

# A sensitivity analysis for sulfur-driven two-step denitrification model

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**Abstract:** A local sensitivity analysis was performed for a S<sup>0</sup>-driven two-step denitrification model, accounting for NO<sub>2</sub><sup>-</sup> accumulation, biomass growth and S<sup>0</sup> solubilization. The model sensitivity was aimed at verifying the model stability, understanding the identifiability of the model structure and evaluating the model parameters to be further optimized. The sensitivity analysis identified the mass specific area of the sulfur particles ( $a^*$ ) and hydrolysis kinetic constant ( $k_I$ ) as the dominant parameters. Additionally, the maximum growth rate of the denitrifying biomass on NO<sub>3</sub><sup>-</sup> ( $\mu^{max}_{2,3}$ ) and NO<sub>2</sub><sup>-</sup> ( $\mu^{max}_{2,4}$ ) were detected as the most sensitive kinetic parameters. Further calibration would be performed for the sensitive model parameters to optimize the quality of the model.

**Keywords:** biological surface based hydrolysis; elemental sulfur; modeling; sensitivity analysis; two-step autotrophic denitrification.

## INTRODUCTION

Both nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) are considered as environmental pollutants in groundwater and surface water (Kilic et al., 2014). High NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> concentrations are associated with various negative environmental and human health impacts. An elevated NO<sub>3</sub><sup>-</sup> concentration in fresh waters leads to many environmental problems, such as eutrophication, loss of crop productivity and aquatic biodiversity. Also, a high NO<sub>3</sub><sup>-</sup> concentration has an adverse effect on human health. It might cause methemoglobinemia (also known as "blue baby" disease) and bring higher risk of cancer (Liu et al., 2016). Thus, the guidance value of 50 mg/l for NO<sub>3</sub><sup>-</sup> was set for drinking water (WHO, 2011). The highly toxic nature of NO<sub>2</sub><sup>-</sup> to both humans and aquatic life resulted in a stringent limit as low as 3 mg/l in drinking water (WHO, 2011).

$\text{NO}_3^-$  and  $\text{NO}_2^-$  removal from wastewaters and drinking water can be performed by physico-chemical or biological processes. However, physico-chemical processes, including ion exchange, reverse osmosis and chemical reduction, are quite costly and energy-demanding (Sierra-Alvarez et al., 2007). Therefore, biological removal of  $\text{NO}_3^-$  (denitrification) and  $\text{NO}_2^-$  (denitritation) from waters could be applied.

Depending on the type of energy source used, denitrification can be maintained heterotrophically by using organic compounds, or autotrophically with inorganic compounds. Heterotrophic denitrification is a proven technology, widely applied at the industrial scale. However, autotrophic denitrification has been suggested as an alternative treatment for those waters poor in carbon content (e.g. drinking water) due to the costly supplementation of organics (Sierra-Alvarez et al., 2007). Moreover, autotrophic denitrification shows the following advantages 1) reduced sludge production and handling; 2) decreased risk of bacterial contamination; and 3) lower operating cost of the process due to use of inorganic carbon compounds as a carbon source (Sierra-Alvarez et al., 2007; Zhou et al., 2015). Therefore, autotrophic denitrification is a promising and sustainable process to treat  $\text{NO}_3^-$  and  $\text{NO}_2^-$  pollution not only from industrial wastewaters, but also small drinking water systems.

Elemental sulfur ( $\text{S}^0$ ) is one of the most promising electron donor for autotrophic denitrification due to its low cost, non-toxicity and easy handling (Christianson et al., 2015; Di Capua et al., 2015; Kilic et al., 2014; Soares, 2002). However, chemically produced  $\text{S}^0$  has a low solubility, which limits its application in autotrophic denitrification and denitritation and the scale-up of the processes. To guarantee higher denitrification rates, smaller  $\text{S}^0$  particles, with a higher specific surface area, can be used (Di Capua et al., 2016). Therefore, the specific surface area of the  $\text{S}^0$  particles is one of the key parameters to be considered during  $\text{S}^0$  solubilization in the autotrophic denitrification and denitritation processes (Sierra-Alvarez et al., 2007).

When  $\text{NO}_3^-$  and  $\text{NO}_2^-$  are removed in the presence of elemental  $\text{S}^0$ , pH decreases and this may result in an inhibitory effects for autotrophic denitrifiers if the system is not adequately buffered (Soares, 2002). Therefore, the use of limestone as a buffering agent and inorganic carbon source is a common practice within the so-called 'sulfur-limestone autotrophic denitrification (SLAD)' process (Sahinkaya et al., 2015).

