Sensitivity analysis for an elemental sulfur-based two-step denitrification model

A. Kostrytsia, S. Papirio, M. R. Mattei, L. Frunzo, P. N. L. Lens and G. Esposito

ABSTRACT

A local sensitivity analysis was performed for a chemically synthesized elemental sulfur (S\textsubscript{0})-based two-step denitrification model, accounting for nitrite (NO\textsubscript{2}/C\textsubscript{0}) accumulation, biomass growth and S\textsubscript{0} hydrolysis. The sensitivity analysis was aimed at verifying the model stability, understanding the model structure and individuating the model parameters to be further optimized. The mass specific area of the sulfur particles (a\textastermath) and hydrolysis kinetic constant (k\textsubscript{1}) were identified as the dominant parameters on the model outputs, i.e. nitrate (NO\textsubscript{3}/C\textsubscript{0}), NO\textsubscript{2}/C\textsubscript{0} and sulfate (SO\textsubscript{4}\textsuperscript{2-}/C\textsubscript{0}) concentrations, confirming that the microbially catalyzed S\textsubscript{0} hydrolysis is the rate-limiting step during S\textsubscript{0}-driven denitrification. Additionally, the maximum growth rates of the denitrifying biomass on NO\textsubscript{3}/C\textsubscript{0} and NO\textsubscript{2}/C\textsubscript{0} were detected as the most sensitive kinetic parameters.

Key words | biological surface-based hydrolysis, elemental sulfur, mathematical modeling, sensitivity analysis, two-step autotrophic denitrification

INTRODUCTION

Globally, up to 80% of wastewater is released into the environment without adequate treatment (UN-Water 2015) affecting the water quality in ground and surface water bodies. Contamination by nitrate (NO\textsubscript{3}) and nitrite (NO\textsubscript{2}), due to the excessive use of N-based fertilizers and uncontrolled discharge of wastewaters, is one of the main environmental concerns (Kilic et al. 2014). Elevated NO\textsubscript{3} concentrations result in eutrophication and ecological disturbance, while NO\textsubscript{2} leads to toxicity towards aquatic life. Also, a high NO\textsubscript{3} concentration imposes an adverse effect on human health such as methemoglobinemia (also known as ‘blue baby’ syndrome) or higher risk of cancer (Liu et al. 2016). Thus, the guidance value of 50 mg/l for NO\textsubscript{3} was set for drinking water (WHO 2011).

NO\textsubscript{3} and NO\textsubscript{2} removal from wastewaters and drinking water can be performed by physico-chemical or biological processes. However, due to high costs and energy demand of the physico-chemical methods (Sierra-Alvarez et al. 2007), biological removal of NO\textsubscript{3} (denitrification) and NO\textsubscript{2} (denitritation) represents a valuable alternative technology (Mattei et al. 2015). Heterotrophic denitrification which uses organic compounds as energy source is a proven technology (Papirio et al. 2014; Zou et al. 2014), widely applied at the industrial scale. Notwithstanding, autotrophic denitrification has been suggested as an alternative and environmentally sustainable treatment for waters poor in carbon content, due to the costly supplementation of organics (Zhou et al. 2015). The use of S\textsubscript{0} as electron donor is easy handling and results in low operational costs and a low N\textsubscript{2}O production (Soares 2002; Christianson et al. 2015). Therefore, reduced capital and operational costs, a decreased sludge production and limited greenhouse gas emissions (Kilic et al. 2014; Di Capua et al. 2015) make S\textsubscript{0}-based autotrophic denitrification an appropriate technology to be applied in decentralized and small-scale wastewater treatment systems.
However, chemically synthesized $S^0$ has a low solubility, which limits its application in autotrophic denitrification and denitritation systems. To obtain higher denitrification rates, the use of smaller and more porous $S^0$ particles, with a higher specific surface area, has previously been suggested (Di Capua et al. 2016). The specific surface area of the $S^0$ particles, thus, becomes one of the key parameters that control the autotrophic denitrification and denitritation rates (Sierra-Alvarez et al. 2007).

