Estimation of kinetic parameters of a model for deammonification in biofilms and evaluation of the model

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Abstract A systematic approach to estimate and evaluate parameters for deammonification in biofilms from available experimental data was evaluated. Parameter estimation was based on a regional steady state sensitivity analysis to select relevant parameters and design of experiments based on a local identifiability analysis. The calibrated model was evaluated under different experimental conditions. Nine of the 16 kinetic and stoichiometric parameters had a significant influence on model predictions. Of these nine parameters only six kinetic parameters were identifiable from batch experiments regardless of the experimental design. More parameters were not identifiable due to high correlations between growth rates and the corresponding affinity constant for oxygen. Data from a batch experiment at 2 mg/L dissolved oxygen (DO) were used to estimate inactivation rates and affinity constants for oxygen for ammonium oxidisers (AO), nitrite oxidisers (NO) and anaerobic ammonium oxidisers (AN). In addition, it was found that not only kinetic and stoichiometric parameters but also the external mass transfer resistance significantly affected model predictions. The resulting model was able to reproduce batch test and continuous reactor operation where DO concentrations were similar to those in the batch experiment used for parameter estimation. However, the model overestimated deammonification for a batch experiment at a much higher DO concentration (5 mg/L). Thus, parameter values that are identifiable and are estimated for given environmental conditions may not necessarily be valid for significantly different experimental conditions.

Keywords Biofilm; deammonification; identifiability; mathematical modelling; nitrogen removal; parameter estimation

Introduction Single-stage autotrophic nitrogen elimination processes are suitable for the treatment of wastewaters with high contents of ammonium but low contents of organic carbon. Autotrophic nitrogen elimination processes called deammonification (Hippen et al., 1997), autotrophic denitrification (Koch et al., 2000), CANON (completely autotrophic nitrogen removal over nitrite; Strous, 2000), and OLAND (oxygen limited autotrophic nitrification denitrification; Kuai and Verstraete, 1998) combine partial oxidation of ammonium to nitrite and subsequent anaerobic oxidation of ammonium with nitrite to dinitrogen gas (Anammox; Strous et al., 1997 and van de Graaf et al., 1997). In comparison to classical nitrogen elimination by nitrification and denitrification, autotrophic nitrogen elimination has the advantage that no external carbon source and less energy for the aeration system are required. Moreover, less sludge is produced due to the low growth rates and biomass yields of the autotrophic microorganisms. Both steps of autotrophic nitrogen elimination can be combined in biofilms into a single-stage operation (Helmer et al., 1999; Pynaert et al., 2002).
Using biofilms to achieve complete autotrophic ammonium oxidation is attractive as it requires only a single tank. However, process performance will depend on carefully adjusting bulk phase conditions to allow for the development of redox zones inside of the biofilm. Mathematical modelling is an important tool to evaluate the influence of process operation and model parameters on model predictions. Previous studies have focused on local sensitivity analysis of the model and model-based evaluation of different influences on the conversion processes (Hao et al., 2002a, b; Hao and van Loosdrecht, 2004; Van Hulle, 2005), and on experimental design for parameter estimation of the inhibition constant for nitrite of the anaerobic ammonium oxidisers (Van Hulle, 2005). However, these studies did not test or calibrate their model using experimental data. Koch et al. (2000) compared their model results to experimental data but focused on a qualitative evaluation of model predictions.

The purpose of this paper was to estimate parameters of a model for deammonification in biofilm systems from experimental data of a batch experiment. A systematic procedure for selecting appropriate parameters and a suitable experimental layout for parameter estimation are presented. Additional experimental data were used to verify the calibrated model.

