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# Investigating the kinetics of autotrophic denitrification with thiosulfate: Modeling the denitritation mechanisms and the effect of the acclimation of SO-NR cultures to nitrite

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## HIGHLIGHTS

- Two-step autotrophic denitrification was modeled with combined Monod–Haldane kinetics.
- FIM method revealed a high sensitivity of the kinetic parameters estimated.
- Biomass acclimated to nitrite presented a 7-fold increase of the $K_i$.
- The biomass presented good resistance and resilience to nitrite up to 150 mg N $L^{-1}$.

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## ABSTRACT

In this work the kinetics of a number of sulfide-oxidizing nitrate-reducing (SO-NR) cultures acclimated and not acclimated to nitrite were characterized. Anoxic respirometry coupled to kinetic modeling of respirometric profiles was the methodology used to study the two-step denitrification associated to thiosulfate oxidation. Autotrophic denitritation was initially studied in a non-acclimated SO-NR culture to confirm that nitrite reduction kinetics could be described through a Haldane-type equation. Afterwards, a kinetic model describing the two-step denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow N_2$) was calibrated and validated through the estimation of several kinetic parameters from the fitting of experimental respirometric profiles obtained using either nitrate or nitrite as electron acceptors for both acclimated and non-acclimated biomass. The model proposed was a multi-substrate model that considered all the species implicated in the process as well as the stoichiometry associated particularly to the biomass studied in this work. A comparison between the kinetic parameters with the biomass acclimated and non-acclimated to nitrite revealed a 7-fold increase of the Haldane nitrite inhibition constant in the acclimated biomass with respect to the non-acclimated while the nitrite half-saturation constant and the maximum specific growth rate remained almost unchanged. The Fisher Information Matrix method was used to obtain the confidence intervals and also to evaluate the sensitivity and the identifiability in model calibration of each kinetic parameter estimated.



## 1. Introduction

Different techniques have been developed to study the biodegradation characteristics of microbial cultures [1,2]. In particular, respirometry has been demonstrated as a powerful technique to gain insight on kinetics and stoichiometrics of biological processes [3–5] since biomass growth and substrate removal are directly associated with the electron acceptor consumption. Moreover, this technique allows obtaining biokinetic characteristics by modeling the respirometric profiles [6] as well as evaluating the inhibitory and/or toxic effects that a particular microbial population may suffer [7,8].

Kinetic characterization of autotrophic denitrifying biomass through respirometry has already been reported for suspended cultures obtained from wastewater treatment processes [9] and for SO-NR cultures obtained from anoxic desulfurizing biotrickling filters (BTF) [10]. However, in both cases the stoichiometric coefficients used and the kinetic parameters obtained were significantly different. This means that the use of some kinetic or stoichiometric

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data reported in literature for SO-NR biomass entails an inaccurate characterization of a specific biomass since the experimental conditions as well as the microbial diversity of biomass are hardly the same. In the case of immobilized biomass an additional bottle-neck is the estimation of biomass growth yield, biomass concentration and the active fraction of such microbial culture.

Some authors have used single-substrate kinetic models taking into account microbial growth rates associated only to a single pollutant biodegradation (Monod, Haldane and other kinetic equations) [11,12] to describe biological processes. A drawback of single-substrate kinetic models is the disability to describe the potential limitations of other species such as nutrients or the electron acceptor. Also, models based on single-substrates can hardly describe the formation of multiple end-products in complex biological processes such as biological denitrification and desulfurization processes [13]. For this reason, a multi-substrate model has to be proposed to describe the two-step denitrification since both electron acceptors (nitrate and nitrite) are implicated in the process. Moreover, the confidence intervals of the kinetic parameters are not commonly assessed even if they are as important as the estimation of the parameters themselves. The Fisher Information Matrix (FIM) method is a proven tool that accurately provides confidence intervals for kinetic parameters. This method is based on the calculation of the covariance matrix inverse, which is directly associated to the uncertainty of the model parameters estimated and the quantity and quality of the experimental data. Many authors have successfully used this mathematical method to evaluate the reliability of the parameters estimated both in wastewater and in polluted gas treatment processes [14,15]. The FIM method allows evaluating the sensitivities of the parameters and the quality of estimations.