Modeling has proven to be an important tool for the understanding, design and control of autotrophic denitrification. Over the last years, several mathematical models accounting for $S^0$-driven autotrophic denitrification have been proposed. In most of them, zero- or half-order reactions have been applied to describe the simplified $S^0$-driven autotrophic denitrification kinetics without accounting for microbial growth or $NO_2$ evolution (Koenig & Liu 2001; Moon et al. 2004; Qambrani et al. 2015). Xu et al. (2016) established a kinetic model for two-step autotrophic denitrification with hydrogen sulfide ($H_2S$) that accurately predicted the concentration of the intermediate $NO_2$. Recently, Liu et al. (2016) have developed a model for three-step autotrophic denitrification linked to $H_2S$ and $S^0$ oxidation with a focus on $N_2O$ accumulation.

However, none of the above mentioned models distinctly focused on $S^0$ hydrolysis as a step prior to autotrophic denitrification and denitritation (Sierra-Alvarez et al. 2007). In a recent study, a mechanistic model accounting for $NO_2$ accumulation, biomass growth and $S^0$ hydrolysis has been proposed (Kostrytsia et al. 2018). As demonstrated through numerical simulations, the developed model could serve as a tool to predict the performance of autotrophic denitrification biofilm systems and assess their process efficiency when compared to other denitrification systems. In the present work, the model developed by Kostrytsia et al. (2018) was subjected to a sensitivity analysis to verify the model stability as well as identify the model parameters to be further optimized. Specifically, the focus was to use a local sensitivity analysis to better understand the dominant parameters of the process.

**METHODS**

**Mathematical model overview**

A mathematical model was developed by Kostrytsia et al. (2018) to dynamically simulate the main processes occurring during the two-step denitrification with $S^0$ ($S_1$). The model takes into account the activities of a hydrolytic biomass ($X_1$) growing on $S^0$ lentils and an autotrophic denitrifying biomass ($X_2$) using $NO_3$ ($S_3$) or $NO_2$ ($S_4$) as electron acceptor and reducing them to dinitrogen gas ($N_2$) ($S_5$), and evaluates the interactions between $S^0$ hydrolysis and $S^0$-based denitrification and denitritation. $S^0$ uptake was modeled by introducing a new variable, the bioavailable sulfur ($S_2$), which is the soluble compound directly taken up by the denitrifying bacteria for further oxidation to $SO_2^-$ ($S_6$). A modified surface-based kinetic equation was introduced to account for the hydrolysis of $S^0$ (Esposito et al. 2008). The model equations were derived from mass balances and expressed as double-Monod kinetics (Equations (1)–(8)), as reported below or in the matrix in Table 1.

\[
\frac{dS_1}{dt} = - k_1 \frac{S_1}{K_1 + S_1} X_1, \quad (1)
\]

\[
\frac{dS_2}{dt} = k_1 \frac{S_1}{K_1 + S_1} X_1 - \frac{r_1 \mu_{\text{max}}}{Y_{\text{X}1} S_1} K_{S,2} + S_2 \frac{(S_3 - S_3)}{K_{S,2} + (S_3 - S_3) S_3 + S_4} \frac{r_2 \mu_{\text{max}}}{Y_{\text{X}2} S_2} S_2 \frac{(S_4 - S_4)}{(S_4 - S_4) S_4 + S_4} X_2, \quad (2)
\]

\[
\frac{dS_3}{dt} = - \frac{1}{Y_{\text{X}1} S_1} \frac{r_1 \mu_{\text{max}}}{K_{S,2} + S_2} S_2 \frac{(S_3 - S_3)}{(S_3 - S_3) S_3 + S_4} X_2, \quad (3)
\]

