This is an Accepted Manuscript of an article published by Taylor & Francis in Journal of Environmental Science and Health, Part A on 2020, available online:

<http://www.tandfonline.com/> with DOI <https://doi.org/10.1080/10934529.2020.1852011> .

Modeling of the effect of zeolite concentration on the biological nitrification process in the presence of sulfide and organic matter

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Abstract

The evaluation of the nitrification kinetics in the simultaneous presence of sulfide and organic matter using zeolite as improver was the main goal of this work. According to the sensitivity and collinearity analyses, five parameters were the most sensitive in the model, whose calibrated values were: $\mu_{max, AOB} = 0.02642 \pm 0.002 \text{ h}^{-1}$; $\mu_{max, NOB} = 0.3307 \pm 0.416 \text{ h}^{-1}$; $K_{S, NOB} = 1.65 \cdot 10^{-6} \pm 2.85 \cdot 10^{-6} \text{ mgHNO}_2\text{-N/L}$; $k_{S2} = 0.8213 \pm 0.076$, and $n = 0.6537 \pm 0.030$.

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A good fit between the experimental data and the model's results including the effect of zeolite on the kinetic parameters was obtained, with Theil inequality coefficient values between 0.109 and 0.007 for all the variables studied, with all of these values lower than 0.3. Thus, the model proposed is robust and can simulate the nitrification process in the presence of sulfide and organic matter when zeolite was used as improver.

KEYWORDS: kinetic parameters, modeling, nitrification kinetics, zeolite.

Nomenclature

c_{TAN}	: Total ammonia nitrogen concentration NH_3 -N+ NH_4^+ -N, mg N L ⁻¹
$c_{NO_2^-}$	: Total nitrite nitrogen concentration HNO_2 -N+ NO_2^- -N, mg N L ⁻¹
$c_{NO_3^-}$	: Total nitrate nitrogen concentration HNO_3 -N+ NO_3^- -N, mg N L ⁻¹
c_{NH_3}	: Concentration of NH_3 -N, mg N L ⁻¹
c_{HNO_2}	: Concentration of HNO_2 -N, mg N L ⁻¹
c_{DO}	: Concentration of dissolved oxygen, mg O ₂ L ⁻¹
c_{H_2S}	: Concentration of sulfide, mg S L ⁻¹
c_{COD}	: Concentration of organic matter as chemical oxygen demand (COD), mg L ⁻¹ :
x_{AOB}	: Concentration of ammonia-oxidizing bacteria (AOB), mg VSS L ⁻¹
x_{NOB}	: Concentration of nitrite-oxidizing bacteria (NOB), mg VSS L ⁻¹
x_H	: Concentration of heterotrophic bacteria (HB), mg VSS L ⁻¹
k_d	: Constant of death for nitrifying bacteria, h ⁻¹
$\mu_{max,i}$	: Maximum specific growth rate of i : AOB, NOB, HB, h ⁻¹
μ_i	: Specific growth rate of i : AOB, NOB, HB, h ⁻¹
$K_{s,AOB}$	: Saturation constant for ammonia-oxidizers, mg NH_3 -N L ⁻¹
$K_{s,NOB}$	: Saturation constant for nitrite-oxidizers, mg HNO_2 -N L ⁻¹
$K_{S,COD}$	: Saturation constant for chemical oxygen demand, mg COD L ⁻¹

$K_{IS,AOB}$	: Substrate inhibition for ammonia-oxidizers, mg NH ₃ -N L ⁻¹
$K_{IS,NOB}$	: Substrate inhibition for nitrite-oxidizers, mg HNO ₂ -N L ⁻¹
$K_{DO,i}$	: Saturation constant of oxygen concentration for <i>i</i> : AOB, NOB, HB, mg O ₂ L ⁻¹
$K_{I,S,AOB}$	: Noncompetitive inhibition constant for AOB, mg HS ⁻ -S L ⁻¹
$K_{I,S,NOB}$	: Noncompetitive inhibition constant for NOB, mg HS ⁻ -S L ⁻¹
$K_{ISu,NOB}$	: Competitive inhibition constant for NOB, mg HS ⁻ -S L ⁻¹
$K_{d,NH3}$	: Equilibrium constant of NH ₃
$K_{d,HNO2}$	: Equilibrium constant of HNO ₂
$Y_{AOB,TAN}$	: Yield coefficient of ammonia-oxidizing bacteria over to TAN, mg VSS/mg TAN
$Y_{NOB,NO2-}$	: Yield coefficient of nitrite-oxidizing bacteria over to NO ₂ ⁻ , mg VSS/mg NO ₂ ⁻
$Y_{H,COD}$	: Yield coefficient of heterotrophic bacteria over to COD, mg VSS/mg COD
$f_{NO2-/TAN}$	: Stoichiometric coefficient of component NO ₂ ⁻ over TAN, mgN/mgN
$f_{NO3-/NO2-}$	: Stoichiometric coefficient of component NO ₃ ⁻ over NO ₂ ⁻ , mgN/mgN
k_{S2}	: Kinetic constant for sulfide oxidation under abiotic conditions, dimensionless
n	: order constant, dimensionless
TAN	: Total ammonia nitrogen
T	: Temperature, °C
t	: Time, h

Introduction

Nitrification is a well-known biological process for oxidizing ammonia coming from industrial and municipal wastewaters. It is a two-step reaction: first, the ammonium is oxidized to nitrite by ammonium-oxidizing bacteria (AOB), and then nitrite is oxidized to nitrate by nitrite-oxidizing bacteria (NOB). ^[1-3] These processes are sensitive to inhibitors, including sulfide and organic matter. ^[4] Such compounds are present in many kinds of wastewaters together with ammonia; however, there are few studies of the nitrification

processes in the presence of both sulfide and organic matter simultaneously, those studies basically using organic matter coming from petrochemical wastewater. ^[5, 6] On the other hand, the kinetics of nitrification in the simultaneous presence of sulfide and organic has not been proposed up to date. There are studies which reported the single effect of sulfide on nitrification ^[7-9] and also several studies about nitrification in the presence of organic matter. ^[10, 11] However, the evaluation of a kinetic model involving the simultaneous effects of sulfide and organic matter has not been proposed and has never been reported in the literature. Therefore, the evaluation of nitrification kinetics in the presence of sulfide and organic matter simultaneously is one of the main goals of this work.

