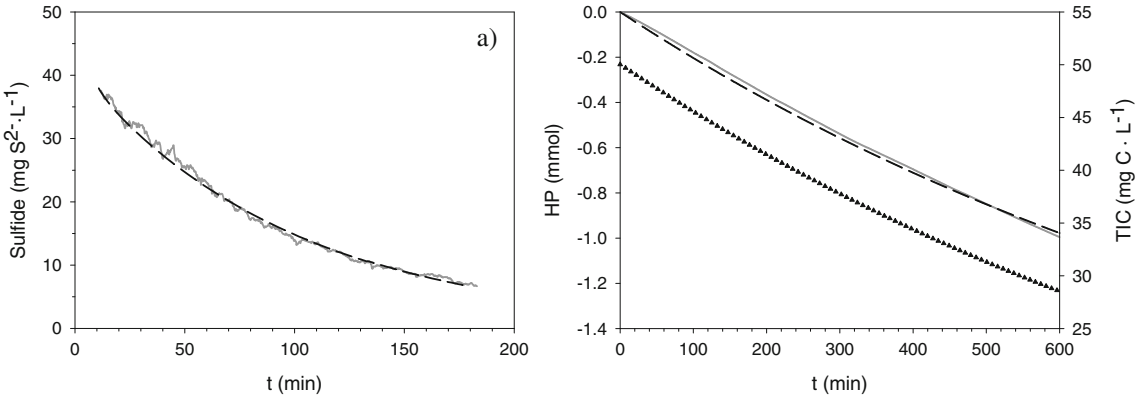

Fig. 2 Abiotic stripping tests profiles. **a** CO_2 stripping characterization. **b** Sulfide stripping test at 1.5 mM S^{2-}. Experimental data (*solid line*), model data (*dashed line*), and TIC predicted profile (*symbol*)

accumulation was not observed during the respirometric tests, the nitrite effect was studied since many authors have reported inhibition caused by nitrite over denitrification (Fajardo et al. 2014; Soto et al. 2007) and, consequently, on desulfurization. Sulfide inhibition on desulfurization has already been reported (Cardoso et al. 2006; Gonzalez-Sanchez et al. 2009; Reyes-Avila et al. 2004), but there is a lack of knowledge on kinetic inhibition parameters; hence, further investigation is necessary. In this study, the inhibition kinetics caused by the presence of these compounds was modeled through a Haldane-type expression using the experimental data obtained from tests I-1 and I-2.

Specific sulfide and nitrite uptake rates obtained in tests I-1 and I-2 were successfully predicted by the model using the kinetic parameters shown in the subpanels a and b of Fig. 3, respectively. As can be observed, the model accurately described the inhibition caused by both compounds on the corresponding biodegradation rates.

Sulfide oxidation using nitrate as the electron acceptor

Experimental respirometric data corresponding to the sulfide oxidation test performed with nitrate (S-1) is presented in Fig. 4a. As can be observed, sulfide was initially oxidized to elemental sulfur since sulfate concentration remained almost constant during the first 20 min. Moreover, as mentioned above, nitrite was not detected during the experiment, hence the partial denitrification of nitrate was not considered in this work. From these results, the stoichiometry of the two-step sulfide oxidation associated to denitritation was solved (Eqs. 9 and 10) using the overall biological reaction previously solved (Eq. 8), and the nitrate to sulfate ratio obtained from the last 20 min of the respirometric test (N/S=0.35) in which the unique reaction taking place was the oxidation of elemental sulfur.

$$HS^- + 0.350\ NO_3^- + 1.40\ H^+ + 0.059\ HCO_3^- + 0.004\ CO_2 + 0.013\ NH_4^+ \rightarrow 0.013\ C_5H_7O_2N + 1.21\ H_2O + 0.175\ N_2 + S^0 \quad (9)$$

$$S^0 + 0.876\ NO_3^- + 0.343\ H_2O + 0.379\ HCO_3^- + 0.023\ CO_2 + 0.080\ NH_4^+ \rightarrow 0.080\ C_5H_7O_2N + 0.824\ H^+ + 0.44\ N_2 + SO_4^{2-} \quad (10)$$

In Fig. 4a, the predicted profiles and experimental data for all species, including the expected profile for elemental sulfur, are shown. Haldane kinetic parameters obtained previously (Fig. 3) and the affinity constant related to nitrate (K_N= 1.30 mg N L^{-1}) reported by Artiga et al. (2005) were used to estimate the remaining parameters ($\mu_{max1,N}$, $\mu_{max2,N}$, K, and K_{S0}) for experiment S-1. As can be observed in Fig. 4a, a satisfactory prediction of the experimental data was obtained with the model calibration. The estimated parameters (Table 2) were used to validate the kinetic model with the experimental data obtained from test S-3, which is presented in Fig. 4b. Again, the model calibrated in experiment S-1 effectively predicted the experimental profiles of experiment S-3 performed at a different S/N ratio.