\[
\frac{dS_4}{dt} = \frac{1}{Y_{\text{X}2} S_2} \frac{r_2 \mu_{\text{max}}}{K_{S,2} + S_2} K_{S,2} + S_2 \frac{(S_3 - S_3)}{(S_3 - S_3) S_3 + S_4} X_2 \quad (4)
\]

\[
\frac{dS_5}{dt} = \frac{1}{Y_{\text{X}1} S_1} \frac{r_1 \mu_{\text{max}}}{K_{S,2} + S_2} S_2 \frac{(S_4 - S_4)}{(S_4 - S_4) S_4 + S_4} X_2, \quad (5)
\]

\[
\frac{dS_6}{dt} = \frac{1}{Y_{\text{X}2} S_2} \frac{r_2 \mu_{\text{max}}}{K_{S,2} + S_2} K_{S,2} + S_2 \frac{(S_4 - S_4)}{(S_4 - S_4) S_4 + S_4} X_2 \quad (6)
\]

\[
\frac{dX_1}{dt} = K_0 k_1 \frac{S_1}{K_1 + S_1} X_1, \quad (7)
\]

\[
\frac{dX_2}{dt} = \frac{r_1 \mu_{\text{max}}}{K_{S,2} + S_2} S_2 \frac{(S_3 - S_3)}{(S_3 - S_3) S_3 + S_3} X_2 \quad (8)
\]

where $K_0$ denotes the efficiency growth coefficient for hydrolytic biomass; $r_1$ and $r_2$ are the stoichiometric $S_2$ to
S₃ and S₂ to S₄ ratios, respectively; Y₂,₃ and Y₂,₄ represent the denitrifying biomass yield coefficients on NO₃ and NO₂, respectively; a° denotes the mass specific area of the sulfur particles; k₁ denotes the hydrolysis kinetic constant; Kᵢ indicates the volume specific half-saturation constant for S°; μ²,₃ max and μ²,₄ max represent the maximum growth rates for denitrifying biomass on NO₃ and NO₂, respectively; k₉,₁ and k₉,₂ represent the decay constants for X₁ and X₂ biomass, respectively; K₂,₂, K₂,₃ and K₂,₄ denote the half-saturation constants for S°, NO₃ and NO₂, respectively; S° and S°ₐ indicate the lowest NO₂ and NO₃ concentrations that enable metabolic activities of X₂. The obtained ordinary differential equations (ODEs) were integrated by using an original software developed on the MATLAB platform and based on the numerical differentiation formulas.

The general structure of the described model is illustrated in Figure 1. Two main compartments can be individuated: the first one is related to the two-step denitrification kinetic reaction system, the second one represents the microbially catalyzed hydrolysis reaction system. The model parameters have been classified in three main groups: kinetic (μ²,₃ max, μ²,₄ max, k₉,₁, K₂,₂, K₂,₃, K₂,₄, S° and S°ₐ) and stoichiometric (Y₂,₃, Y₂,₄, r₁ and r₂), which directly affect the two-step denitrification kinetic reaction system (‘biological kinetic reaction system’ in Figure 1), and hydrolysis parameters (K₀, a°, k₁, K₁ and k₉,₁) which are related to the S° hydrolysis compartment. K₀ represents the efficiency growth coefficient for X₁; r₁ and r₂ are the stoichiometric S₂ to S₃ and S₂ to S₄ ratios, respectively. The values of Y₂,₃, Y₂,₄, K₂,₂, K₂,₃, r₁, r₂, k₉,₁ and k₉,₂ were adopted from previous studies (Sierra-Alvarez et al. 2007; Sin et al. 2008; Liu et al. 2016; Xu et al. 2016) (Table 2). The optimal values of μ²,₃ max and μ²,₄ max were deduced from both the denitrification and denitritation experiments (Table 2).