Nitrification inhibition due to the presence of both organic matter and sulfide could be avoided with the use of improvers of the process. “Improvers can reduce the effect of inhibition of some compounds on biological processes either by acting as a direct antagonist ^[12] or by delaying the effect of inhibition and helping the system to recover after being inhibited. They can also cause the system to increase resistance to toxicity from these compounds or decrease the time required to stabilize the systems. For instance, Chen *et al.* ^[12] indicated that nickel can be used for decreasing the inhibition caused by cadmium and copper in anaerobic digestion processes and that sodium (Na^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) can be used as antagonists of ammonium inhibition of the anaerobic digestion process. In the case of nitrification, Young *et al.* ^[13] used activated carbon improvers for nitrification in order to decrease the inhibition by phenol and free cyanide on the nitrification process. In this sense, zeolite is another improver that has been used in many biological processes such as: anaerobic digestion, nitrification, heterotrophic autotrophic denitrification ^[14-19]. In these processes, the use of zeolite improves the biological performance based on its ability to retain ammonia and form biofilm, in addition to its high porosity and large specific surface area ^[20]. Zeolite has the ability to adsorb molecules with a cross-sectional diameter

suitable for its pore size, which have small diameters (3 to 10 Å) and are capable of exchanging their cations without significantly changing their structure ^[21].”

Recently, Huiliñir *et al.* ^[4] proposed the use of natural zeolite for improving nitrification in discontinuous systems in the presence of sulfide and organic matter. Zeolite is a crystalline, hydrated aluminosilicate of alkali and alkaline earth cations, consisting of three-dimensional-frameworks of SiO_4^{4-} and AlO_4^{5-} tetrahedrally linked through shared oxygen atoms. ^[14, 15] In the presence of zeolite, Huiliñir *et al.* ^[4] obtained a faster TAN removal rate and higher nitrate production compared to controls (systems without zeolite), because the inhibitory effect of both sulfur and organic matter in the nitrification process was reduced by 53% when zeolite was used as improver. Although the positive effect of zeolite on nitrification has been observed, a kinetic analysis of its effects through modelling of the process has not been presented until now. The use of modelling can elucidate which process could be stimulated or inhibited by zeolite, providing support in decision making for plant operation or developing new control schemes. ^[22]

According to these backgrounds, there is still a need to determine the kinetics of nitrification in the presence of sulfide and organic matter using an improver such as natural zeolite. Therefore, the main goal of this study was to determine and propose a kinetic model that incorporates explicitly the effect of zeolite on the nitrification process. The kinetic model was analyzed through sensitivity and collinearity analysis of the parameters, and this proposed model was then calibrated. According to Kostrysia *et al.* ^[23], the sensitivity analysis allows to verify the stability of the model and identify which parameters must be optimized. To the best of our knowledge, this is the first time that the effect of zeolite as improver is incorporated explicitly in the kinetics of the nitrification process in the presence of both sulfide and organic compounds.

Material and methods

Kinetic Model

The proposed kinetic model involves the following steps or processes: Total ammonia nitrogen (TAN) degradation by ammonium-oxidizing biomass (AOB), nitrite degradation by nitrite-oxidizing biomass (NOB), and COD removal by heterotrophic biomass (HB). Because of these degradation processes, the corresponding specific growth rates of all the biomass species (AOB, NOB and HB) involved were taken into account and included in the model. Furthermore, the inhibition of TAN and nitrite oxidation by sulfide was also included based on the work of Bejarano *et al.* ^[7] i.e. a non-competitive inhibition of sulfide on AOB and both competitive and noncompetitive inhibition of NOB. According to Huiliñir *et al.* ^[4] sulfide was mainly oxidized by chemical reaction. Thus, the chemical oxidation of sulfide was also added. Therefore, the model included three biological species (AOB, NOB and HB) and 5 soluble species: TAN, nitrate (NO_3^-), nitrite (NO_2^-), organic matter (COD) and sulfide (H_2S). It is important to note that oxygen was not included as a state variable in the model because according to previous experimental results, ^[4] the oxygen concentration was always greater than 2 mg/L, and was therefore not a limiting substrate.

