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Review

Modelling enhanced groundwater denitrification in batch micrococosm tests

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ABSTRACT

In the last few years, enhanced *in situ* denitrification has gained a lot of interest as a reliable bioremediation option to remove nitrate from groundwater. However, denitrification modelling in the subsurface environment is less developed than in other fields like wastewater treatment, due to the complexity of describing microbial processes in natural systems and the lack of proper kinetic and stoichiometric parameters.

In this study, a mathematical model describing nitrate, oxygen and organic carbon consumption coupled with the growth and decay of a heterotrophic microbial population was developed. The model has the aim of explaining experimental data that was obtained in microcosm batch tests containing groundwater and subsoil from a nitrate-contaminated aquifer stimulated with glucose as an external carbon source.

The most sensitive parameters (heterotrophic maximum growth rate, decay rate constant and initial heterotrophic biomass concentration) were calibrated by experimental data fitting. Two experimental designs, a single dentrification test and a fedbatch-operation test, were performed in order to calibrate these parameters. The fedbatch-operation experiment, consisting of four consecutive pulses of nitrate and a carbon source, resulted in a more appropriate calibration of model parameters than the single denitrification test, based on the practical identifiability study. Parameter confidence intervals were calculated by means of the Fisher Information Matrix (FIM). Results indicated that the model, with the optimal estimated parameters, could properly fit experimental data. The presented model constitutes a first approach for modelling enhanced denitrification in aquifer systems.

1. Introduction

During the past few decades, nitrate contamination of groundwater has become a growing environmental concern worldwide. It usually results from the excessive use of fertilizers in crops, livestock, sewage waste and septic tanks [1,2]. The worldwide trend towards rising nitrate concentrations has led the focus of attention to obtain the most effective method for its *in situ* removal, the so-called denitrification process, in which nitrate is biologically transformed to nitrogen gas and removed from water.

Most published studies about denitrification in groundwater are based on the investigation of the influence of different environmental conditions on the process, which comprises of: the type of electron donor, nutrient availability or pH and temperature ranges, amongst others [3]. However, recent research has pointed out the need to develop mathematical models in order to predict *in situ* biological processes, which may be useful to design and suitably apply bioremediation technologies such as *in situ* denitrification [4].

The use of Monod kinetics has been the most common approach for modelling biological processes and, in particular, denitrification [1]. Monod mathematically relates the growth of a microbial population to the concentration of the substance limiting its growth. A more sophisticated kinetic model applied in denitrification is known as the multiple-Monod kinetics, which is an extension of the Monod equation, where the rate of microbial growth is limited by the concentration of one or more species other than a single growth substrate. Within this approach, Bae and Rittman [5] supported the dual-limitation kinetics in which, both the electron acceptor and the electron donor substrates limit the overall cell-growth rate. This kinetics has successfully been applied in several works to describe denitrification and other biological processes [6–8].

Modelling of microbial processes is characterised by two important features. On the one hand, models are usually high-order non-linear systems including a large number of state variables and

Abbreviations: ASM1, Activated Sludge Model No. 1; DOC, dissolved organic carbon; FIM, Fisher Information Matrix; MPN, Most Probable Number; OC, organic carbon; OF, objective function.

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Nomenclature

$\mu_{max,H}$	heterotrophic maximum growth rate (d^{-1})
η	reduction factor for anoxic conditions
K_{NO_3}	saturation coefficient for nitrate ($mg\,NO_3^-\,L^{-1}$)
K_{O_2}	saturation coefficient for oxygen ($mg\,O_2\,L^{-1}$)
$K_{O_2,I}$	inhibition coefficient for oxygen ($mg\,O_2\,L^{-1}$)
K_{OC}	saturation coefficient for organic carbon ($mg\,OC\,L^{-1}$)
b_H	heterotrophic decay rate constant (d^{-1})
Y_H	heterotrophic growth yield ($mg\,cells\,mg\,OC^{-1}$)
$X_{H,0}$	initial heterotrophic biomass concentration ($mg\,biomass\,L^{-1}$)
X_H	heterotrophic biomass concentration ($mg\,biomass\,L^{-1}$)

parameters (e.g. multiple-Monod models) and, on the other hand, there is a lack of reliable techniques for measurement of all the state variables of interest [9]. In addition, an impediment when modelling biological processes in environmental systems is the selection of the proper parameter values.