Materials and methods

Pilot and laboratory experiments

Continuous reactor operation. A pilot-scale moving-bed biofilm plant was used for deammonification of sludge digester liquids as described previously in Hippen (2001). The pilot plant consisted of a preliminary denitrification reactor with topped settling tank and three in-line moving-bed biofilm reactors. Each biofilm reactor had a volume of 40 L and was filled to 20% with Kaldnes® support material resulting in a total biofilm surface area of 4 m². In this study, only data of the second moving-bed reactor are used for simulations. The influent flow to this reactor was 0.24 m³/d and contained on average 148 mg NH₄⁺-N/L, 15 mg NO₂⁻-N/L and 7 mg NO₃⁻-N/L. The concentration of dissolved oxygen (DO) in the bulk liquid was on average 0.8 mg O₂/L. The average temperature was 28°C and the pH was 8.1.

Batch experiments. Helmer et al. (1999, 2001) carried out several batch experiments with Kaldnes® material sampled from the continuous reactor. The bulk volume and the biofilm surface of the batch reactor were 1.8 L and 0.36 m², respectively. The initial ammonium concentration was 170 mg NH₄⁺-N/L for experiments at DO concentrations of 0.7 and 2 mg O₂/L, and 140 mg NH₄⁺-N/L for the experiment at a DO concentration of 5 mg O₂/L. The temperature was 28°C and the pH was 8.

Mathematical modelling

Model structure. The model considered growth and inactivation of aerobic ammonium oxidisers (XNH₄), aerobic nitrite oxidisers (XNO) and anaerobic ammonium oxidisers (XAN). Growth processes were described with Monod kinetics for substrate utilisation where the growth of XAN was inhibited by oxygen. Ammonium consumption for biomass growth was neglected due to low production of autotrophic biomass. Inactivation processes reduced the amount of active biomass and formed inert biomass. Default values for the parameters were taken from Hao et al. (2002a) except for the value of the affinity constant for oxygen of the nitrite oxidisers (K_{O₂,NO}). K_{O₂,NO} was assumed as 0.7 mg/L based on initial steady state model evaluations compared with experimental data. The biofilm thickness was set to 450 μm based on measured biofilm thicknesses ranging from 420 to 510 μm (Tromm, 1999). The external mass transfer resistance was
modelled assuming a concentration boundary layer ($L_i$) with a default thickness of 10 $\mu$m considering high flow velocity in the reactor due to intensive mixing. Increased $L_i$ were evaluated for parameter estimation as well as after calibrating kinetic model parameters. All simulations were performed using AQUASIM (Reichert, 1998).

**Sensitivity, identifiability analysis, and design of experiments.** In this study, sensitivity analysis as well as identifiability analysis and design of experiment focused on kinetic parameters. Other parameters, such as diffusion coefficients, external mass transfer resistance and biomass density, can be important as well, but they were fixed to default values to reduce the computational effort. A regional steady state sensitivity analysis was conducted for continuous reactor operation based on a factorial design as described by Box et al. (1978). Identifiability of parameters was evaluated for batch experiments by calculation of the collinearity index $\gamma_k$ as defined by Brun et al. (2001). The collinearity index is a measure of the degree of correlation between parameters and was calculated from the matrix of the scaled local sensitivity functions.

$$\gamma_k = \frac{1}{\min_{||\beta||=1} ||S\beta||} = \frac{1}{\sqrt{\min (EV[S^{T}S])}}$$

where $S$ is the matrix of the normalised local sensitivity functions evaluated for the default parameters, $\beta$ is a vector with $||\beta|| \neq 0$ such that $S\beta = 0$, $S^{T}S$ is the so-called Fisher information matrix (FIM) and $EV$ is the eigenvalue. Parameters and parameter subsets with a collinearity index $\gamma_k$ below a threshold of 15 are considered to be identifiable whereas values of $\gamma_k$ larger than a threshold of 15 indicate non-identifiability of the parameters. As a basis for choosing optimal experimental conditions, identifiability analyses were performed for six different types of batch experiments. The batch experiments differed in the initial ammonium and nitrite concentrations, the bulk phase oxygen concentrations, the addition of a second ammonia pulse and partial anaerobic conditions.