According to these assumptions, the specific growth rates of AOB and NOB can be expressed as follows: ^[7, 24]

$$\mu_{AOB} = \mu_{max,AOB} \cdot \frac{c_{NH_3}}{K_{S,AOB} + c_{NH_3} + \frac{c_{NH_3}^2}{K_{IS,AOB}}} \cdot \frac{K_{I,S,AOB}}{c_{H_2S} + K_{I,S,AOB}} \quad (1)$$

$$\mu_{NOB} = \mu_{max,NOB} \cdot \frac{c_{HNO_2}}{K_{S,NOB} \cdot \left[1 + \frac{c_{H_2S}}{K_{ISu,NOB}} \right] + c_{HNO_2} + \frac{c_{HNO_2}^2}{K_{IS,NOB}}} \cdot \frac{K_{I,S,NOB}}{c_{H_2S} + K_{I,S,NOB}} \quad (2)$$

It is important to note that specific growth rates are in terms of only no ionized species. Thus, $\text{NH}_4^+/\text{NH}_3$ and $\text{NO}_2^-/\text{HNO}_2$ equilibria as a function of pH and temperature were also included in the kinetic model. The concentration of NH_3 and HNO_2 are related with TAN concentration and nitrite concentration by equations (3) and (4), respectively: ^[25]

$$c_{\text{NH}_3} = \frac{c_{\text{TAN}}}{1 + \frac{10^{-\text{pH}}}{K_{d,\text{NH}_3}}} \quad (3)$$

$$c_{\text{HNO}_2} = \frac{c_{\text{NO}_2^-}}{1 + 10^{\text{pH}} \cdot K_{d,\text{HNO}_2}} \quad (4)$$

where:

$$K_{d,\text{NH}_3} = e^{-\frac{6344}{(T+273)}} \quad (5)$$

$$K_{d,\text{HNO}_2} = e^{-\frac{2300}{(T+273)}} \quad (6)$$

The specific growth rate of HB was expressed as:

$$\mu_H = \mu_{\text{max},H} \cdot \frac{c_{\text{COD}}}{K_{S,\text{COD}} + c_{\text{COD}}} \quad (7)$$

On the other hand, equations (8) to (16) present the mass balances in a batch system:

- Total ammonia nitrogen (TAN):

$$\frac{dc_{\text{TAN}}}{dt} = -\frac{\mu_{\text{AOB}}}{Y_{\text{AOB}/\text{TAN}}} \cdot x_{\text{AOB}} \quad (8)$$

- Total nitrite nitrogen (NO_2^-):

$$\frac{dc_{\text{NO}_2^-}}{dt} = \frac{\mu_{\text{AOB}}}{Y_{\text{AOB}/\text{TAN}}} \cdot x_{\text{AOB}} \cdot f_{\text{NO}_2^-/\text{TAN}} - \frac{\mu_{\text{NOB}}}{Y_{\frac{\text{NOB}}{\text{NO}_2^-}}} \cdot x_{\text{NOB}} \quad (9)$$

- Total nitrate nitrogen (NO_3^-):

$$\frac{dc_{\text{NO}_3^-}}{dt} = \frac{\mu_{\text{NOB}}}{Y_{\frac{\text{NOB}}{\text{NO}_2^-}}} \cdot x_{\text{NOB}} \cdot f_{\text{NO}_3^-/\text{NO}_2^-} \quad (10)$$

- Organic matter as chemical oxidation demand (COD):

$$\frac{dc_{COD}}{dt} = -\frac{\mu_H}{Y_{H/COD}} \cdot x_H \quad (11)$$

- AOB:

$$\frac{dx_{AOB}}{dt} = (\mu_{AOB} - k_d) \cdot x_{AOB} \quad (12)$$

- NOB

$$\frac{dx_{NOB}}{dt} = (\mu_{NOB} - k_d) \cdot x_{NOB} \quad (13)$$

- HB

$$\frac{dx_H}{dt} = (\mu_H - k_d) \cdot x_H \quad (14)$$

- Sulfide: “The kinetic of sulfide oxidation was assumed as chemical reaction, support by our own experiments (see supplementary data) and other researchers ^[4]. The sulfate was not considered because it does not affect the nitrification process.” According to Mora *et al.* ^[26] sulfide oxidation under abiotic conditions can be represented by a *n*th-order kinetics:

$$\frac{dc_{H_2S}}{dt} = -k_{H_2S} \cdot (c_{H_2S})^n \quad (15)$$

where k_{H_2S} is the kinetic constant for sulfide oxidation under abiotic conditions and n is an order constant (dimensionless).

Model implementation

Model implementation, parameter identification, and calibration were carried out by a systematic procedure in order to obtain reliable model response and parameter uncertainty, ^[27] based on available experimental data (Figure 1).

The first step of the systematic procedure consists of a local sensitivity analysis, which was carried out using the method of Brun *et al.* ^[28] The sensitivity analysis allows distinguishing the most sensitive parameters affecting the state variables (model outputs). According to Brun *et al.*, ^[28] the sensitivity of the model's output variable y_i to small changes in the parameter values p_j was considered. The sensitivity matrix is determined in equation 16, where every column v_j , $j = 1, 2, \dots, m$, represents the change in the model's outcome vector y_i caused by a small change in p_j (Equation 18). The scaled sensitivity matrix (Equation 17) proposed by Brun *et al.* ^[28] to obtain sensitivity without dimensions was also considered.

$$V = \frac{\partial y_i}{\partial p_j} \quad (16)$$

$$s_{ij} = v_{ij} \frac{p_j}{y_i} \quad (17)$$

Where v_{ij} denotes an element of V ,

p_j : a priori measure of the reasonable parameter range,

y_i : scale factor with the same physical dimension as the corresponding observation (model output), accounting mainly for different scales of different output signals.