In order to overcome the above-mentioned problems, recent literature points out the need to include a study of the possibility of identifying unique values for model parameters; this is known as the identifiability study. Two types of identifiability studies are described: the theoretical or structural identifiability which is related to the model structure and the available measured outputs, and the practical identifiability which is based on the experimental conditions and, therefore, on the available data [10]. Some examples of applying these types of analyses were found in wastewater literature [11–13], but no references were found in the field of groundwater bioremediation modelling.

The main objective of this work was to apply a mathematical model in order to explain the evolution of nitrate, oxygen and organic carbon when stimulating indigenous aquifer bacteria with an organic carbon source. In order to achieve this objective, proper experiments were designed to allow model calibration. Furthermore, the present work was aimed to apply a novel procedure in groundwater bioremediation modelling to study the quality of the model parameters: the practical identifiability study and the consequent calculation of the parameter confidence intervals.

2. Materials and methods

2.1. Experimental design

Soil and groundwater samples were collected from a nitrate-contaminated aquifer located in Argentona, Catalonia (Spain). Batch microcosm tests were carried out by suspending 35 g of subsoil material in 325 mL of groundwater in a 400 mL methacrylate flask provided with an adjustable lid that prevented the formation of a headspace in the reactor [14]. Glucose was added as an external carbon source to promote denitrification.

Tests were magnetically stirred and maintained in a dark thermostatic chamber (Medilow, Selecta) at 17 °C to simulate natural aquifer conditions.

Dissolved oxygen was continuously monitored by using a dissolved oxygen electrode (Crison No. 6050) coupled with a Crison OXI 49. Samples for nitrate and dissolved organic carbon (DOC) measurements were taken twice daily.

2.2. Analytical methods

Nitrate was determined by High Performance Liquid Chromatography (HPLC, Agilent 2100 series) with a Waters 432 non-suppressed conductivity detector. A Hamilton PRP-X110 column packed with a polymeric anion exchanger was used. The mobile phase consisted of a 2 mM p-hydroxybenzoic acid solution where the pH was adjusted to 9.2 with NaOH. The analytical procedure followed conformed with method UNE-EN ISO 10304-1. The detection limit of this method was 1.5 $mg\,NO_3^-\,L^{-1}$.

DOC was measured using a Shimadzu TOC 5050 analyzer according to the standard method [15]. The detection limit of the method was 1.0 $mg\,DOC\,L^{-1}$.

Denitrifiers in soil were measured by the Most Probable Number (MPN) according to the method described by Tiedje [16].

3. Model development

3.1. Model description

The mathematical model was built according to the activated sludge models developed by Henze et al. [18]. The model included the aerobic oxidation of organic carbon and the denitrification, processes mediated by the same microbial group, heterotrophs. Process kinetics and stoichiometry are detailed in Table 1.

Heterotrophic microbial growth was modelled as the sum of two equations. The first one representing the growth rate under aerobic conditions (oxygen acts as a terminal electron acceptor) and the second one representing the growth rate under anoxic conditions (nitrate acts as a terminal electron acceptor). Each growth equation was derived following the multiple-Monod kinetics and, therefore, included terms for organic carbon (OC) and the electron acceptor (i.e. oxygen or nitrate). An additional inhibition term was considered in the equation for anoxic conditions. This term suppresses anoxic growth as long as oxygen concentration exceeds a certain threshold value, which is expressed with the inhibition coefficient for oxygen ($K_{O_2,I}$).

The maximum growth rate of heterotrophic bacteria ($\mu_{max,H}$) was considered to be a constant regardless of oxygen conditions. However, the parameter η was included in the growth equation of heterotrophs under anoxic conditions as a correction factor to adjust either the change in heterotrophic growth associated with anoxic conditions or the fact that only a portion of the heterotrophic biomass can denitrify [17].