**Sensitivity analysis**

The model proposed by Kostrytsia et al. (2018) requires a high dimensional (stoichiometric, kinetic and hydrolysis-related) parameter space to be explored. To estimate each parameter, excess experimental results should be provided to avoid ill-conditioning of the parameter estimation (Kesavan & Law 2005). Therefore, a model sensitivity analysis was performed to identify the parameter targets for further experimental exploration (Jarrett et al. 2015). The low-sensitive parameters have a negligible effect on the predictions, whereas the highly sensitive parameters require some level of certainty to make robust model predictions (Croicu et al. 2017).

In this model, a local sensitivity analysis was performed to compute sensitivity functions for the dynamic simulations with the initial conditions of 30 mg/l NO₂-N and 210 mg/l NO₃-N, originating from the kinetic experiments (Kostrytsia et al. 2018). The stoichiometric ratios (r₁ and r₂) were
calculated on the biotransformation mechanism and obtained by previous experimental studies (Sierra-Alvarez et al. 2007). Thus, $r_1$ and $r_2$ were not re-estimated in the current study. Additionally, the influence of the biomass decay rates ($k_{d,1}$ and $k_{d,2}$) of the microorganisms performing S$^0$-driven denitrification and denitrification was out of investigation, as $k_{d,1}$ and $k_{d,2}$ were very low (Liu et al. 2016). Also, the threshold values for NO$_3$ and NO$_2$ ($S_3^\ast$ and $S_4^\ast$, 

Table 2 | Stoichiometric and kinetic parameters (with nominal values) of the developed model for two-step autotrophic denitrification with S$^0$ (adopted from Kostrytsia et al. 2018)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoichiometric parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Y_{2,3}$ Yield coefficient for $X_2$ on $S_3$</td>
<td>0.25</td>
<td>mg VS/mg N</td>
<td>Xu et al. (2016)</td>
</tr>
<tr>
<td>$Y_{2,4}$ Yield coefficient for $X_2$ on $S_4$</td>
<td>0.28</td>
<td>mg VS/mg N</td>
<td>Xu et al. (2016)</td>
</tr>
<tr>
<td>$r_1$ $S_2$ to $S_3$ stoichiometric ratio</td>
<td>1.2</td>
<td>mg S/mg N</td>
<td>Sierra-Alvarez et al. (2007)</td>
</tr>
<tr>
<td>$r_2$ $S_2$ to $S_4$ stoichiometric ratio</td>
<td>0.55</td>
<td>mg S/mg N</td>
<td>Sierra-Alvarez et al. (2007)</td>
</tr>
<tr>
<td>Kinetic parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{0}$ Efficiency growth coefficient for $X_1$</td>
<td>0.1</td>
<td>mg VS/mg S</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$\mu_{2,3}^{max}$ Maximum growth rate for $X_2$ on $S_3$</td>
<td>0.0067</td>
<td>1/d</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$\mu_{2,4}^{max}$ Maximum growth rate for $X_2$ on $S_4$</td>
<td>0.0058</td>
<td>1/d</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$K_{2,2}$ Half-saturation constant for $S_2$</td>
<td>0.215</td>
<td>mg S/l</td>
<td>Liu et al. (2016)</td>
</tr>
<tr>
<td>$K_{2,3}$ Half-saturation constant for $S_3$</td>
<td>36</td>
<td>mg N/l</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$S_3^\ast$ The threshold value for $S_3$</td>
<td>35</td>
<td>mg N/l</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$K_{2,4}$ Half-saturation constant for $S_4$</td>
<td>40</td>
<td>mg N/l</td>
<td>Xu et al. (2016)</td>
</tr>
<tr>
<td>$S_4^\ast$ The threshold value for $S_4$</td>
<td>37</td>
<td>mg N/l</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$K_1$ Volume specific half-saturation constant for $S_1$</td>
<td>5.1</td>
<td>1/dm</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$k_1$ Hydrolysis kinetic constant</td>
<td>0.12</td>
<td>mg S/mg VS d</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$a^\ast$ Mass specific area</td>
<td>0.0008164</td>
<td>dm$^2$/mg</td>
<td>Kostrytsia et al. (2018)</td>
</tr>
<tr>
<td>$k_{d,1}$ Decay rate coefficient for $X_1$</td>
<td>0.0006</td>
<td>1/d</td>
<td>Sin et al. (2008)</td>
</tr>
<tr>
<td>$k_{d,2}$ Decay rate coefficient for $X_2$</td>
<td>0.0006</td>
<td>1/d</td>
<td>Sin et al. (2008)</td>
</tr>
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</table>