To obtain an order of importance of the parameters the three summaries proposed by Brun *et al.* ^[28] were calculated.

$$\delta_j^{msqr} = \sqrt{\frac{1}{n} \sum_{i=1}^n s_{ij}^2} \quad (18)$$

$$\delta_j^{mabs} = \frac{1}{n} \sum_{i=1}^n |s_{ij}| \quad (19)$$

$$\delta_j^{mean} = \frac{1}{n} \sum_{i=1}^n s_{ij} \quad (20)$$

According to the sensitivity analysis, the parameters with the largest sensitivity indices (mean, mabs, and mean) are selected, but there may be linear dependence on them. Because of it, a collinearity analysis was used. This was the second step of the procedure in order to detect the parameters with less linear dependence and/or lower eigenvalues based on their local sensitivity profile. ^[28] The collinearity index (γ_k) can be determined as follows:

$$\gamma_k = \frac{1}{\sqrt{\lambda_k}} \quad (21)$$

with λ_k being the smallest eigenvalue of $\widetilde{S_K^T} S_K$

Once more-sensitive parameters were obtained, calibration with experimental data was performed. The initial values of the parameters were taken from the literature in order to start from a feasible real initial state point (Table 1). The adjustment was performed via a minimization of the NMSE error function presented in Eq. 22:

$$NMSE = \sqrt{\sum_{i=1}^N \frac{((y_i - y_{i,m})/(y_{i,o} - y_i))^2}{N}} \quad (22)$$

where y_i represents the concentration of the species measured experimentally, $y_{i,m}$ represents the model's calculated results, $y_{i,o}$ represents the species' inlet concentration, and N represents the amount of data. The error function was minimized with the Nonlinear Least squares (*lsqcurvefit* function in Matlab), and a numerical optimization method (*optimset* function in Matlab) determined the model's optimal parameters. Both calculation procedures were implemented using the Matlab R2014a. The numerical method used to solve the system of ordinary differential equations was the Gear method, which is included in Matlab R2014a for resolving stiff type equations. ^[29]

The Theil inequality coefficient, TIC ^[30] was used as the criterion to compare the results of both models as suggested by Hvala *et al.* ^[31] The value of TIC varies between 0 and 1, where values closer to zero indicate a better model validity. The TIC is given by:

$$TIC = \frac{\sqrt{\sum_i (y_i - y_{m,i})^2}}{\sqrt{\sum_i y_i^2} + \sqrt{\sum_i y_{m,i}^2}} \quad (23)$$

where y_i represents the experimentally measured point and $y_{m,i}$ represents the model's prediction of the same point. The value of TIC lies between zero and one, and TIC values below 0.3 indicate good agreement with measured data. ^[31]

To determine the confidence interval or degree of uncertainty of the parameters, the bootstrap technique ^[32] was applied, with a 95% confidence. Finally, the randomization analysis (residual error distribution) was performed using Statgraphics Centurion XVII.

The main parameters used in the model are listed in Table 1.

The initial biomass fractions used to solve the model were 0.67 and 0.33 for the active biomass and inert biomass, respectively. The active biomass (0.67 in fraction) was divided into nitrifying biomass (0.6) and heterotrophic biomass (0.07). ^[33] Finally, the nitrifying biomass was divided into 0.77 for AOB and 0.23 for NOB. ^[34] Therefore, the initial fractions of AOB, NOB, and HB were 0.462, 0.138, and 0.07, respectively. The model does not incorporate the generation of inert biomass.

Experimental data

The experimental data for calibration of the model were obtained from Huiliñir *et al.* ^[4] Data of TAN, nitrite, nitrate, sulfide, sulfate, volatile suspended solids, and COD were used for this task. All the tested conditions and systems, as well as the experimental setup used, were described in detail in the mentioned paper. It is important to note that Huiliñir *et al.* ^[4] indicated that in these experiments there was not biofilm formation on the zeolite. Therefore,

the transport phenomena associated to biofilms (e.g., mass transfer) was not analyzed in this work, focusing mainly in the kinetic without mass transfer effect.

For the case of chemical sulfide oxidation, new experiments in abiotic conditions were performed in order to obtain the kinetic parameters. For this reason, the possibility of biological oxidation of sulfide was neglected.

. The experimental setup was the same presented by Huiliñir *et al.* ^[4] Summarizing, 8 reactors of 300 mL each were used, with a liquid volume of 200 mL of synthetic wastewater. These bioreactors were sealed with rubber stoppers. All the assays were carried out at 25 ± 2 °C, and the dissolved oxygen concentration was kept at 4.0 ± 0.5 mg/L by continuous aeration. The air was injected through the bottom of the reactor using a porous ceramic diffuser that also kept the agitation inside the bioreactors. “Furthermore, the possible stripping of sulfide was neglected because the airflow rate used in all the experiments were small values, in order to prevent the stripping. Furthermore, the production of sulfate agreed with the sulfide oxidized, accomplishing the mass balance”.

Five sulfide concentrations were used: 40, 60, 80, 100 and 160 mg/L. In the kinetic assays, the sulfide concentration was measured every hour. Profiles obtained for each sulfide concentration are shown in the supplementary data (Figure S1). In order to determine n and k_{S2} , for each sulfide concentration, the maximum slope of the sulfide concentration vs. time plot was determined using Excel 2010 software. Then, each maximum slope was plotted against sulfide concentration. The values of the n and k_{S2} parameters shown in Table 1 were determined by minimizing the error between the experimental maximum slope and the slope obtained with the kinetic model (Equation 15) through the *solver analysis* tool, included in Excel 2010 software. Figure 2 shows the results of the experimental slope and the model slopes, presenting a good fit with the model.

Results and discussions

Sensitivity analysis

Tables 2 and 3 show the results of the parameter importance screening. Table 2 shows the results of the parameter ranking by varying the parameters (+50%).

Table 3 shows the results of the parameter ranking by varying the parameters (-50%).