**Parameter estimation.** A subset of identifiable parameters was estimated based on data of a batch experiment carried out at a DO of 2 mg O$_2$/L. Measurements of bulk concentrations of ammonium, nitrite and nitrate were used as data basis. Default values were used as initial values for the selected parameters. The minimum and maximum parameter values indicating the boundaries of the defined parameter ranges were the same as for calculating the regional steady state sensitivities for continuous reactor operation. Parameters were estimated in AQUASIM by minimising the sum of squares of the deviations between measurements and calculated model results ($\chi^2$).

**Model evaluation.** Experimental data of two other batch experiments carried out at DO concentrations of 0.7 and 5 mg O$_2$/L, respectively, were available to verify the previously calibrated model. In addition, data of a period of approximately four months of continuous reactor operation of the laboratory-scale pilot plant for deammonification were used as well for verification of the model.

**Results and discussion**

**Sensitivity analysis.** Results from the regional steady state sensitivity analysis are shown in Figure 1. Regional steady state sensitivities are plotted as ratio of the main effect of a parameter on the model output to the average value of the model output. Positive values indicate that the model output increases when the parameter moves from
the minimum to the maximum value of the parameter range, while negative values indicate that the model output decreases. The growth and decay rates of aerobic ammonium oxidisers ($\mu_{\text{NH}_b \text{NH}}$, $b_{\text{NH}}$), nitrite oxidisers ($\mu_{\text{NO}_b \text{NO}}$, $b_{\text{NO}}$) and anaerobic ammonium oxidisers ($\mu_{\text{AN}_b \text{AN}}$, $b_{\text{AN}}$) as well as the affinity constants for oxygen of all three microorganisms ($K_{O_2,\text{NH}}$, $K_{O_2,\text{NO}}$ and $K_{O_2,\text{AN}}$) had a very large influence on the model output. The impact of the affinity constants for nitrite ($K_{\text{NO}_2,\text{NO}}$ and $K_{\text{NO}_2,\text{AN}}$) was comparably small. The affinity constants for ammonium ($K_{\text{NH}_4,\text{NH}}$ and $K_{\text{NH}_4,\text{AN}}$) affected the output only marginally. Ammonium was provided in excess so that the concentration of ammonium in the bulk liquid and within the entire biofilm was mostly much higher than the affinity constant. Growth and decay rates and affinity constants for oxygen were selected for identifiability analysis due to their large impact on the model output.

Identifiability analysis. Results from the identifiability analysis are shown in Figure 2. For five of the six investigated conditions of batch experiments, several parameter subsets of size 6 were identifiable from combined measurements of ammonium, nitrite and nitrate (NH$_4$/NO$_2$/NO$_3$) in the bulk liquid. However, more than six of the nine selected parameters were not simultaneously identifiable regardless of the experimental design due to a significant correlation between growth rates and corresponding affinity constant for oxygen ($\mu_{\text{NH}} - K_{O_2,\text{NH}}$, $\mu_{\text{NO}} - K_{O_2,\text{NO}}$, $\mu_{\text{AN}} - K_{O_2,\text{AN}}$; Brockmann (2006) and Brockmann et al. (2006)).

Parameter estimation. The parameter estimation was performed based on measurements of concentrations of ammonium, nitrite and nitrate of a batch experiment carried out at a DO of 2 mg O$_2$/L ( = batch experiment (c) in Figure 2). Under these conditions eight parameter subsets of size 6 were identifiable where all three inactivation rates and either the growth rate or the corresponding affinity constant for oxygen for each of the three organism groups could be estimated (Table 1). It was assumed that the growth rates of aerobic ammonium and nitrite oxidisers as well as of anaerobic ammonium oxidisers are relatively well known compared with the inactivation rates and the affinity constants for oxygen. Based on this assumption, parameter subset No. 4 in Table 1 was selected for parameter estimation. This parameter subset included the inactivation rates and the affinity constants for oxygen for all three groups of organisms. The three growth rates were fixed to their default values.
Table 1 Parameter subsets of size 6 identifiable from NH₄/NO₂/NO₃ measurements from a batch experiment carried out at a concentration of DO of 2 mg O₂/L