Sulfide oxidation using nitrite as the electron acceptor

In the case of nitrite, thermodynamically calculated biomass growth yields reported by Dogan et al. (2012) were used in order to solve the total reaction stoichiometry (Eqs. 11–13) since the CSTR was not operated with this electron acceptor.

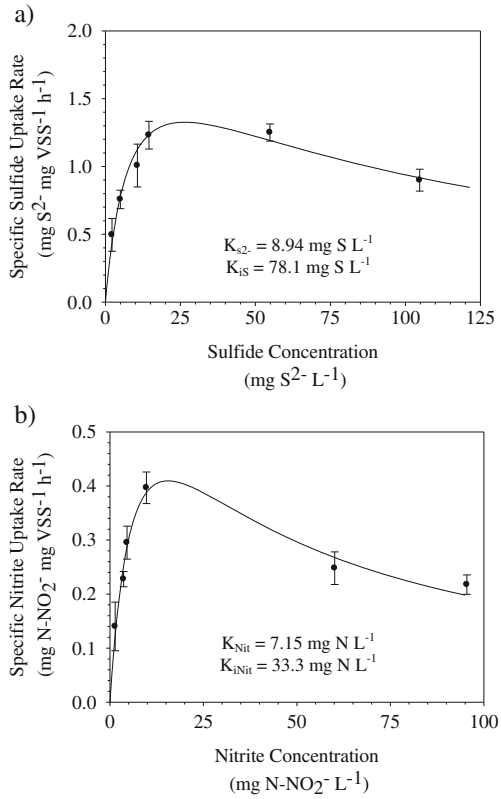

Fig. 3 Haldane-type kinetics modeling: **a** sulfide inhibition and **b** nitrite inhibition. Experimental data (*symbol*) and model data (*solid line*)

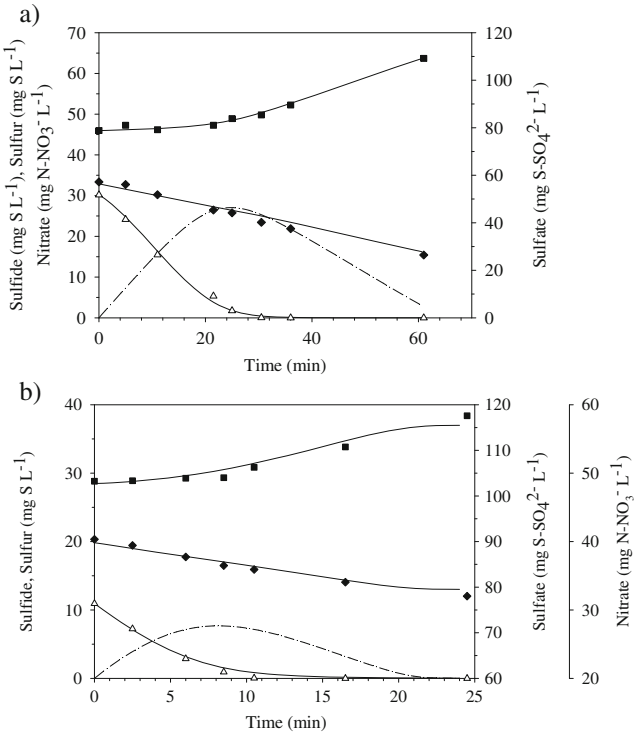

Fig. 4 Kinetic modeling of sulfide oxidation using nitrate as electron acceptor (tests S-1 and S-3). **a** Model calibration. **b** Model validation. Sulfate (*black square*), nitrate (*black diamond*), sulfide (*white triangle*), elemental sulfur (*dash-dot line*), and model data (*solid line*)