The sensitivity threshold was set at 10% of the maximum $\delta msqr$ value calculated over all parameters according to Brun *et al.* [28] With this value, 11 and 10 parameters were determined as insensitive, as shown in Table 2 and Table 3, respectively. The values of $\delta msqr$ reflect the overall sensitivity of a parameter j and allow identifying the order of importance of the parameters when the set of parameters to analyze is very large, as in this case. According to Brun *et al.* [28] a comparison of $\delta mabs$ and $\delta mean$ shows whether all the elements of sensitivity have the same sign, and $\delta mean$ gives information on the sign of the averaged effect that a change in a parameter has on the model output. According to this, Table 2 and Table 3 show that only in the case of $K_{I,S,NOB}$ (both tables) and $\mu_{max,NOB}$ (Table 3) all the elements of sensitivity have the same sign, indicating that these parameters increased the value of the model output (according to sensitive parameters).

The results of the sensitivity analysis lead to establish the preliminary set of potential identifiable parameters: n , k_{S2} , $\mu_{max,AOB}$, $\mu_{max,NOB}$, $K_{I,S,AOB}$, $K_{I,S,NOB}$. This set presents the high sensitivity and presumably reflects higher model output perturbation.

Collinearity analysis

Results obtained from sensitivity analysis reflect that 6 parameters presented the highest sensitivity and reflect the highest model output perturbation. However, to complement this sensitivity analysis it is necessary to perform a collinearity analysis, since in the set of highly sensitive parameters (according to parameter importance ranking) there may be parameters that share their sensitivity profiles over time, thus balancing the effect of a change of value of

a parameter with the modification of other parameters. Hence, the collinearity analysis allows selecting those most sensitive parameters that have the least linear dependence (Brun *et al.* [28]). Collinearity analysis is important due to the linear dependence between the parameters, which results in problems during optimization, increasing the uncertainty of the calibrated parameters. The results of the collinearity analysis are presented in Table 4.

The parameters subset selected to carry out the calibration was chosen taking into account sensitivity analysis and collinearity results simultaneously. The parameter subset used for calibration comprises the subset parameters with most sensitivity (according to parameter importance ranking) and with a collinearity index lower than 10 ($\gamma_k = 6.62$). This means that not necessarily the lowest γ_k of this subset dimension must be selected as parameters to calibrate. Therefore, the chosen subset is shown next (subset 1): n , k_{S2} , $\mu_{max, AOB}$, $\mu_{max, NOB}$, K_S , NOB .

According to this analysis, even though the sulfide and organic matter are very important inhibitors, the more sensitivities parameters obtained are associated to the AOB and NOB growth. On the other hand, parameters associated to the chemical oxidation of sulfide produce a stronger effect on the process than the parameters associated to the inhibition. That means that the degradation (chemical or biological) of sulfide must be included in models that involve the presence of sulfide in a nitrifying bioreactor. On the other hand, the NOB kinetic has more sensitive parameters than AOB microorganism, because both $\mu_{max, NOB}$ and $K_{S, NOB}$ affect in an important way the output's model. This result show the importance of NOB and the NO_2^- variables on the development of nitrification kinetic models. Finally, according to this analysis, the organic matter removal by heterotrophic biomass has not influence on the model's output of nitrification, at least at low organic matter concentrations. Therefore, standard values of kinetic parameters associated to heterotrophic biomass growth

could be used in models that involve simultaneously the nitrification and organic matter removal in aerobic bioreactors.

Model calibration and effect of zeolite on the parameters

Once the sensitivity analysis showed which parameters should be calibrated, the calibration of the model was performed. Calibration was done with data coming from Huiliñir *et al.* ^[4] in the presence of sulfide and organic matter, but without zeolite. The agreement between the calibrated model and the experimental data is shown in Figure 3 and the determined parameters are shown in Table 5. According to Figure 3, the model was able to represent qualitatively the experimental behavior of the system for all the variables studied in the system without zeolite. The model could represent both parts of the dynamic of the system, i.e. the slow degradation of TAN and production of NO_3^- in the first 5 hours and then the fast part of the process, with a higher TAN degradation rate and NO_3^- production. It is important to note that, according to experimental data, a very low concentration of nitrite was obtained, with a very strong activity of NOB. This behavior also was obtained by the model (data not shown).

The quantification of the good agreement was made by the TIC calculation, whose values for all the species were lower than 0.3 (0.01, 0.0018 and 0.04 for TAN, NO_3^- and H_2S , respectively), confirming that the model is able to represent the activity of the nitrification process in the presence of sulfide and organic matter. To determine if the error has a normal distribution, the Shapiro Wilk test was applied using Statgraphics Centurion XVII, and it showed that the error has a normal distribution with 95% confidence, with values of P higher than 0.05 (0.7220, 0.3419 and 0.1160 for TAN, NO_3^- and H_2S concentration, respectively). The calibrated values of the parameters are presented in Table 5. The values of $\mu_{max, AOB}$ and $K_{S, NOB}$ are within the range reported in the literature, ^[5, 25, 36-39] with values closer to that reported by Wett & Rauch. ^[37, 40] On the contrary, the values obtained for NOB parameters are different from those reported in the literature, especially the value of $\mu_{max, NOB}$. Indeed,

$\mu_{max, NOB}$ was much greater than that reported in the literature. [25, 37-40] The values reported in the literature varied between 0.0208 and 0.075 h⁻¹, while the value obtained here was 0.3307 ± (0.416) h⁻¹. Nevertheless, this high value is in agreement with the experimental results obtained, because the nitrite concentration was very low in all the recorded tests. A possible explanation of the fast removal of nitrite and then the high value of $\mu_{max, NOB}$ could be the high oxygen concentration that allowed a very good performance of NOB in this experieeces.

Once the parameters where calibrated for the experiments without zeolite, the effect of zeolite on the calibrated kinetic parameters was studied. The results are presented in Figure 4.