Over the last decades, several mathematical models accounting for  $\text{S}^0$ -driven autotrophic denitrification have been proposed. In most of them, zero- or half-order reactions have been applied to describe the simplified  $\text{S}^0$ -driven autotrophic denitrification kinetics without accounting for microbial growth (Koenig and Liu, 2001; Moon et al., 2004; Qambrani et al., 2015; Zhang and Zeng, 2006). The two-step autotrophic denitrification with thiosulfate ( $\text{S}_2\text{O}_3^{2-}$ ) and  $\text{S}^0$  was described by Monod equations (Mora et al., 2015). However,  $\text{NO}_2^-$  evolution has been poorly predicted in the models. Xu et al. (2016) established a kinetic model for two-step autotrophic denitrification with hydrogen sulfide ( $\text{H}_2\text{S}$ ) that accurately predicted the concentration of intermediate  $\text{NO}_2^-$  as well as  $\text{SO}_4^{2-}$  as end product. Recently, Liu et al. (2016) have developed a model for three-step autotrophic denitrification linked to  $\text{H}_2\text{S}$  and  $\text{S}^0$  oxidation with a focus on  $\text{N}_2\text{O}$  accumulation. However, none of the developed models distinctly accounted for  $\text{S}^0$  solubilization as a prior step to autotrophic denitrification and denitritation (Sierra-Alvarez et al., 2007).

In a recent study, a mechanistic model accounting for  $\text{NO}_2^-$  accumulation, biomass growth and  $\text{S}^0$  solubilization has been proposed (Kostrzytsia et al., 2017). As demonstrated through numerical simulations, the developed model could serve as a tool to predict the performance of SLAD biofilm systems and assess their process efficiency when compared to other denitrification systems. In the present work, the model developed in (Kostrzytsia et al., 2017)

was recalled and a sensitivity analysis was applied to verify the model stability as well as define the model parameters to be further optimized. Specifically, our focus was to use local sensitivity analysis to better understand the dominant parameters of the process and possibly reduce the complexity of the model.

## MATERIALS AND METHODS

### Mathematical model overview

A mathematical model was developed in (Kostrysia et al., 2017) to dynamically simulate the main processes occurring during the two-step denitrification with  $S^0$  ( $S_1$ ). The proposed model takes into account the activities of autotrophic denitrifying bacteria ( $X_2$ ) using  $NO_3^-$  ( $S_3$ ) or  $NO_2^-$  ( $S_4$ ) as electron acceptors and a hydrolytic biomass ( $X_1$ ) growing on  $S^0$  lentils. It evaluates the interactions between the related processes:  $S^0$  solubilization,  $S^0$ -based denitrification and denitrification.  $S^0$  uptake was modeled by introducing a new variable, the bioavailable sulfur ( $S_2$ ), a soluble compound that can be directly uptaken by denitrifying bacteria for further oxidation to  $SO_4^{2-}$  ( $S_6$ ). A modified surface based kinetics equation was introduced to account for the hydrolysis of  $S^0$  (Esposito et al., 2011). The model equations were derived from mass balances and expressed as double-Monod kinetics (Eq. 1-8). They can be written as follows:

$$\frac{dS_1}{dt} = -k_1 \frac{S_1}{\frac{K_1}{a^*} + S_1} X_1, \quad (1)$$

$$\frac{dS_2}{dt} = k_1 \frac{S_1}{\frac{K_1}{a^*} + S_1} X_1 - \frac{r_1}{Y_{2,3}} \mu_{2,3}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_3 - S_3^*)}{K_{2,3} + (S_3 - S_3^*)} \frac{S_3}{S_3 + S_4} X_2 - \frac{r_2}{Y_{2,4}} \mu_{2,4}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_4 - S_4^*)}{K_{2,4} + (S_4 - S_4^*)} \frac{S_4}{S_3 + S_4} X_2, \quad (2)$$

$$\frac{dS_3}{dt} = -\frac{1}{Y_{2,3}} \mu_{2,3}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_3 - S_3^*)}{K_{2,3} + (S_3 - S_3^*)} \frac{S_3}{S_3 + S_4} X_2, \quad (3)$$