Two-step sulfide oxidation

$$HS^- + 0.587\ NO_2^- + 1.63\ H^+ + 0.057\ HCO_3^- + 0.004\ CO_2 + 0.012\ NH_4^+ \rightarrow 0.012\ C_5H_7O_2N + 1.326\ H_2O + 0.293\ N_2 + S^0 \quad (11)$$

$$S^0 + 1.78\ NO_2^- + 0.021\ H_2O + 0.158\ HCO_3^- + 0.010\ CO_2 + 0.034\ NH_4^+ \rightarrow 0.034\ C_5H_7O_2N + 0.099\ H^+ + 0.888\ N_2 + SO_4^{2-} \quad (12)$$

Overall sulfide oxidation

$$HS^- + 2.36\ NO_2^- + 1.53\ H^+ + 0.215\ HCO_3^- + 0.013\ CO_2 + 0.046\ NH_4^+ \rightarrow 0.046\ C_5H_7O_2N + 1.30\ H_2O + 1.18\ N_2 + SO_4^{2-} \quad (13)$$

As shown in Fig. 5, the kinetic model together with the calculated stoichiometric coefficients properly described the respirometric profile corresponding to test S-2, which was performed uniquely with nitrite as the electron acceptor. From the fitting of the experimental data, the maximum specific growth rates corresponding to each of the process considered ($\mu_{max1,Nit}$, $\mu_{max2,Nit}$) (Eqs. 11 and 12) were estimated (Table 2).

Discussion

Assessment of the SO-NR in continuous culture

Cultivation of the SO-NR biomass withdrawn from the packing material of the anoxic desulfurizing BTF was successfully performed in a CSTR. During the operation, the maximum elemental sulfur formed was around 6 %, which is probably due to

Table 2 Biokinetic parameters determined from the fitting of the experimental respirometric profiles with the kinetic models proposed (T=30 °C, pH=7.5)

Coefficients	Values	Units
$\mu_{max_{1,N}}$	0.125	h^{-1}
$\mu_{max_{2,N}}$	0.218	h^{-1}
$\mu_{max_{1,Nit}}$	0.187	h^{-1}
$\mu_{max_{2,Nit}}$	0.093	h^{-1}
K_{Nit}	7.15	mg N L^{-1}
$K_{i,Nit}$	33.3	mg N L^{-1}
$K_{S^{2-}}$	8.94	mg S L^{-1}
$K_{i,S^{2-}}$	78.1	mg S L^{-1}
K_{S^0}	0.609	mg S L^{-1}
K	3.04	mg S L^{-1}

nitrate limitation. Nitrite was neither observed during the fed-batch nor during the continuous operations, indicating that the sulfide and nitrate were consumed in stoichiometric ratio and that neither the denitritation nor the elemental sulfur oxidation was limited at a dilution rate of 0.01 h^{-1}. The stoichiometry solved using the experimental $Y_{x/s}$ (0.813 mol HS^-/mol NO_3^-) was different compared to yields in the range of 0.56 and 0.75 g VSS/g S obtained by other authors for the complete oxidation to sulfate (Cardoso et al. 2006; Vaiopoulou et al. 2005; Yavuz et al. 2007), but similar to that proposed by Campos et al. (2008) (0.842 mol S/mol NO_3^-). This result indicates that stoichiometric coefficients, which are essential to characterize a microbial culture, should be solved for each specific biomass in order to accurately estimate the corresponding kinetic coefficients. The DGGE analysis showed that the microbial diversity was preserved during the CSTR operation which indicates that the immobilized SO-NR mixed culture from the biotrickling filter acclimated well to suspended culture conditions.

Carbon source and sulfide stripping

The K_La value obtained from the characterization of CO_2 stripping was very low if compared with those of liquid bubbled systems, which helped to minimize the carbon source stripping (López et al. 2013). This result is also reflected in the TIC profile (Fig. 2b), where a 40 % of the initial concentration is lost after 600 min of experiment, confirming that no carbon source limitation nor slowed respirometric rates would appear during biotic tests. Regarding sulfide stripping, the K_La obtained indicated that the stripping of sulfide was also minimized since Gonzalez-Sanchez et al. (2009) a value of 1.2 h^{-1} was obtained for the same respirometric system when the liquid was bubbled into the liquid phase with an air flow of 18 mL min^{-1}. As expected, K_La values obtained for CO_2 and H_2S using an Argon flow of 50 mL min^{-1} were similar since this parameter depends on the hydrodynamics of the system being practically not influenced by the characteristics of the species in diluted solutions.