According to Figure 4, the saturation constant of NOB ($K_{S, NOB}$, Figure 4a) decreases with increasing zeolite concentration, which means that the presence of zeolite improves the affinity of NOB for the substrate. This parameter decreased by 44.78% (2.5 g/L of zeolite), by 62.24% (5 g/L of zeolite), by 76.15% (10 g/L of zeolite) and by 93.40% (15 g/L of zeolite) respect to the value obtained without zeolite. A possible explanation could be the higher availability of nitrite by the higher activity of AOB (see Fig. 4b), allowing then a higher NOB activity. Regarding to $\mu_{max, AOB}$, Figure 4b shows that there is an increasing of its value with respect to the values without zeolite. The increase varied between 16.57% at 2.5 g/L of zeolite and 387% at 15 g/L of zeolite, showing that zeolite concentration has a strong effect on the specific growth rate. That situation can be explained by the adsorption of NH₄⁺ on the zeolite, allowing a higher consumption of ammonium by AOB. In the case of $\mu_{max, NOB}$ (Figure 4c), it decreases strongly in the presence of zeolite, from 24.19% at a zeolite concentration of 2.5 g/L to 71.15% at a zeolite concentration of 15 g/L. This can be explained by the higher presence of nitrite that could affect the growth of NOB by inhibition. However, the value of $\mu_{max, NOB}$ at a zeolite concentration = 15 g/L is still quite high (around 0.1 h⁻¹), situation that together to the lower $K_{S, NOB}$ values, allowing the fast consumption of nitrite and avoiding the presence of nitrite under these conditions.

Finally, n (Figure 4d) and k_{S2} (Figure 4e) increased with the use of zeolite, but their increase was not large. Parameter n increased 3.10% (2.5 g/L of zeolite), 4.51% (5 g/L of zeolite), 11.20% (10 g/L of zeolite), and 19.63% (15 g/L of zeolite) with respect to the system without zeolite; for k_{S2} , it increased 3.09% (2.5 g/L of zeolite), 4.38% (5 g/L of zeolite), 7.26% (10 g/L of zeolite), and 9.02% (15 g/L of zeolite) with respect to the system without zeolite. This showed that the chemical oxidation of sulfide is not dependent on zeolite.

From the results of the calibrated parameters obtained for different concentrations of zeolite, a function was adjusted according to their tendencies. Figure 4 shows the equations of the adjusted functions and their corresponding R^2 . It was found that the dependence on zeolite of the parameters $K_{S, NOB}$, n , $\mu_{max, AOB}$, $\mu_{max, NOB}$ can be described by an exponential function, while the dependence of the parameter k_{S2} is linear with respect to zeolite concentration. In all cases, the adjustments were adequate, since R^2 values were close to 1.

With the functions obtained for the different parameters, new simulations were made including these functions in the parameters, i.e.:

$$K_{S,NOB} = 2 \cdot 10^{-6} e^{-0.167 \cdot c(zeolite)} \quad (24)$$

$$\mu_{max,AOB} = 0.0256 \cdot e^{0.1075 \cdot c(zeolite)} \quad (25)$$

$$\mu_{max,NOB} = 0.3186 \cdot e^{-0.086 \cdot c(zeolite)} \quad (26)$$

$$k_{S2} = 0.0047 \cdot c(zeolite) + 0.8297 \quad (27)$$

$$n = 0.6508 \cdot e^{0.0118 \cdot c(zeolite)} \quad (28)$$

Figure 5 shows the results obtained by simulations using the dependence of the parameters on zeolite (equations 24 to 28), while Table 6 shows the TIC values. Good agreement was obtained between the data and model results, including the effect of zeolite on the kinetic parameters, with values of TIC between 0.109 and 0.007, with all these values being lower than 0.3. It is interesting to note that the model was able to simulate the process behavior

when the system changed its rate rapidly. For instance, at a zeolite concentration of 10 g/L (Figure 5c), the TAN degradation rate increased very fast between the 7th and the 9th hours, a situation that was represented very well by the model, with TIC values for TAN, nitrate, and sulfide concentrations of 0.024, 0.0056, and 0.0863, respectively. The same situation was observed at a zeolite concentration of 15 g/L (Figure 5d), where the TAN degradation rate and nitrate production rate increased very fast between the 5th and 7th hours and whose TIC values were 0.098, 0.005, and 0.21 for TAN, nitrate, and sulfide concentrations, respectively. Another characteristic of the model was its capacity of simulating the long lag phase at a zeolite concentration of 2.5 g/L (Figure 5a), where the reaction rate started from the 5th hour onward. Under this condition, the TIC values were 0.09, 0.103, and 0.007 for TAN, nitrate, and sulfide concentrations, respectively. Finally, at a zeolite concentration of 5 g/L (Figure 5b), the slight drop of TAN and the increase of NO₃⁻-N also were very well simulated by the kinetic model, even though the TIC value for nitrate concentration was the highest obtained in all the tests (TIC = 0.109). Thus, the function obtained for quantifying the effect of zeolite on the kinetics of nitrification can simulate properly the behavior and performance of the system under sulfide and organic matter inhibition.

The last point that was evaluated with the model was its capacity to obtain the values of COD and VSS reported experimentally. Table 7 shows the comparison of the final concentrations of VSS and COD predicted with the model and those reported in the literature ^[4]. The error between the model and experimental data was calculated as follows:

$$Error (\%) = \frac{y_{literature} - y_{model}}{y_{literature}} \cdot 100 \quad (29)$$

As can be seen in Table 7, the error values for both parameters (VSS and COD) and all the tested conditions were lower than 3.5%, showing that the model was able to predict the concentrations of VSS and COD in the system. In the case of VSS, the model predicted

values lower than the experimental data under all the conditions, with errors lower than 2.0%. For the case of soluble COD concentration, the values obtained by the model were also lower than the experimental data with all the tested zeolite concentrations. The largest error was obtained for the test without zeolite, although this error was lower than 3.5%. Thus, according to these results, the model was able to predict both the VSS and COD concentrations in the tests under all the conditions studied.