VS: volatile solids.
respectively), which represent the concentration values below which the microorganisms are not able to grow, were not considered for the sensitivity analysis. Therefore, the sensitivity analysis was conducted for the following 11 parameters: $\mu_{23}^{\text{max}}$, $\mu_{24}^{\text{max}}$, $K_{2,2}$, $K_{2,3}$ and $K_{2,4}$ (kinetic), $Y_{2,3}$ and $Y_{2,4}$ (stoichiometric) and $K_0$, $a^*$, $k_1$ and $K_1$ (hydrolysis-related). NO$_3$-N, NO$_2$-N and SO$_4^{2-}$-S concentrations were set as the focused variables to measure the sensitivity.

The sensitivities were calculated as the effect of the change in the input parameters on the model output over a time span of 22 days. An automatic differentiation tool SENS_SYS coupled with the ODE solver of MATLAB was used to predict the local sensitivity. The SENS_SYS tool is an extension of the ODE15s tool that allows the solving of the ODE system while computing derivatives (sensitivities) of the solution with respect to parameters (Molla & Padilla 2002). The accuracy of the SENS_SYS tool is controlled by the default relative tolerance of $1 \times 10^{-6}$. The sensitivity analysis of the system $F$ was calculated by differentiating the system with respect to the kinetic parameter $u$, as illustrated in Equation (9).

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

RESULTS AND DISCUSSION

Parameter sensitivity overview on NO$_3$-N, NO$_2$-N and SO$_4^{2-}$-S concentrations

The absolute sensitivities of the 11 parameters ($K_0$, $Y_{2,3}$, $Y_{2,4}$, $a^*$, $k_1$, $\mu_{23}^{\text{max}}$, $\mu_{24}^{\text{max}}$, $K_{2,2}$, $K_{2,3}$ and $K_{2,4}$) to the input state variables (i.e. 210, 0 and 0 mg/L of NO$_3$-N, NO$_2$-N and SO$_4^{2-}$-S, respectively) are shown in Figure 2. The nominal values of the parameters used for the model (Equations (1)–(8)) are listed in Table 2.

The $Y_{2,3}$, $Y_{2,4}$, $a^*$, $k_1$, $\mu_{23}^{\text{max}}$, $\mu_{24}^{\text{max}}$ and $K_{2,2}$ parameters were sensitive to some extent to at least one of the model outputs, i.e. NO$_3$-N, NO$_2$-N and SO$_4^{2-}$-S (Figure 2). Both NO$_3$-N and NO$_2$-N process variables were highly sensitive to $a^*$, $\mu_{23}^{\text{max}}$ and $\mu_{24}^{\text{max}}$. It is noteworthy to highlight that a negative value of the absolute sensitivity refers to the reduction of the process variables with parameter perturbation. For example, the

Figure 2 | Absolute (or local) sensitivities of kinetic parameters during the simulation time for the degradation of (a) NO$_3$-N and (b) NO$_2$-N and the production of (c) SO$_4^{2-}$-S.
negative value of $\mu_{2,3}^{\text{max}}$ sensitivity for NO$_3$-N indicated NO$_3$-N consumption (Figure 2(a)), while the positive value of $\mu_{2,3}^{\text{max}}$ sensitivity for NO$_2$-N corresponded to NO$_2$-N production (Figure 3(a)). The NO$_3$ and NO$_2$ reduction was coupled to $S^0$ biooxidation in order to produce energy and facilitate microbial growth. Therefore, the use of NO$_3$ or NO$_2$ as a substrate for the microbial cultures resulted in a high sensitivity of the $\mu_{2,3}^{\text{max}}$ and $\mu_{2,3}^{\text{max}}$ to the model outputs (Figure 2).