$$\frac{dS_4}{dt} = \frac{1}{Y_{2,3}} \mu_{2,3}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_3 - S_3^*)}{K_{2,3} + (S_3 - S_3^*)} \frac{S_3}{S_3 + S_4} X_2 - \frac{1}{Y_{2,4}} \mu_{2,4}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_4 - S_4^*)}{K_{2,4} + (S_4 - S_4^*)} \frac{S_4}{S_3 + S_4} X_2, \quad (4)$$

$$\frac{dS_5}{dt} = \frac{1}{Y_{2,4}} \mu_{2,4}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_4 - S_4^*)}{K_{2,4} + (S_4 - S_4^*)} \frac{S_4}{S_3 + S_4} X_2, \quad (5)$$

$$\frac{dS_6}{dt} = \frac{r_1}{Y_{2,3}} \mu_{2,3}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_3 - S_3^*)}{K_{2,3} + (S_3 - S_3^*)} \frac{S_3}{S_3 + S_4} X_2 + \frac{r_2}{Y_{2,4}} \mu_{2,4}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_4 - S_4^*)}{K_{2,4} + (S_4 - S_4^*)} \frac{S_4}{S_3 + S_4} X_2, \quad (6)$$

$$\frac{dX_1}{dt} = K_0 k_1 \frac{S_1}{\frac{K_1}{a^*} + S_1} X_1 - k_{d,1} X_1, \quad (7)$$

$$\frac{dX_2}{dt} = \mu_{2,3}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_3 - S_3^*)}{K_{2,3} + (S_3 - S_3^*)} \frac{S_3}{S_3 + S_4} X_2 + \mu_{2,4}^{max} \frac{S_2}{K_{2,2} + S_2} \frac{(S_4 - S_4^*)}{K_{2,4} + (S_4 - S_4^*)} \frac{S_4}{S_3 + S_4} X_2 - k_{d,2} \cdot X_2, \quad (8)$$

where  $K_0$  denoted the efficiency growth coefficient for hydrolytic biomass;  $Y_{2,3}$  and  $Y_{2,4}$  represented the denitrifying biomass yield coefficients on  $NO_3^-$  and  $NO_2^-$  respectively;  $a^*$  denoted the mass specific area of the sulfur particles;  $k_l$  denoted the hydrolysis kinetic constant;  $K_l$  indicated the volume specific half-saturation constants for  $S^0$ ;  $\mu_{2,3}^{max}$  and  $\mu_{2,4}^{max}$

represented the maximum growth rate for denitrifying biomass on  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , respectively;  $k_{d,1}$  and  $k_{d,2}$  represented the decay constants for  $X_1$  and  $X_2$  biomass, respectively;  $K_{2,2}$ ,  $K_{2,3}$  and  $K_{2,4}$  denoted the half-saturation constants for  $\text{S}^0$ ,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , respectively;  $S_3^*$  and  $S_4^*$  indicated the lowest  $\text{NO}_2^-$  and  $\text{NO}_3^-$  concentrations that enable metabolic activities of  $X_2$ . The obtained ordinary differential equations were integrated by using an original software developed on the MATLAB platform and based on the Runge-Kutta method.

### Sensitivity analysis

The proposed model requires a high dimensional (stoichiometric and kinetic) parameter space to be explored. To estimate each parameter, excessive experimental results should be provided to avoid ill-conditioning of the parameter estimation (Kesavan and Law, 2005). Therefore, a model sensitivity analysis was performed to reduce the parameter space of the model as well as identify parameter targets for experimental exploration (Jarrett et al., 2015). The change of the dominant parameters resulted in a high sensitivity of the model outputs. In contrast, the variation of the non-significant parameters indicated a low sensitivity.

In this model, a local sensitivity analysis was applied to compute sensitivity functions for the dynamic simulations. The sensitivities were calculated in terms of the effect of the perturbation on the model output over a time span because of the change in the input parameters.