Conclusions

A kinetic model that included the effect of sulfide and organic matter on the nitrification process was successfully calibrated and simulated in the presence of zeolite. According to the sensitivity and collinearity analysis, five parameters were the most sensitive in the model, whose calibrated values were: $\mu_{max, AOB} = 0.02642 \pm 0.002 \text{ h}^{-1}$; $\mu_{max, NOB} = 0.3307 \pm 0.416 \text{ h}^{-1}$; $K_{S, NOB} = 1.65 \cdot 10^{-6} \pm 2.85 \cdot 10^{-6} \text{ mgHNO}_2\text{-N/L}$; $k_{S2} = 0.8213 \pm 0.076$, and $n = 0.6537 \pm 0.030$. Zeolite affected mainly the parameters associated with the nitrification process, whose dependence on the $K_{S, NOB}$, $\mu_{max, AOB}$ and $\mu_{max, NOB}$ parameters can be described by an exponential function. A good agreement between the experimental data and the model's results including the effect of zeolite on the kinetic parameters was obtained, with TIC values between 0.109 and 0.007 for all the variables studied, with all these values being lower than 0.3.

Acknowledgements

The authors acknowledge gratefully the financial support provided by FONDECYT (Chile) under Project 1170103 and PhD fellowship 21170147 awarded to L. Acosta-Cordero. This work is dedicated to the memory of our well esteemed and wonderful colleague Dr. Silvio Montalvo Martinez who recently passed away.

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TABLES

Table 1: Model initial parameters before optimization for a nitrification process in the presence of sulfide and organic matter

Parameter	Value	Unit	Reference
$\mu_{max. AOB}$	0.032	h^{-1}	[25]
$K_{S.AOB}$	0.028	mgNH ₃ -N/L	[25]
$K_{IS.AOB}$	540	mgNH ₃ -N/L	[25]
$K_{DO. AOB}$	0.3	mgO ₂ /L	[25]
$K_{I. S. AOB}$	2.54±0.01	mgHS ⁻ -S/L	[7]
$\mu_{max. NOB}$	0.045	h^{-1}	[25]
$K_{S.NOB}$	3.2·10 ⁻⁵	mg HNO ₂ -N/L	[25]
$K_{IS.NOB}$	0.26	mg HNO ₂ -N/L	[25]
$K_{DO. NOB}$	1.1	mgO ₂ /L	[25]
$K_{I. S.NOB}$	1.03±0.06	mgHS ⁻ -S/L	[7]
$K_{ISu. NOB}$	0.22±0.03	mgHS ⁻ -S/L	[7]
$\mu_{max. H}$	0.25	h^{-1}	[17]
$K_{S.COD}$	20	mgCOD/L	[17]
$K_{DO. H}$	0.2	mgO ₂ /L	[17]
$k_{d.A}$	0.002	h^{-1}	[25]
$k_{d. H}$	0.0083	h^{-1}	[26]
k_{S2}	0.7355	-	-
n	0.6369	-	-
$Y_{AOB/TAN}$	0.147	mg SSV/mgTAN	[25]
$f_{NO2-/TAN}$	0.98	mgNO ₂ ⁻ /mgTAN	[25]
$Y_{NOB/NO2-}$	0.042	mgSSV/mgNO ₂ ⁻	[25]
$f_{NO3-/NO2-}$	1	mgNO ₃ ⁻ / mgNO ₂ ⁻	[25]
$Y_{H/COD}$	0.28	mg SSV/mgCOD	[26]

Table 2: Parameter importance ranking (+50% parameters)

Parameters	$\delta msqr$	$\delta mabs$	$\delta mean$
n	4.91	2.76	-0.12
k_{S2}	4.19	2.17	-0.08
$\mu_{max.AOB}$	1.84	0.89	$-8.00 \cdot 10^{-3}$
$\mu_{max.NO B}$	1.31	0.54	$-4.08 \cdot 10^{-6}$
$K_{I.S.AOB}$	1.28	0.66	$-6.04 \cdot 10^{-3}$
$K_{I.S.NO B}$	0.72	0.32	$1.49 \cdot 10^{-5}$
$K_{S.NO B}$	0.51	0.25	$-3.01 \cdot 10^{-6}$
$K_{ISu NO B}$	0.42	0.19	$2.21 \cdot 10^{-5}$
$K_{O2.NO B}$	0.20	0.08	$-8.94 \cdot 10^{-7}$
$K_{O2.AOB}$	0.10	0.04	$4.34 \cdot 10^{-4}$
$k_{d.A}$	0.03	0.02	$7.49 \cdot 10^{-5}$
$K_{IS.AOB}$	0.02	0.01	$-1.08 \cdot 10^{-4}$
$\mu_{max.HET}$	0.01	0.01	$2.78 \cdot 10^{-5}$
$K_{S.AOB}$	0.01	$6 \cdot 10^{-3}$	$6.96 \cdot 10^{-5}$
$K_{S.COD}$	$2.19 \cdot 10^{-3}$	$9 \cdot 10^{-4}$	$2.46 \cdot 10^{-7}$
$K_{IS.NO B}$	$1.16 \cdot 10^{-3}$	$4.3 \cdot 10^{-4}$	$1.23 \cdot 10^{-6}$
$K_{O2.HET}$	$6.60 \cdot 10^{-4}$	$2.80 \cdot 10^{-4}$	$1.66 \cdot 10^{-5}$
$k_{d.H}$	$4.70 \cdot 10^{-4}$	$1.95 \cdot 10^{-4}$	$-2.16 \cdot 10^{-7}$