The sensitivity analysis results suggest that the parameter vector can be reduced to $a^*$, $k_1$, $\mu_{2,3}^{\text{max}}$ and $\mu_{2,4}^{\text{max}}$, based on the minimum magnitude of significance considered (200 as absolute sensitivity). The parameters deemed not to be sufficiently significant can be fixed at their nominal values (Table 2). Further investigation might be related to such a reduced model both in terms of assumed parameter distribution and experimental calibration. Further calibration is required for the most sensitive model parameters to improve the quality of the model. However, the values of absolute sensitivity for $K_0$, $K_1$, $K_{2,3}$ and $K_{2,4}$ for each process variable were significantly lower than those of $Y_{2,3}$, $Y_{2,4}$, $a^*$, $k_1$, $\mu_{2,3}^{\text{max}}$, $\mu_{2,4}^{\text{max}}$ and $K_{2,2}$. The effect of each sensitive parameter on the process variables should be investigated in more detail due to their crucial role in the model calibration.

### Sensitivity analysis for kinetic ($\mu_{2,3}^{\text{max}}$, $\mu_{2,4}^{\text{max}}$, $K_{2,2}$, $K_{2,3}$ and $K_{2,4}$) and stoichiometric ($Y_{2,3}$ and $Y_{2,4}$) model parameters

To investigate the effect of each parameter on the process variables, a series of sensitivity curves was obtained by changing the five kinetic ($\mu_{2,3}^{\text{max}}$, $\mu_{2,4}^{\text{max}}$, $K_{2,2}$, $K_{2,3}$ and $K_{2,4}$), and the two stoichiometric ($Y_{2,3}$ and $Y_{2,4}$) parameters one by one during the simulation. The effect of each parameter on the input state variables NO$_3$-N, NO$_2$-N and SO$_4^{2-}$-S is illustrated in Figure 3. The greater parameter line slope indicates the more significant role of the parameter in the autotrophic denitrification process.

The most sensitive kinetic parameters were the maximum growth rate of the denitrifying biomass on NO$_3$ ($\mu_{2,3}^{\text{max}}$) and NO$_2$ ($\mu_{2,4}^{\text{max}}$) as illustrated in Figures 2(a)–2(c) and 3(d)–3(e), with a more significant effect on NO$_3$-N from day 10 to day 15 when the denitrification rate was higher (Kostrytsia et al. 2018). The SO$_4^{2-}$-S absolute sensitivity was 10,000 and 7,500 for $\mu_{2,3}^{\text{max}}$ and $\mu_{2,4}^{\text{max}}$ (Figure 2), respectively. This was likely attributed to the higher metabolic rates, in particular $S^0$ oxidation to SO$_4^{2-}$, of the denitrifying bacteria growing on NO$_3^-$ ($\mu_{2,3}^{\text{max}}$) rather than on NO$_2$. On the other hand, the half-saturation constants for $S_2$ ($K_{2,2}$), $S_3$ ($K_{2,3}$) and $S_4$ ($K_{2,4}$) had a minimal impact on the model outputs (Figure 3(c), 3(f) and 3(g)). A larger set of experimental data would be required to get an accurate evaluation of the half-saturation constants. More data could be obtained from kinetic tests with different initial substrate concentrations for a reliable model calibration of the half-saturation constants.

Regarding the stoichiometric parameters, the denitrifying biomass yield coefficient on NO$_3$ ($Y_{2,3}$) showed a high sensitivity for NO$_3$-N, NO$_2$-N and SO$_4^{2-}$-S outputs (Figure 3(a)). The highest sensitivity of the $Y_{2,3}$ was observed between days 10 and 15 due to the higher denitrification rate.