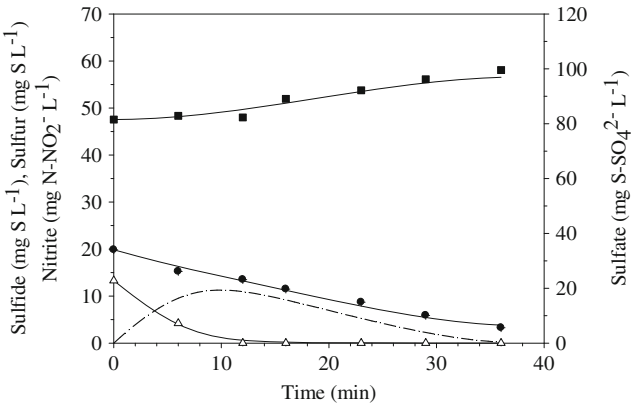

Fig. 5 Kinetic modeling of sulfide oxidation using nitrite as electron acceptor (test S-2). Sulfate (*black square*), nitrite (*black circle*), sulfide (*white triangle*), elemental sulfur (*dash-dot line*), and model data (*solid line*)

Kinetics analysis of sulfide and nitrite inhibitions

From Fig. 3, it can be observed that denitritation as well as sulfide oxidation is well described with the Haldane model, confirming the existence of both inhibitions. On the one hand, kinetic coefficients obtained for nitrite inhibition were in agreement with those reported by Fajardo et al. (2014) (K_{Nit}= 10.7 mg N L^{-1} and K_{iNit}=34.7 mg N L^{-1}) for autotrophic denitrifying biomass, who also observed less than a 40 % of nitrite reduction at a concentration over 80 mg N-NO_2^- L^{-1}. Other authors have also reported satisfactory description of nitrite inhibition considering different kinetic models (Soto et al. 2007; Wild et al. 1995), indicating that nitrite inhibition mechanisms require to be further investigated. On the other hand, kinetic coefficients obtained from the sulfide inhibition test (I-2) were especially higher (K_s=8.94 mg S L^{-1} and K_{is}= 78.1 mg S L^{-1}) than those obtained by Gonzalez-Sanchez et al. (2009) for aerobic desulfurizing cultures (K_s=0.032 mg S L^{-1} and K_{is}=32.48 mg S L^{-1}). This indicates that the biomass developed in the anoxic BTF presented less activity at significantly higher sulfide concentrations (under 22.5 mg S L^{-1}). Conversely, the anoxic biomass was less inhibited by sulfide. This result was obtained probably because the anoxic BTF was operating at both high empty bed residence time and sulfide concentration. Results also confirm that inhibitory limits and, consequently the inhibition constants estimated, largely depend on the exposure and acclimation of microbial cultures to their environment and must be determined case by case.

Stoichiometry and kinetics of sulfide oxidation under anoxic conditions

With the respirometric profile of test S-1, it was confirmed that nitrite was not accumulated as an intermediate of denitrification, thus indicating that the denitritation rate was not the limiting step. Furthermore, nitrite reduction was neither influenced by an excess of nitrate nor by sulfide under