An automatic differentiation tool SENS\_SYS coupled with the ordinary differential equation (ODE) solver of MATLAB was used to predict local sensitivity. SENS\_SYS tool is an extension of the ODE15s tool that allows to solve ODE system while compute derivatives (sensitivities) of the solution with respect to parameters (Molla and Padilla, 2002). The accuracy of the SENS\_SYS tool is controlled by the default relative tolerance of  $1e^{-6}$ . The sensitivity analysis of the system  $F$  (Eqs. 3-4,6) was calculated by differentiating the system with respect to kinetic parameter  $u$ , as illustrated in Eq. (9).

$$F(t, y, y', u) = 0 \quad (9),$$

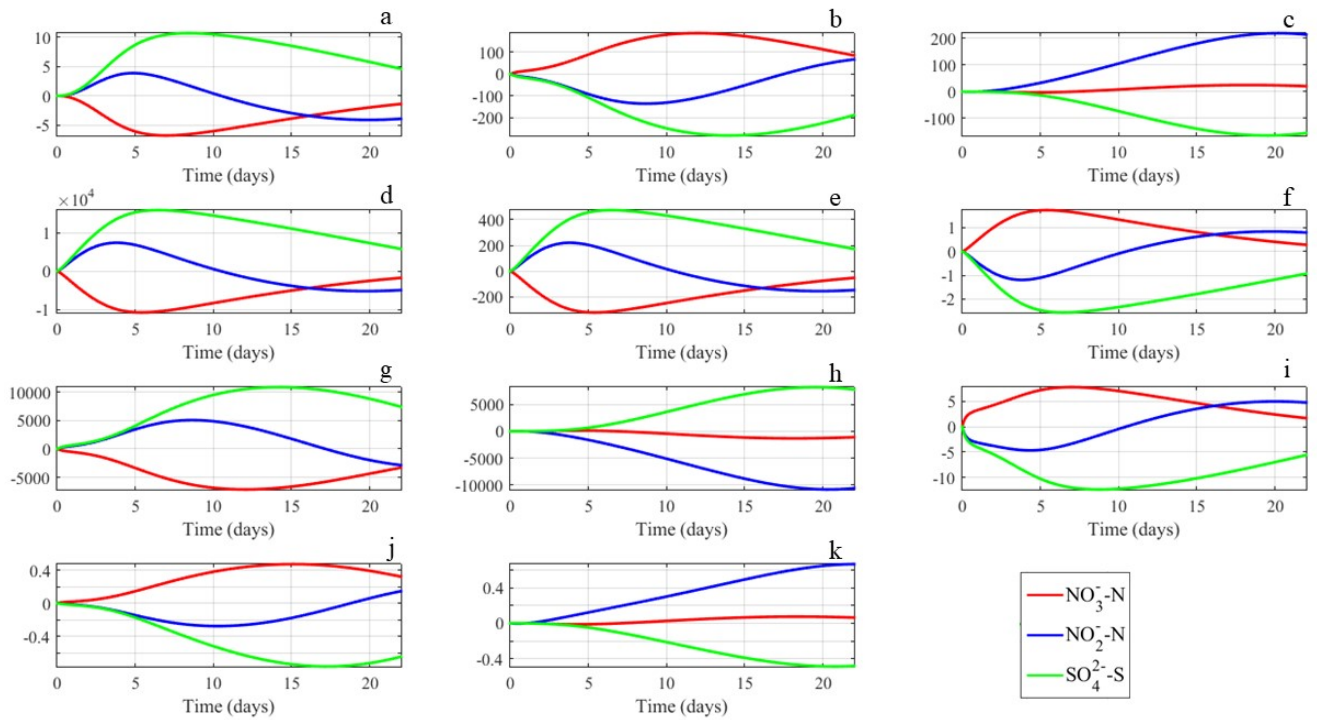
where  $t$  denoted the time interval for the integration (days),  $y$  represented input state variables,  $y'$  the first derivative of  $y$  with respect to  $t$  and  $u$  denoted the parameter.

The stoichiometric parameters of the model were based on the biotransformation mechanism and determined in the previous experimental studies (Sierra-Alvarez et al., 2007). The biomass decay rate for most autotrophic microorganisms are very low. The threshold values for substrates ( $\text{NO}_3^-$  and  $\text{NO}_2^-$ ), which represented the inability of microorganisms to grow below these values, described the characteristics of the enriched microbial community and were determined experimentally. Therefore, in the proposed model the above-mentioned parameters were not considered for the sensitivity analysis and the remaining 11 kinetic parameters were tested.