Previous works have reported a methodology and an experimental setup of the anoxic respirometer used in this study. In addition, denitrification and thiosulfate oxidation rates of a SO-NR biomass from an anoxic biotrickling filter have been obtained as well as the stoichiometry of the two-step denitrification process has been solved [16,17]. However, kinetic equations and parameters that characterize the biological process have not been already reported. Consequently, the aim of the present study was to determine the kinetic parameters of the autotrophic denitrification mechanisms based on thiosulfate oxidation. The effect of culture acclimation to nitrite was also investigated and evaluated in this work through the changes in the kinetic parameters, which are directly related with the operating conditions of the reactor in which the biomass was grown and the history of the culture. For a reliable characterization of the kinetic model and parameters, the stoichiometric coefficients corresponding to the culture used in this study were those previously calculated through respirometric and titrimetric techniques in Mora et al. [17]. The acclimation of the biomass to nitrite was also assessed through kinetic parameters estimation and proposed to face nitrite inhibition problems in denitrifying reactors.

## 2. Materials and methods

### 2.1. Biomass

The SO-NR biomass used in this work was obtained from an anoxic biogas desulfurizing biotrickling filter (BTF) at different operation times (175 d and 325 d of operation) [18]. The first sampling of the biomass from the BTF (175 d) was used to inoculate a 2.8 L continuous stirred tank reactor (CSTR). Once assessed the steady state, the study of denitritation kinetics was performed using the SO-NR biomass cultured in the CSTR (Operation A). This biomass was then discarded for further studies. The second sample of biomass obtained from the BTF (325 d) was cultured in the CSTR (Operation B) during 22 weeks, without nitrite accumulation, for assessing the two-step denitrification process. Later, the biomass was acclimated to nitrite 2 weeks before the end of the CSTR operation by decreasing stepwise the hydraulic retention time. A detailed description of the start-up and the continuous operation of the reactors are reported elsewhere [16].

### 2.2. Respirometric profiles

Two sets of respirometric tests were performed with the biomass cultured in the CSTR in order to evaluate the denitrification kinetics associated to thiosulfate oxidation (Table 1). During the first set of tests (Set A) nitrite was used as the electron acceptor in order to define the kinetic model describing denitritation. The second set (Set B) was performed to study the kinetics of the whole denitrification process and to assess the impact of biomass acclimation to nitrite. Biomass used in Set A and B had the same origin (anoxic desulfurizing BTF) but were collected and cultivated in different periods of time (see Section 2.1 – Operation A and B of the CSTR). Thus, impact of biomass acclimation was only assessed with biomass from Set B before and after biomass acclimation to nitrite. Moreover, the use of thiosulfate allowed studying denitrification and nitrite acclimation clearly since the effects of many additional reduced sulfur compounds reactions were avoided. It would not be the case of sulfide since elemental sulfur can be formed as an intermediary product affecting consequently the denitrification rates [10].

To obtain the respirometric profiles a certain volume of the biomass cultured in the CSTR was previously washed and poured into the respirometer. Subsequently, known pulses of thiosulfate, nitrate and nitrite were added to the respirometer after overcoming both the endogenous and the wake up phases. The continuous sampling of the system allowed monitoring the concentration of the species involved in the process, which enables the estimation of the corresponding kinetic parameters by modeling the respirometric profiles. The protocol used for biomass preparation prior to the anoxic respirometric tests as well as the set up of the respirometer and the procedure to obtain the respirometric profiles were previously optimized and properly described in Mora et al. [16].

### 2.3. Chemical analysis

Nitrite ($NO_2^-$), sulfate ($SO_4^{2-}$), nitrate ($NO_3^-$) and thiosulfate ($S_2O_3^{2-}$) concentrations were determined by ion chromatography with conductivity detection using a Dionex ICS-2000. The biomass concentration was determined according to Standard Methods [19]. The inorganic carbon concentration was measured with an OI Analytical TIC/TOC Analyzer (Model 1020A). The dissolved oxygen concentration and the pH were continuously monitored in situ with sensors (CellOx® 325 and SenTix® 82 from WTW, respectively) connected to a bench-top multimeter (Inolab® Multi 740 – WTW).

## 3. Development of the kinetic model

According to literature [20], nitrate reduction could be represented by a Monod-type equation while nitrite reduction can be described by Haldane-type kinetics. The accumulation of nitrite has also been reported as a kinetic affecting factor which could depend of the pH [21], the S/N ratio used [22,23], the competition for each electron acceptor (nitrite or nitrate) [24], etc. In this work none of these considerations were included in the kinetic model since in a previous study [16] it was observed that the factor