30 mg S L^{-1}. Some authors have associated nitrite accumulation with a sulfide limitation (Manconi et al. 2007), although according to McMurray et al. (2004), products arising from stepwise denitrification depend mainly on the bacterial community, their environmental conditions, and the availability of the carbon source. For this reason, the stoichiometry of a biological process must be firstly adapted and solved for each microbial culture to accurately describe the biodegradation mechanisms. In this study, the calculated stoichiometric coefficients corresponding to the two-step sulfide oxidation using nitrate as the electron acceptor were different from those thermodynamically calculated by other authors (Kleerebezem and Mendez 2002; Yavuz et al. 2007). This result indicates that the use of experimental data is necessary in order to obtain specific coefficients; otherwise, some kinetic parameters such as the specific growth rates could be under- or overestimated. Moreover, the stoichiometry in this study does not consider the partial denitrification since the SO-NR culture had a high denitritation activity. Regarding sulfide oxidation, elemental sulfur was not oxidized until sulfide was almost depleted (K=3.04 mg S L^{-1}), which indicates the existence of a noncompetitive inhibition of sulfide over the elemental sulfur oxidation rate. This observation is in agreement with that observed by An et al. (2010). Nonetheless, Can-Dogan et al. (2010) observed the formation of elemental sulfur under nitrate-limiting conditions and suggested that the end product of sulfide oxidation depended on the ratio of the nitrogen source to sulfide. Manconi et al. (2007) neither observed inhibition by sulfide over elemental sulfur oxidation with SO-NR mixed cultures but detected a milky appearance of the reactor during sulfide excess operating conditions, which does not agree with the results obtained in this study. This leads again to conclude that anoxic sulfide oxidation mechanisms depended mainly on the microbial community composition and its acclimation to different substrates. The stoichiometry solved to express the two-step sulfide oxidation with nitrate combined with the kinetic model proposed satisfactorily described the respirometric profile obtained from test S-1. In the literature, few comparable studies providing the kinetic characterization of autotrophic denitrification using sulfide as the electron donor have been found. An et al. (2010) reported μ_{max} values ranging from 0.08 to 0.17 h^{-1}, by using a mixed culture from the water treatment of oil industry, which were similar to those obtained in this study (Table 2). Gadekar et al. (2006) also reported a μ_{max} of 0.36 h^{-1} with a pure culture of *Thiomicrospira* sp. In any case, Fig. 4b shows that the model was satisfactorily validated with test S-3, which was performed at different sulfide concentration from that used in test S-1, indicating that the kinetic data estimated from the calibration was able to adequately describe the sulfide oxidation under anoxic conditions.

Modeling of the respirometric test S-2, corresponding to sulfide oxidation with nitrite, allowed for obtaining the kinetic parameters which were noticeably different from those found from test S-1 for each electron acceptor used. Parameters estimated by curve fitting to experimental data in test S-2 served to satisfactorily predict the experimental profiles. However, since thermodynamically calculated stoichiometric coefficients were used, the maximum specific growth rates $\mu_{max1,Nit}$ and $\mu_{max2,Nit}$ were less accurately estimated. However, less biomass was produced per mole of sulfide even if the desulfurizing activity with nitrite was comparable to that obtained using nitrate under noninhibiting concentrations. These findings are in agreement with Dogan et al. (2012) who proposed the use of nitrite as an advantageous option in order to reduce the biomass production as well as to improve the sulfide oxidation rates. A direct consequence of such finding is that the use of noninhibiting concentrations of nitrite in an anoxic desulfurizing BTF could minimize or even avoid the clogging of the trickling bed due to an excessive biomass growth as well as enhance the elimination capacity of the system.

Overall, the results obtained in this study demonstrated that respirometry and titrimetry can effectively be applied to investigate the mechanisms and kinetics of anoxic desulfurization. The complete kinetic model proposed in this work satisfactorily fitted the experimental respirometric profiles. The model considered the inhibition caused by nitrite and sulfide over the biological process (substrate inhibition kinetic) as well as the noncompetitive inhibition caused by sulfide on elemental sulfur oxidation, since sulfate production was not observed until sulfide was almost depleted. Moreover, the partial denitrification of nitrate was not observed, indicating that the microbial community had a high denitritating activity. Kinetic parameters were accurately estimated and validated; a future challenge would be the optimization of the BTF operation by incorporating the kinetic model in the general model describing the desulfurization under anoxic conditions.

Acknowledgments The Spanish government provided financial support through the CICYT project CTM2009-14338-C03 and CTM2012-37927-C03. 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. The authors are grateful to Martin Ramirez, Antonio Valle, and Fernando Almengló for their great contribution to this work.

References

Almenglo F, Ramírez M, Gómez JM, Cantero D (2013) H_2S removal from biogás by a pilot anoxic biotrickling filter. A comparison between cocurrent and countercurrent flow operation mode increasing the loading rate. In: 5th IWA Specialized Conference on Odors and Air Emissions Jointly Held With 10th Conference on Biofiltration for Air Pollution Control, San Francisco, California, USA

An S, Tang K, Nemati M (2010) Simultaneous biodesulphurization and denitrification using an oil reservoir microbial culture: effects of sulphide loading rate and sulphide to nitrate loading ratio. Water Res 44(5):1531–1541. doi:10.1016/j.watres.2009.10.037

APHA-AWWA-WPCF (2005) Standard methods for the examination of water and wastewater. American Publication Health Association, Washington