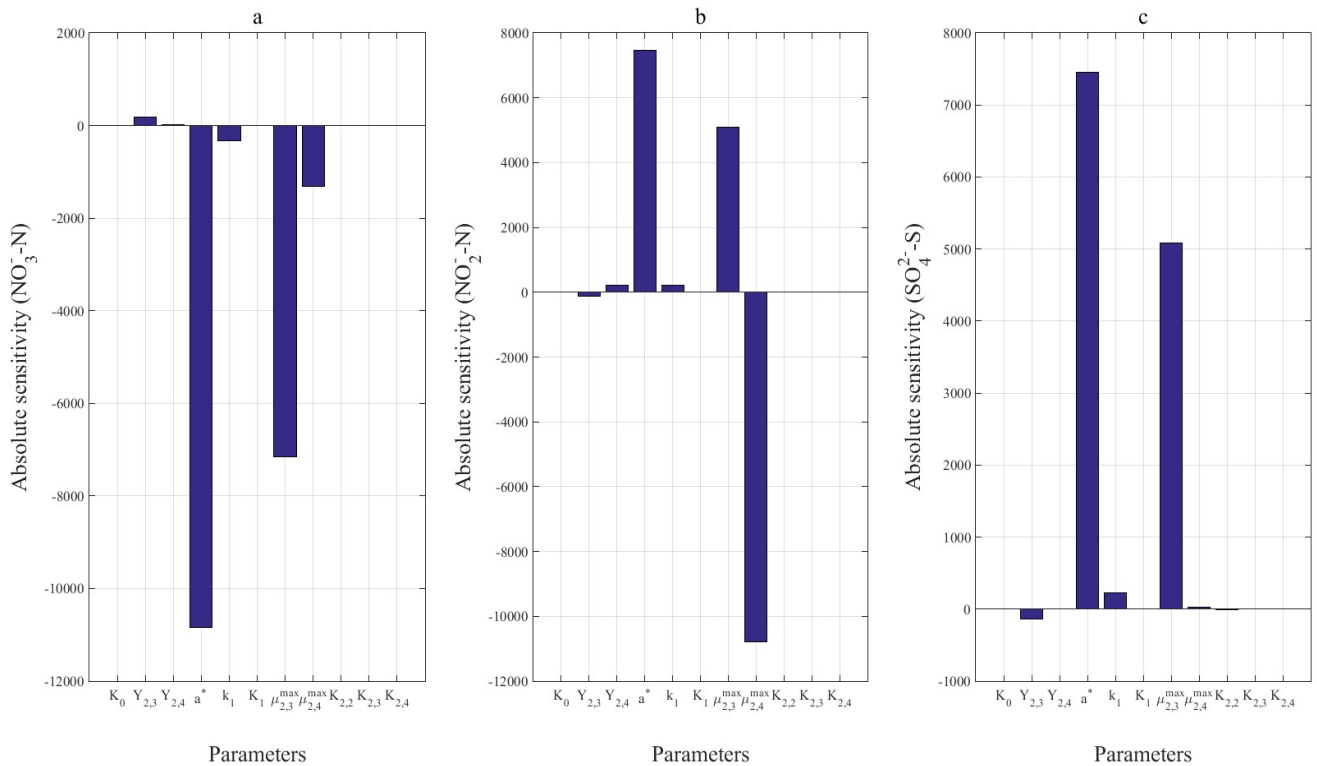
## RESULTS AND DISCUSSION

A series of sensitivity curves were obtained by changing the 11 kinetic parameters one by one in the simulation and absolute sensitivity was calculated, as illustrated in **Figure 1**. The greater parameter line slope indicates the more significant role of the parameter on the autotrophic denitrification process. **Figure 2** demonstrates the absolute sensitivities of the kinetic parameters for the input state variables of 210, 0 and 0, for  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N and  $\text{SO}_4^{2-}$ -

S, respectively. The values of the parameters (stoichiometric and kinetic) used for the model (Eqs.1 - 8) are reported in (Kostrysia et al., 2017).



**Figure 1.** Output absolute (or local) sensitivity of different parameters: (a)  $K_0$ , (b)  $Y_{2,3}$ , (c)  $Y_{2,4}$ , (d)  $a^*$ , (e)  $k_1$ , (f)  $K_1$ , (g)  $\mu^{max}_{2,3}$ , (h)  $\mu^{max}_{2,4}$ , (i)  $K_{2,2}$ , (j)  $K_{2,3}$  and (k)  $K_{2,4}$ .



**Figure 2.** Absolute (or local) sensitivities of kinetic parameters during the simulation time for the degradation of (a)  $\text{NO}_3^-$ -N, (b)  $\text{NO}_2^-$ -N and (c)  $\text{SO}_4^{2-}$ -S production.