Table 1
Kinetic and stoichiometric model for enhanced denitrification in aquifer systems.

Process	Component				
	OC ($mg\,OC\,L^{-1}$)	X_H ($mg\,bacteria\,L^{-1}$)	NO_3^- ($mg\,NO_3^-\,L^{-1}$)	O_2 ($mg\,O_2\,L^{-1}$)	Process rate ($mg\,L^{-1}\,d^{-1}$)
1. Growth of X_H					
Growth of X_H under aerobic conditions	$-(1/Y_H)$	1	$-(R/Y_H)$	$-(W/Y_H)$	$\mu_{max,H}\cdot\frac{OC}{OC+K_{OC}}\cdot\frac{O_2}{O_2+K_{O_2}}\cdot X_H$
Growth of X_H under anoxic conditions	$-(1/Y_H)$	1	$-(Z/Y_H)$		$\mu_{max,H}\cdot\frac{OC}{OC+K_{OC}}\cdot\frac{NO_3^-}{NO_3^-+K_{NO_3}}\cdot\frac{K_{O_2,I}}{O_2+K_{O_2,I}}\cdot\eta\cdot X_H$
2. Decay of X_H		−1			$b_H\cdot X_H$

Table 2
Initial kinetic and stoichiometric parameters for the enhanced denitrification model at 17 °C (parameters derived from Henze et al. [18]).

Parameter	Symbol	Value	Units
Heterotrophic maximum growth rate	$\mu_{max,H}$	4.90	d^{-1}
Reduction factor for anoxic conditions	η	0.8	–
Saturation coefficient for nitrate	$K_{NO_3^-}$	2.21	mg NO_3^- L^{-1}
Saturation coefficient for oxygen	K_{O_2}	0.20	mg O_2 L^{-1}
Saturation coefficient for organic carbon	K_{OC}	7.41	mg OC L^{-1}
Inhibition coefficient for oxygen	$K_{O_{2,I}}$	0.20	mg O_2 L^{-1}
Heterotrophic decay rate constant	b_H	0.44	d^{-1}
Heterotrophic growth yield	Y_H	0.91	mg cells mg OC^{-1}
Ratio NO_3^-/OC in denitrification	Z	1.86	mg NO_3^- mg OC^{-1}
Ratio O_2/OC in aerobic process	W	0.88	mg O_2 mg OC^{-1}
Ratio NO_3^-/OC in aerobic process	R	0.49	mg NO_3^- mg OC^{-1}

Another process considered was the decay of heterotrophic bacteria. Decay was modelled as a first order process with respect to heterotrophic biomass concentration (X_H). It was considered that this process was not dependent on aerobic/anoxic conditions and therefore a constant decay rate for heterotrophic bacteria was taken into account (b_H).

3.2. Kinetic and stoichiometric parameters

Initial guesses of all parameter values involved in the model were taken from the IWA Activated Sludge Model No. 1 (ASM1) at 20 °C [18]. It is well known that the kinetics of microbial mediated reactions is influenced by temperature, therefore, the temperature dependence of $\mu_{max,H}$ and b_H was included using an Arrhenius-type function:

$$\mu_{max,H}(T) = \mu_{max,H}(20\ ^\circ\mathrm{C}) \times 1.07^{(T-20)} \quad (1)$$

$$b_H(T) = b_H(20\ ^\circ\mathrm{C}) \times 1.12^{(T-20)} \quad (2)$$

in which 20 °C is the reference temperature and 1.07 and 1.12 are the constants describing the temperature influence on the maximum specific growth rate and the decay rate constant, respectively [18].

The ASM1 parameters are expressed in units of chemical oxygen demand (COD), which are typically used units in the wastewater field. However, when modelling biodegradation processes in environmental systems the use of COD units is generally not accepted, therefore mass units is the preferred method. Consequently, in this work two conversion factors, 0.38 g OC g^{-1} COD and 0.49 g cells g^{-1} COD, were used to convert COD units to equivalent mass units of organic carbon and cell biomass, respectively. These conversion factors were obtained from the stoichiometric oxidation reactions of glucose and of cells (cells considered as $C_5H_7O_2N$), respectively [19].