<table>
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<th>Subset</th>
<th>Parameters</th>
<th>Collinearity index γ_{k} [-]</th>
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<td>b_{AN}, b_{NH}, b_{NO}, K_{O₂,AN}, pH_{AN}, μ_{NO}</td>
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<td>2</td>
<td>b_{AN}, b_{NH}, b_{NO}, K_{O₂,AN}, K_{O₂,NO}, pH_{AN}</td>
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</tr>
<tr>
<td>3</td>
<td>b_{AN}, b_{NH}, b_{NO}, K_{O₂,AN}, K_{O₂,NO}, μ_{NO}</td>
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</tr>
<tr>
<td>4</td>
<td>b_{AN}, b_{NH}, b_{NO}, K_{O₂,AN}, K_{O₂,NO}, K_{O₂,NO}</td>
<td>10.1</td>
</tr>
<tr>
<td>5</td>
<td>b_{AN}, b_{NH}, b_{NO}, K_{O₂,AN}, K_{O₂,NO}, K_{O₂,NO}, μ_{NO}</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>b_{AN}, b_{NH}, b_{NO}, K_{O₂,NO}, pH_{AN}, μ_{NO}</td>
<td>10.3</td>
</tr>
<tr>
<td>8</td>
<td>b_{AN}, b_{NH}, b_{NO}, K_{O₂,NO}, K_{O₂,NO}, K_{O₂,NO}, pH_{AN}</td>
<td>10.3</td>
</tr>
</tbody>
</table>

Figure 2 Influence of experimental design on the collinearity index (γ_{k}) for different experimental conditions in batch experiments. (a) Initial NH₄⁺ concentration of 150 mgN/L and DO = 0.7 mg/L; (b) like (a) but in addition an initial NO₂⁻ concentration of 60 mgN/L; (c) like (a) but DO = 2 mg/L; (d) initial NH₄⁺ concentration of 50 mgN/L and second ammonia addition (50 mgN/L), DO = 2 mg/L; (e) like (a) but DO = 5 mg/L; (f) like (a) but aeration turned off after 180 min. Symbols in the figure correspond to pair wise combinations of measurements of ammonium (NH₄⁺), nitrite (NO₂⁻) and nitrate (NO₃⁻) or measurement of all three parameters.
An initial parameter estimation predicted ammonium concentrations very well but overestimated nitrite and nitrate concentrations (data not shown). A second parameter estimation was conducted for the same set of parameters, starting from the same initial parameter values but with an increased thickness of the concentration boundary layer of $L_l = 50 \mu m$. Increasing the external mass transfer resistance significantly improved the model fit and reduced $\chi^2$ from 1,741 to 343. As shown in Table 2, increasing $L_l$ significantly affected the estimated value of $K_{O_2,NO}$ and $K_{O_2,NH}$. It should be noted that all parameter values estimated assuming $L_l = 50 \mu m$ were on the boundaries of the defined parameter ranges. This means that the ideal parameter values for the regarded experimental conditions were most likely outside of the defined parameter ranges. Definition of new parameter ranges would be necessary to determine the parameter values resulting in the smallest deviations between experimental and simulation results. However, one should keep in mind that one motivation for defining parameter ranges in the first place was to keep estimated parameters within realistic boundaries.