Apparently, the mass specific area of the sulfur particles ( $a^*$ ) possessed a major influence on the model outputs and was ranked as a first dominant parameter (**Figure 2**). Parameter  $a^*$  accounts for the overall surface area of the sulfur particles to be microbially solubilized prior to denitrification and denitritation. Additionally, the parameter considers the particle size distribution. As illustrated in **Figure 1d**,  $\text{NO}_3^-$ -N output is more sensitive to the change in parameter  $a^*$ , compared to  $\text{NO}_2^-$ -N. The latter might be attributed to the higher stoichiometric S/N ratio required for complete denitrification than for denitritation.

These results were consistent with the literature, where the impact of the specific surface area of sulfur particles was suggested as a prerequisite of  $\text{S}^0$  oxidation coupled to denitrification. Therefore, the proposed model was able to successfully describe  $\text{S}^0$  solubilization as an inevitable aspect and limiting step in the denitrification and denitritation processes.

Among the other parameters related to the  $\text{S}^0$  solubilization step, the hydrolysis kinetic constant ( $k_1$ ), being dependent on the nature of the sulfur, could be considered as a sensitive one. The absolute sensitivity of both parameters,  $a^*$  and  $k_1$ , showed a peak between 5 and 10 days, and then slowly dropped. Consequently, the model predictions are crucial during that phase. On the other hand, the efficiency growth coefficient for hydrolytic biomass ( $K_0$ ) and volume specific half-saturation constant for  $\text{S}^0$  ( $K_1$ ) did not significantly influence the model outputs.

The second and third most sensitive kinetic parameters were the maximum growth rate for denitrifying biomass on  $\text{NO}_3^-$  ( $\mu^{max}_{2,3}$ ) and  $\text{NO}_2^-$  ( $\mu^{max}_{2,4}$ ), as illustrated in **Figure 2**. As expected,  $\mu^{max}_{2,3}$  played a crucial role for model outputs (**Figure 1g**). However,  $\mu^{max}_{2,4}$  had a high sensitivity only for  $\text{NO}_2^-$ -N and  $\text{SO}_4^{2-}$ -S outputs (**Figure 1h**). The  $\text{SO}_4^{2-}$ -S absolute sensitivity of 10000 was reached for  $\mu^{max}_{2,3}$  compared to that of 7500 for  $\mu^{max}_{2,4}$ . That might be attributed to the higher metabolic activities rates, in particular  $\text{S}^0$  oxidation to  $\text{SO}_4^{2-}$ , of denitrifying bacteria growing on  $\text{NO}_3^-$  ( $\mu^{max}_{2,3}$ ) than  $\text{NO}_2^-$ .

Denitrifying biomass yield coefficient on  $\text{NO}_3^-$  ( $Y_{2,3}$ ) possessed high sensitivity for  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N and  $\text{SO}_4^{2-}$ -S outputs (**Figure 1b**). Instead, yield coefficients on  $\text{NO}_2^-$  ( $Y_{2,4}$ ) affected only  $\text{NO}_2^-$ -N and  $\text{SO}_4^{2-}$ -S outputs (**Figure 1b**). On the other hand, half-saturation constants for  $\text{S}^0$  ( $K_{2,2}$ ),  $\text{NO}_3^-$  ( $K_{2,3}$ ) and  $\text{NO}_2^-$  ( $K_{2,4}$ ) had a minimal impact for the model outputs (**Figure 1i-k**). To get an accurate evaluation of the half-saturation constants, a larger set of experimental data would be required; for example, they could be obtained from kinetic tests with different initial substrate concentrations.

Therefore, the sensitive parameters, such as  $a^*$ ,  $k_1$ ,  $\mu^{max}_{2,3}$  and  $\mu^{max}_{2,4}$  were indicated for model outputs by sensitivity analysis. Further calibration is required for the most sensitive model parameters to improve the quality of the model.

## CONCLUSIONS

In this work, the results of a local sensitivity analysis performed for the newly developed model (Kostrysia et al., 2017) were presented. The model was able to dynamically describe the biological and physico-chemical processes occurring during autotrophic denitrification with  $\text{S}^0$ , such as  $\text{NO}_2^-$  accumulation, biomass growth and  $\text{S}^0$  surface based solubilization. The sensitivity analysis demonstrated that the model was more sensitive to  $a^*$ ,  $k_1$ ,  $\mu^{max}_{2,3}$  and  $\mu^{max}_{2,4}$ . The model calibration will be performed only for the dominant parameters to increase the quality of model and reduce its complexity.

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