The derivation of the stoichiometric overall reaction for aerobic oxidation of organic carbon and denitrification was reached by thermodynamic and bioenergetic principles [19]. This approach describes the mass balance in terms of the fraction of electrons transferred from donor to acceptor (f_e) and to biomass (f_s). The fraction f_s and f_e sum up to 1. In this work, f_s and f_e values were considered to be 0.67 and 0.33, respectively, for heterotrophic bacteria growing under aerobic and anoxic conditions. These values were calculated from the yield coefficient for heterotrophs (Y_H) given by ASM1, 0.67 [18]. It should be emphasized that f_s is the dimensionless form of Y_H (g cell COD formed·(g COD oxidized)$^{-1}$) [19]. The stoichiometric reactions of microbial growth under aerobic and anoxic conditions are given in Eqs. (3) and (4), respectively:

$$0.042C_6H_{12}O_6 + 0.094O_2 + 0.022NO_3^- + 0.022H^+ \rightarrow 0.022C_5H_7O_2N + 0.138CO_2 + 0.183H_2O \quad (3)$$

$$0.042C_6H_{12}O_6 + 0.097NO_3^- + 0.097H^+ \rightarrow 0.037N_2 + 0.022C_5H_7O_2N + 0.138CO_2 + 0.220H_2O \quad (4)$$

Table 2 summarizes initial kinetic and stoichiometric parameter values with the appropriate units used in this work.

3.3. Simulation and parameter calibration

The simulation model was built from the model equations (Table 1). The software used for simulation was Simulink® (The Mathworks, Natick, MA), which is a software package for modelling, simulating, and analyzing dynamic systems. It supports non-linear systems, modelled in continuous time and allows an easy construction of the model, keeping the structure and dealing with algebraic loops.

The set of parameters obtained from the literature (Table 2) were introduced using Matlab® (The Mathworks, Natick, MA). This software allows for programming and generating a series of simulations and includes some toolboxes that are useful for parameter estimation. The function 'fmincon.m' from Matlab was used for parameter calibration. This function was programmed to work as follows: call simulink to generate the simulated values, calculate the objective function (OF), propose new parameter values and decide when the convergence is reached.

Parameter values were sought to minimise the least square objective function, which compares the predicted model values with the experimentally measured data. Nitrate and oxygen measurements were considered as indicated in the following equation:

$$OF = \left(\frac{1}{n}\sum_{i=1}^{n}(NO_{3,\mathrm{exp},i}^- - NO_3^-(\bar{p})_i)^2\right)^{1/2} + C \times \left(\frac{1}{m}\sum_{i=1}^{m}(O_{2,\mathrm{exp},i} - O_2(\bar{p})_i)^2\right)^{1/2} \quad (5)$$

in which $NO_{3,\mathrm{exp},i}^-$ and $NO_3^-(p)_i$ are vectors of n measured values and model predictions for nitrate at times t_i (i from 1 to n), $O_{2,\mathrm{exp},i}$ and $O_2(p)_i$ are vectors of m measured values and model predictions for oxygen at times t_i (i from 1 to m), and $\bar{p}$ is the vector of the parameters of the model. The factor C, that multiplies the oxygen term, took a value of 10. This factor was included in the objective function to make the nitrate and oxygen measures comparable, since nitrate concentrations were an order of magnitude higher than the oxygen concentrations. In the objective function, both nitrate and oxygen measures had the same weight, although n and m were different.

It must be emphasized that the described model (Table 1) is fourth order. It is an internal model where all parameters keep their physical meaning. Furthermore, it is a non-linear continuous system and any of the available simulation algorithms converged.

3.4. Practical identifiability study and confidence intervals determination

Practical identifiability study was performed using contour plots of the objective function calculated after the modification of pairs of parameters around the optimum predicted values.

Confidence intervals of the estimated parameters were calculated through a numerical method based on the Fisher Information Matrix (FIM). This approach has been previously used in modelling other biological processes. For example, Dorado et al. [20] used the FIM in a model simulating toluene abatement in gas biofilters and Guisasola et al. [21] in a model describing nitrification in wastewater.