**Model evaluation.** Model parameters estimated from a batch experiment operated with 2 mg O$_2$/L and assuming $L_l$ of 50 $\mu m$ were further evaluated. Simulation results of the calibrated model were compared with batch experiments under different conditions as well as with continuous reactor operation (pilot plant). A batch experiment conducted at a DO concentration of 0.7 mg O$_2$/L could be reproduced quite well using estimated model parameters (Figure 4(a)). In Figure 3, modelling results are compared with continuous reactor operation at an average concentration of DO of 0.8 mg O$_2$/L using the previously estimated kinetic parameters. For the pilot plant, simulations were performed assuming $L_l = 100 \mu m$, which resulted in a much improved model fit compared with simulations with $L_l = 50 \mu m$. As mixing conditions in the pilot-scale reactor and in the much smaller batch experiment are significantly different, it is quite reasonable to assume that external mass transfer in these two systems will be different while stoichiometric and kinetic parameters remain the same. For most of the experimental period, the model reasonably predicts the observed dynamics.

In Figure 4(b), model predictions are compared with experimental results for a batch experiment at high bulk phase DO (5 mg O$_2$/L). It can be seen that the model predicted nitrogen elimination by deammonification whereas the experimental data show that ammonium is converted to nitrite and nitrate but anaerobic ammonium oxidation is apparently inhibited. Thus, while the estimated parameters were suitable to predict batch or continuous reactor performance at low bulk phase DO (<2 mg O$_2$/L) they were not able to predict system performance under these high bulk phase DO concentrations. The prediction of nitrogen elimination even at a DO concentration of 5 mg O$_2$/L can be explained by the relatively high value of the inhibition constant for oxygen of the

<table>
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<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Min. value</th>
<th>Max. value</th>
<th>Initial value</th>
<th>Estimated values for $L_l = 10 \mu m$</th>
<th>Estimated values for $L_l = 50 \mu m$</th>
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<td>0.016</td>
<td>0.003</td>
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<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
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<td>0.5</td>
<td>0.06</td>
<td>0.032</td>
<td>0.03</td>
</tr>
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<td>$K_{O_2,AN}$</td>
<td>Mg O$_2$/L</td>
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<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
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<td>Mg O$_2$/L</td>
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<td>2.0</td>
<td>0.6</td>
<td>0.92</td>
<td>0.4</td>
</tr>
<tr>
<td>$K_{O_2,NO}$</td>
<td>Mg O$_2$/L</td>
<td>0.4</td>
<td>2.0</td>
<td>0.7</td>
<td>0.51</td>
<td>0.4</td>
</tr>
</tbody>
</table>
anaerobic ammonium oxidisers ($K_{O_{2,AN}} = 0.5 \text{ mg} \text{ O}_2/\text{L}$; see Table 2) which is at the maximum of the defined reasonable range.

**Conclusions**

- Parameters of a biofilm model describing deammonification are highly correlated and only six out of 16 model parameters were identifiable.

- Identifiability of model parameters is a prerequisite for estimating model parameters that are applicable under a range of conditions. However, even identifiable parameters that were estimated for given environmental conditions may not be valid for significantly different experimental conditions (e.g., increased bulk phase DO). Thus, model predictions are always most reliable for conditions similar to those used for model calibration. One approach to calibrate a more robust model could be to estimate parameters from a combination of experiments carried out at different experimental conditions.

- While microbial kinetic and stoichiometric parameters are expected to be independent of scale, mixing conditions and reactor scale have a significant influence on mass transfer. For different reactor scales (laboratory-scale batch experiment or pilot-scale continuous reactor), model predictions significantly improved choosing different concentration boundary layer thicknesses ($L_i$) while keeping all stoichiometric and kinetic parameters constant.

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**Figure 3** Continuous reactor operation of the pilot plant for deammonification (average DO 0.8 mg O$_2$/L). Simulated ($L_i = 100 \mu$m) and measured effluent concentrations of ammonium, nitrite, nitrate, and the sum of inorganic nitrogen.

**Figure 4** Batch experiments carried out at (a) DO 0.7 mg/L and (b) DO of 5 mg O$_2$/L. Simulated ($L_i = 50 \mu$m) and measured concentrations of ammonium, nitrite, nitrate and the sum of inorganic nitrogen.
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