Table 3: Parameter importance ranking (-50% parameters)

Parameters	$\delta msqr$	$\delta mabs$	$\delta mean$
n	6.26	3.79	-0.28
k_{S2}	6.04	3.52	-0.23
$\mu_{max.AOB}$	3.12	1.81	-0.02
$\mu_{max.NO2}$	2.44	1.15	$3.48 \cdot 10^{-6}$
$K_{I.S.AOB}$	2.28	1.19	-0.01
$K_{I.S.NO2}$	1.32	0.61	$1.18 \cdot 10^{-5}$
$K_{S.NO2}$	0.81	0.37	$-1.43 \cdot 10^{-5}$
$K_{ISu NO2}$	0.78	0.37	$-1.38 \cdot 10^{-5}$
$K_{O2.NO2}$	0.22	0.09	$-1.68 \cdot 10^{-5}$
$K_{O2.AOB}$	0.10	0.04	$4.80 \cdot 10^{-4}$
$k_{d.A}$	0.03	0.02	$7.24 \cdot 10^{-5}$
$K_{IS.AOB}$	0.07	0.03	$-3.56 \cdot 10^{-4}$
$\mu_{max.HET}$	0.01	0.01	$5.90 \cdot 10^{-5}$
$K_{S.AOB}$	0.01	$6 \cdot 10^{-3}$	$4.00 \cdot 10^{-5}$
$K_{S.COD}$	$2.47 \cdot 10^{-3}$	$1.12 \cdot 10^{-3}$	$-4.59 \cdot 10^{-6}$
$K_{IS.NO2}$	$3.63 \cdot 10^{-3}$	$1.35 \cdot 10^{-3}$	$-1.02 \cdot 10^{-6}$
$K_{O2.HET}$	$5.2 \cdot 10^{-4}$	$2.50 \cdot 10^{-4}$	$-1.787 \cdot 10^{-6}$
$k_{d.H}$	$4.4 \cdot 10^{-4}$	$2.04 \cdot 10^{-4}$	$-3.67 \cdot 10^{-7}$

Table 4: Subsets of parameters with the lower collinearity index value (lower than 10)

Subset	γ_k
1. $n, k_{S2}, \mu_{max, AOB}, \mu_{max, NOB}, K_{S, NOB}$	6.62
2. $n, k_{S2}, \mu_{max, NOB}, K_{I, S, AOB}, K_{S, NOB}$	7.41
3. $n, k_{S2}, \mu_{max, AOB}, K_{I, S, AOB}$	6.53
4. $n, k_{S2}, K_{I, S, AOB}, K_{I, S, NOB}$	7.57
5. $n, k_{S2}, K_{I, S, AOB}, K_{S, NOB}, K_{ISu, NOB}$	7.98

Table 5: Calibrated model parameters values obtained, with their confidence interval (CI)

Parameter	Calibrated value (CI)	Reference value	Author
N	$0.653 \pm (0.030)$	0.6369	-
k_{S2}	$0.821 \pm (0.076)$	0.7355	-
$\mu_{max.AOB}(\text{h}^{-1})$	$0.026 \pm (0.002)$	0.032	[24]
$\mu_{max.NO}(\text{h}^{-1})$	$0.330 \pm (0.416)$	0.045	[24]
$K_{S.NO}(\text{mg HNO}_2\text{-N/L})$	$1.65 \cdot 10^{-6} \pm (2.85 \cdot 10^{-6})$	$3.2 \cdot 10^{-5}$	[24]

Table 6: TIC values for the proposed model using the function of the calibrated parameters with the zeolite concentration

Variable	Zeolite concentration (g/L)			
	2.5	5	10	15
c_{TAN}	0.090	0.034	0.024	0.098
$c_{NO_3^-}$	0.013	0.109	0.0056	0.005
c_{H_2S}	0.007	0.0082	0.0783	0.021

Table 7: Comparison of the final concentrations of VSS and COD calculated with the model and those reported in the literature

Zeolite concentration (g/L)	VSS (mg/L)			Soluble COD (mg/L)		
	Huiliñir <i>et al.</i> [4]	Model	Error (%)	Huiliñir <i>et al.</i> [4]	Model	Error (%)
0 (model calibration)	755	747	1.059	82.773	79.943	3.419
2.5	745	735	1.342	86.183	83.825	2.736
5	567	558	1.587	86.183	83.987	2.548
10	677	664	1.920	82.846	80.876	2.377
15	653	647	0.919	88.481	86.818	1.879

FIGURE CAPTIONS

Figure 1: Systematic procedure applied for model implementation, calibration, and validation (Carrera et al. 2016).

Figure 2: Adjustment of the sulfide chemical oxidation model for the determination of n and k_{S2} .

Figure 3: Model calibration for the system without zeolite. TAN removal, H₂S removal, and nitrate production.

Figure 4: Effect of zeolite on the calibrated kinetic parameters.

Figure 5: Model simulations using functions in terms of zeolite concentrations. a) zeolite concentration = 2.5 g/L; b) zeolite concentration = 5 g/L; c) zeolite concentration = 10 g/L; d) zeolite concentration = 15 g/L.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

SUPPLEMENTARY DATA

Figure S1: Profiles of H₂S-S on the time. a) H₂S-S = 60 mg S/L; b) H₂S-S = 80 mg S/L; c) H₂S-S = 40 mg S/L; d) H₂S-S = 160 mg S/L; e) H₂S-S = 100 mg S/L