Artiga P, Gonzalez F, Mosquera-Corral A, Campos JL, Garrido JM, Ficara E, Mendez R (2005) Multiple analysis reprogrammable titration analyser for the kinetic characterization of nitrifying and autotrophic denitrifying biomass. Biochem Eng J 26:176–183. doi:10.1016/j.bej.2005.04.017

Campos JL, Carvalho S, Portela R, Mosquera-Corral A, Mendez R (2008) Kinetics of denitrification using sulphur compounds: effects of S/N ratio, endogenous and exogenous compounds. Bioresour Technol 99:1293–1299. doi:10.1016/j.biortech.2007.02.007

Can-Dogan E, Turker M, Dagasan L, Arslan A (2010) Sulfide removal from industrial wastewaters by lithotrophic denitrification using nitrate as an electron acceptor. Water Sci Technol 62:2286–2293. doi:10.2166/wst.2010.545

Cardoso RB, Sierra-Alvarez R, Rowlette P, Flores ER, Gomez J, Field JA (2006) Sulfide oxidation under chemolithoautotrophic denitrifying conditions. Biotechnol Bioeng 95:1148–1157. doi:10.1002/Bit.21084

Cox HHJ, Deshusses MA (1998) Biological waste air treatment in biotrickling filters. Curr Opin Biotechnol 9:256–262. doi:10.1016/S0958-1669(98)80056-6

Decostere B, Janssens N, Alvarado A, Maere T, Goethals P, Van Hulle SWH, Nopens I (2013) A combined respirometer-titrimeter for the determination of microalgae kinetics: experimental data collection and modelling. Chem Eng J 222:85–93. doi:10.1016/j.cej.2013.01.103

Dogan EC, Turker M, Dagasan L, Arslan A (2012) Simultaneous sulfide and nitrite removal from industrial wastewaters under denitrifying conditions. Biotechnol Bioproc Eng 17:661–668. doi:10.1007/s12257-011-0677-3

Fajardo C, Mora M, Fernández I, Mosquera-Corral A, Campos JL, Méndez R (2014) Cross effect of temperature, pH and free ammonia on autotrophic denitrification process with sulphide as electron donor. Chemosphere. doi:10.1016/j.chemosphere.2013.10.02

Fernandez M, Ramirez M, Perez RM, Gomez JM, Canter D (2013) Hydrogen sulphide removal from biogas by an anoxic biotrickling filter packed with Pall rings. Chem Eng J 225:456–463. doi:10.1016/j.cej.2013.04.020

Fortuny M, Baeza JA, Gamisans X, Casas C, Lafuente J, Deshusses MA, Gabriel D (2008) Biological sweetening of energy gases mimics in biotrickling filters. Chemosphere 71:10–17. doi:10.1016/j.chemosphere.2007.10.072

Gadekar S, Nemati M, Hill GA (2006) Batch and continuous biooxidation of sulphide by *Thiomicrospira* sp. CVO: reaction kinetics and stoichiometry. Water Res 40:2436–2446. doi:10.1016/j.watres.2006.04.007

Gonzalez-Sanchez A, Tomas M, Dorado AD, Gamisans X, Guisasola A, Lafuente J, Gabriel D (2009) Development of a kinetic model for elemental sulfur and sulfate formation from the autotrophic sulfide oxidation using respirometric techniques. Water Sci Technol 59:1323–1329. doi:10.2166/Wst.2009.110

Guisasola A, Vargas M, Marcelino M, Lafuente J, Casas C, Baeza JA (2007) On-line monitoring of the enhanced biological phosphorus removal process using respirometry and titrimetry. Biochem Eng J 35:371–379. doi:10.1016/j.bej.2007.02.001

Heijnen JJ (2002) Bioenergetics of microbial growth encyclopedia of bioprocess technology. Wiley, New York

Kleerebezem R, Mendez R (2002) Autotrophic denitrification for combined hydrogen sulfide removal from biogas and post-denitrification. Water Sci Technol 45:349–356

Kristensen HG, Jorgensen PE, Henze M (1992) Characterization of functional microorganism groups and substrate in activated sludge and wastewater by AUR, NUR and OUR. Water Sci Technol 25:43–57

López LR, Mora M, Gamisans X, Gabriel D (2013) Application of respirometry and titrimetry under anoxic conditions for the characterization of the carbon dioxide stripping and biological activity of a SO-NR consortium. In: 5th IWA Specialized Conference on Odors and Air Emissions Jointly Held With 10th Conference on Biofiltration for Air Pollution Control, San Francisco, California, USA