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**Figure 3** | Output absolute (or local) sensitivity of kinetic and stoichiometric parameters: (a) $Y_{2,3}$, (b) $Y_{2,4}$, (c) $K_{2,3}$, (d) $\mu_{2,3}^{\text{max}}$, (e) $\mu_{2,4}^{\text{max}}$, (f) $K_{2,2}$ and (g) $K_{2,4}$.
The effect of \( Y_{2.4} \) sensitivity on the NO\(_2\)-N and SO\(_4^{2-}\)-S outputs increased with time due to the accumulation of NO\(_2\)-N as an intermediate product of the NO\(_3\)-N degradation.

**Sensitivity analysis for hydrolysis-related \((K_0, a^*, k_1, \text{and } K_1)\) model parameters**

The effect of hydrolysis-related model parameters \((K_0, a^*, k_1, \text{and } K_1)\) on the input state variables NO\(_3\)-N, NO\(_2\)-N and SO\(_4^{2-}\)-S is illustrated in Figure 4. The mass specific area of the sulfur particles \((a^*)\) posed, apparently, a major influence on the model outputs and was ranked as a first dominant parameter (Figure 2). The parameter \(a^*\) accounts for the overall surface area of the sulfur particles to be microbially solubilized prior to denitrification and denitritation. As illustrated in Figure 4(b), the NO\(_3\)-N output was more sensitive to the change in parameter \(a^*\), compared to NO\(_2\)-N. The latter might be attributed to the higher stoichiometric S/N ratio required for complete denitrification than for denitritation. This is consistent with the literature, where the impact of the specific surface area of sulfur particles was suggested as a prerequisite of S\(^0\) oxidation coupled to denitrification (Wang et al. 2016). The model proposed by Kostrytsia et al. (2018) describes the surface-based S\(^0\) hydrolysis as an inevitable aspect and rate-limiting step in the denitrification and denitritation processes, and the high sensitivity of parameter \(a^*\) to the model outputs confirmed the significance of the used hydrolysis approach.

Among the other parameters related to the S\(^0\) hydrolysis step, the hydrolysis kinetic constant \((k_1)\), being dependent on the nature of sulfur, possessed a high sensitivity for the model output variables (Figure 4(c)). The absolute sensitivity of both parameters \(a^*\) and \(k_1\) showed a peak between days 5 and 10 due to the high denitrification rate, and then slowly dropped (Figure 4(b) and 4(c), respectively). Consequently, the model predictions and calibration 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 S\(^0\) \((K_1)\) did not significantly influence the model outputs (Figure 4(a) and 4(d), respectively).

**CONCLUSIONS**

In this work, the results of a local sensitivity analysis performed on a newly developed model for microbially-catalyzed elemental S\(^0\) hydrolysis and two-step denitrification were presented. The sensitivity analysis provided a few insights on the importance of parameter values and their impact on process dynamics. The sensitivity analysis demonstrated that the model was more sensitive to the mass specific area of the sulfur particles \((a^*)\), hydrolysis kinetic constant \((k_1)\) and the maximum growth rate of the denitrifying biomass on NO\(_3\) \((\mu_{2.3}^{\text{max}})\) and NO\(_2\) \((\mu_{2.4}^{\text{max}})\). The high sensitivity of hydrolysis-related parameters \((a^*\) and \(k_1)\) to the input state variables \((\text{NO}_3\text{-N, NO}_2\text{-N and SO}_4^{2-}\text{-S})\) confirmed the importance of including the microbially catalyzed surface-based S\(^0\) hydrolysis as a limiting process step in the two-step denitrification model. Further experimental and modeling investigations should thus focus on the S\(^0\) hydrolysis.

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NOTES

The authors have no competing interests to declare.

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