The FIM matrix summarizes the quantity and quality of information obtained from experiments because it considers the sensitivity of calibrated parameters and the measurement errors of experimental data [11]. The FIM matrix was obtained as follows:

$$FIM = \sum_{i=1}^{n} (Y(t_i, \bar{p}))^T Q_i (Y(t_i, \bar{p})) \quad (6)$$

where $Y(t_i, \bar{p})$ refers to the output nitrate and oxygen sensitive functions with respect to the optimised parameters at times t_i ($i = 1$–n predicted values). It was expressed as:

$$Y = \begin{pmatrix} \frac{\partial NO_3^-}{\partial \mu_{max,H}}(t_i, \bar{p}) & \frac{\partial NO_3^-}{\partial X_{H,0}}(t_i, \bar{p}) & \frac{\partial NO_3^-}{\partial b_H}(t_i, \bar{p}) \\ \frac{\partial O_2}{\partial \mu_{max,H}}(t_i, \bar{p}) & \frac{\partial O_2}{\partial X_{H,0}}(t_i, \bar{p}) & \frac{\partial O_2}{\partial b_H}(t_i, \bar{p}) \end{pmatrix} \quad (7)$$

where Q_i refers to the inverse of the measurement error covariance matrix. In this case, the Q_i was considered constant with time and was a 2×2 matrix:

$$Q_i = \begin{pmatrix} S_{NO_3}^2 & 0 \\ 0 & S_{O_2}^2 \end{pmatrix}^{-1} \quad (8)$$

where S_{NO_3} and S_{O_2} are the measurement errors for nitrate and oxygen calculated as the standard deviation of different measurements of sample replicates in different days. In this study S values of 1.5 mg L^{-1} for nitrate and 0.04 mg L^{-1} for oxygen were obtained.

Assuming white measurement noise and no model mismatch, the inverse of the FIM provides the lower bound of the parameter estimation covariance matrix, which can be used for estimating the standard errors (σ) of the optimal estimated parameters ($\bar{p}$) [11]:

$$\sigma(\bar{p}) = \sqrt{FIM^{-1}} \quad (9)$$

Confidence intervals were then obtained considering a confidence level of 95% as follows:

$$\bar{p} \pm t_{\alpha;N-p}\sigma(\bar{p}) \quad (10)$$

4. Results and discussion

Two experimental designs were proposed, performed and compared to assess the denitrification model.

4.1. First experimental design: single pulse test

The first test consisted in a three-day experiment, in which groundwater containing an initial concentration of 9 mg oxygen L^{-1} and 76 mg nitrate L^{-1} was amended with 200 mg glucose L^{-1} (80 mg OC L^{-1}) and kept in contact with aquifer soil.

The applicability and accuracy of the model applying initial bibliographic parameter values (Table 2) was first evaluated by comparing model predicted profiles with experimental data. In this simulation an initial biomass concentration of 3.2×10^{-3} mg bacteria L^{-1} was used. This value was based on the MPN of denitrifiers in soil (1×10^5 bacteria g^{-1} dry weight soil), the soil/water ratio used in the experiments and an assumed cell mass of 3×10^{-13} g bacteria^{-1} [22].

Table 3
Obtained parameters and calculated confidence intervals with the single pulse test.

Parameter	Value	Confidence interval	Units
$\mu_{max,H}$	3.24	0.11	d^{-1}
b_H	2.40×10^{-3}	0.09	d^{-1}
$X_{H,0}$	0.92	0.80	mg L^{-1}

First results demonstrated that the model could reasonably explain the experimental behaviour of nitrate, oxygen and DOC although, an obvious time delay was present (data not shown). Therefore, an optimisation of parameter values was required.

A local screening sensitivity analysis was carried out by manually varying parameters. The sensitivity analysis was aimed to identify the model parameters influencing most significantly the model results. This analysis revealed that simulation results were sensitive to changes in primarily three input parameters: $\mu_{max,H}$, b_H and $X_{H,0}$. Especially, $X_{H,0}$ turned out to be crucial. Therefore, only these parameters were calibrated, whereas the others were fixed to the ASM1 model parameters (Table 2).