Manconi I, Carucci A, Lens P (2007) Combined removal of sulfur compounds and nitrate by autotrophic denitrification in bioaugmented activated sludge system. Biotechnol Bioeng 98:551–560. doi:10.1002/Bit.21383

Marcelino M, Guisasola A, Baeza JA (2009) Experimental assessment and modelling of the proton production linked to phosphorus release and uptake in EBPR systems. Water Res 43:2431–2440. doi:10.1016/j.watres.2009.03.003

Martin RW, Li HB, Mihelcic JR, Crittenden JC, Lueking DR, Hatch CR, Ball P (2002) Optimization of biofiltration for odor control: model calibration, validation, and applications. Water Environ Res 74:17–27. doi:10.2175/106143002x139712

McMurray SH, Meyer RL, Zeng RJ, Yuan Z, Keller J (2004) Integration of titrimetric measurement, off-gas analysis and NOx- biosensors to investigate the complexity of denitrification processes. Water Sci Technol 50:135–141

Mora M, Guisasola A, Gamisans X, Gabriel D (2014) Examining thiosulfate-driven autotrophic denitrification through respirometry. Chemosphere (in press)

Munz G, Gori R, Mori G, Lubello C (2009) Monitoring biological sulphide oxidation processes using combined respirometric and titrimetric techniques. Chemosphere 76:644–650. doi:10.1016/j.chemosphere.2009.04.039

Reyes-Avila JS, Razo-Flores E, Gomez J (2004) Simultaneous biological removal of nitrogen, carbon and sulfur by denitrification. Water Res 38:3313–3321. doi:10.1016/j.watres.2004.04.035

Roels JA (1983) Energetics and kinetics in biotechnology. Elsevier, Amsterdam

Sin G, Vanrolleghem PA (2007) Extensions to modeling aerobic carbon degradation using combined respirometric-titrimetric measurements in view of activated sludge model calibration. Water Res 41:3345–3358. doi:10.1016/j.watres.2007.03.029

Soreanu G, Beland M, Falletta P, Edmonson K, Seto P (2008) Laboratory pilot scale study for H_2S removal from biogas in an anoxic biotrickling filter. Water Sci Technol 57:201–207. doi:10.2166/Wst.2008.023

Soreanu G, Beland M, Falletta P, Ventresca B, Seto P (2009) Evaluation of different packing media for anoxic H_2S control in biogas. Environ Technol 30:1249–1259. doi:10.1080/09593330902998314

Soto O, Aspe E, Roeckel M (2007) Kinetics of cross-inhibited denitrification of a high load wastewater. Enzym Microb Technol 40:1627–1634. doi:10.1016/j.enzmictec.2006.11.014

Spanjers H, Vanrolleghem P (1995) Respirometry as a tool for rapid characterization of waste-water and activated-sludge. Water Sci Technol 31:105–114. doi:10.1016/0273-1223(95)00184-O

Spanjers H, Vanrolleghem P, Olsson G, Dold P (1996) Respirometry in control of the activated sludge process. Water Sci Technol 34:117–126. doi:10.1016/0273-1223(96)84211-9

Syed M, Soreanu G, Falletta P, Béland M (2006) Removal of hydrogen sulfide from gas streams using biological processes—a review. Can Biosyst Eng 48:2.1–2.14

Tora JA, Lafuente J, Baeza JA, Carrera J (2010) Combined effect of inorganic carbon limitation and inhibition by free ammonia and free nitrous acid on ammonia oxidizing bacteria. Bioresour Technol 101:6051–6058. doi:10.1016/j.biortech.2010.03.005

Vaiopoulou E, Melidis P, Aivasidis A (2005) Sulfide removal in wastewater from petrochemical industries by autotrophic denitrification. Water Res 39:4101–4109. doi:10.1016/j.watres.2005.07.022

Wild D, Vonschulthess R, Gujer W (1995) Structured modeling of denitrification intermediates. Water Sci Technol 31:45–54. doi:10.1016/0273-1223(95)00179-Q

Yavuz B, Turker M, Engin GO (2007) Autotrophic removal of sulphide from industrial wastewaters using oxygen and nitrate as electron acceptors. Environ Eng Sci 24:457–470. doi:10.1089/ees.2006.0068

Young JC, Cowan RM (2004) Respirometry for environmental science and engineering. SJ Enterprises, Springdale