4.1.1. Model calibration

Minimisation of the objective function (Eq. (5)) gave the optimum parameter values (Table 3). Results were significantly different from the values initially considered (Table 2). $X_{H,0}$ presented the largest deviation from the initial value considered (almost three orders of magnitude). This could be explained by the uncertainty associated with the estimation of this parameter using the MPN and the following conversion to proper model units. Calibrated $\mu_{max,H}$ was observed to differ almost 34% from the initial considered value. Regarding b_H, it should be noted that it was optimised to a practically negligible value and, considering the calculated confidence interval (see Table 3 and discussion in Section 4.1.2), this parameter should be neglected in this parameter estimation. Since the decay rate is not a negligible value for heterotrophs, this indicated that the experimental design was not good enough to calibrate this parameter.

Model predictions by applying the calibrated parameters are shown in Fig. 1. As it can be observed, when using these parameter values, the output model concentrations of nitrate, oxygen and organic carbon fitted well with the experimental data. Concerning the biomass, it can be seen that mortality was almost negligible once the oxygen and nitrate concentrations were depleted, as the calibrated decay constant was almost zero.

4.1.2. Evaluation of estimated parameters quality

As previously mentioned, practical identifiability of the obtained parameters was analysed by means of contour plots of the objective function with respect to different pairs of parameters. Obtained results are depicted in Fig. 2.

A lengthy valley was observed in Fig. 2(A) indicating that $\mu_{max,H}$ and $X_{H,0}$ are parameters that are somewhat correlated. This means that at close to minimum, a deviation of one of these parameters could be compensated by a shift in the other parameter while still producing a satisfying fit between experimental data and model predictions. Fig. 2(B) and (C) shows that any value of b_H within a range near the obtained value produce the same fitting, which means that the objective function does not change.

Confidence intervals assessed through the FIM method were very high compared to the obtained parameter values (Table 3). Even in the case of b_H, the confidence interval was higher than the estimated parameter value. These confidence intervals show the uncertainty associated with the parameter values.

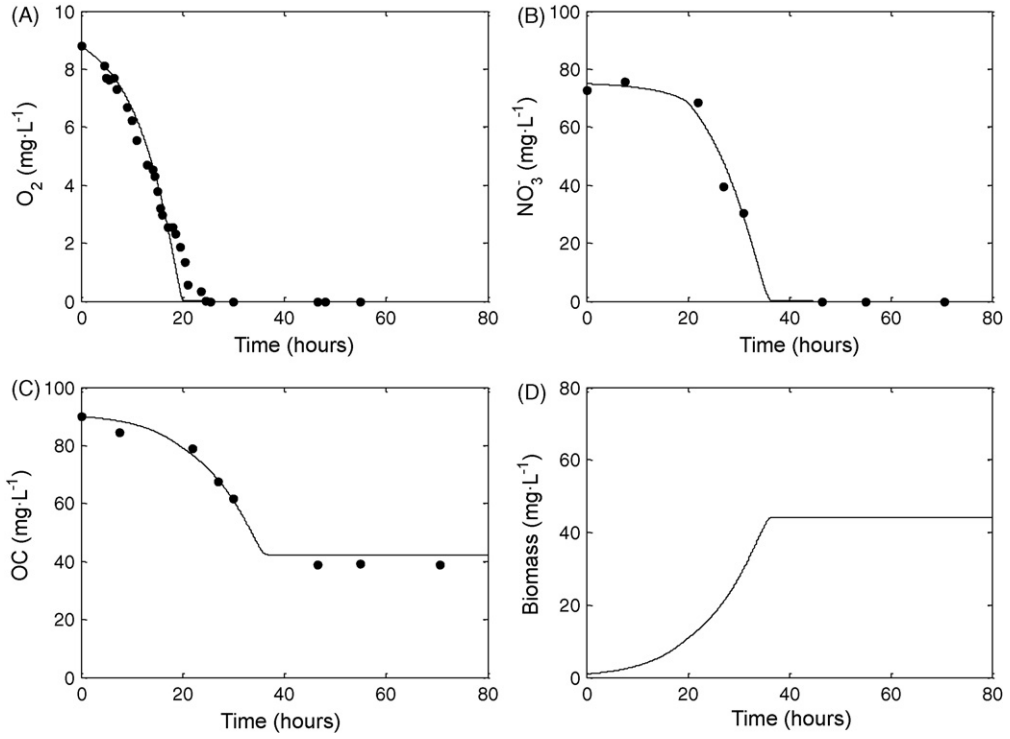

Fig. 1. Single pulse test. Experimental results (●) and model prediction with the estimated parameters (—): (A) dissolved oxygen, (B) nitrate, (C) organic carbon and (D) biomass (only model prediction).

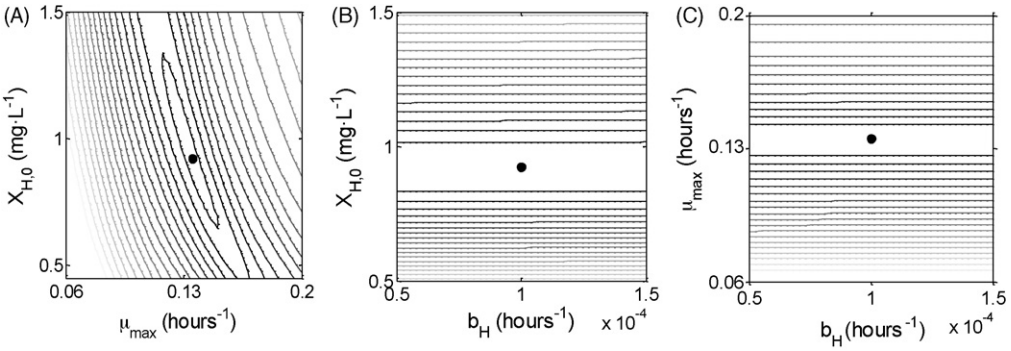

Fig. 2. Contour plots of the objective function for pairs of parameters: (A) μ_{max}–$X_{H,0}$ (B) b_H–$X_{H,0}$ (C) b_H–μ_{max} and optimised parameter values (●) with the single pulse test.

Overall, the analyses of the quality of the estimated parameters validate that the experimental data was not appropriate to calibrate the model. In order to overcome this type of practical identifiability problems, different authors have proposed using experiments with fedbatch operation, that is, with injection of additional substrate at an optimal time in the course of the tests [11,12]. Following this idea, a new batch experiment was performed.

4.2. Second experimental design: multiple pulse test

The new experimental design consisted of four successive nitrate and glucose pulses each time the nitrate concentration was depleted. Especifically, substrate concentrations in each pulse were as follows: 100 mg L^{-1} nitrate and 200 mg L^{-1} glucose in the first pulse, 50 mg L^{-1} nitrate and 100 mg L^{-1} glucose in the second pulse, 180 mg L^{-1} nitrate and 100 mg L^{-1} glucose in the third pulse and 180 mg L^{-1} nitrate and 300 mg L^{-1} glucose in the fourth pulse. Initial oxygen concentration was 9 mg L^{-1}.

Table 4
Obtained parameters and calculated confidence intervals with the multiple pulse test.

Parameter	Value	Confidence interval	Units
$\mu_{max,H}$	4.93	7.0×10^{-6}	d^{-1}
b_H	0.83	9.1×10^{-7}	d^{-1}
$X_{H,0}$	0.47	2.7×10^{-5}	mg L^{-1}

4.2.1. Model calibration

Calibration of the goal parameters was performed following the same procedure than the single pulse test, the obtained values are indicated in Table 4.

A rather important observation is that the obtained $\mu_{max,H}$ and b_H values were in the same order of magnitude than the literature considered values (Table 2). Optimised $\mu_{max,H}$ was practically equal to the ASM1 value (0.6% difference), whereas the obtained b_H differed about 47% from the initial considered value. Therefore, these results seem to indicate that the kinetic parameters from ASM1

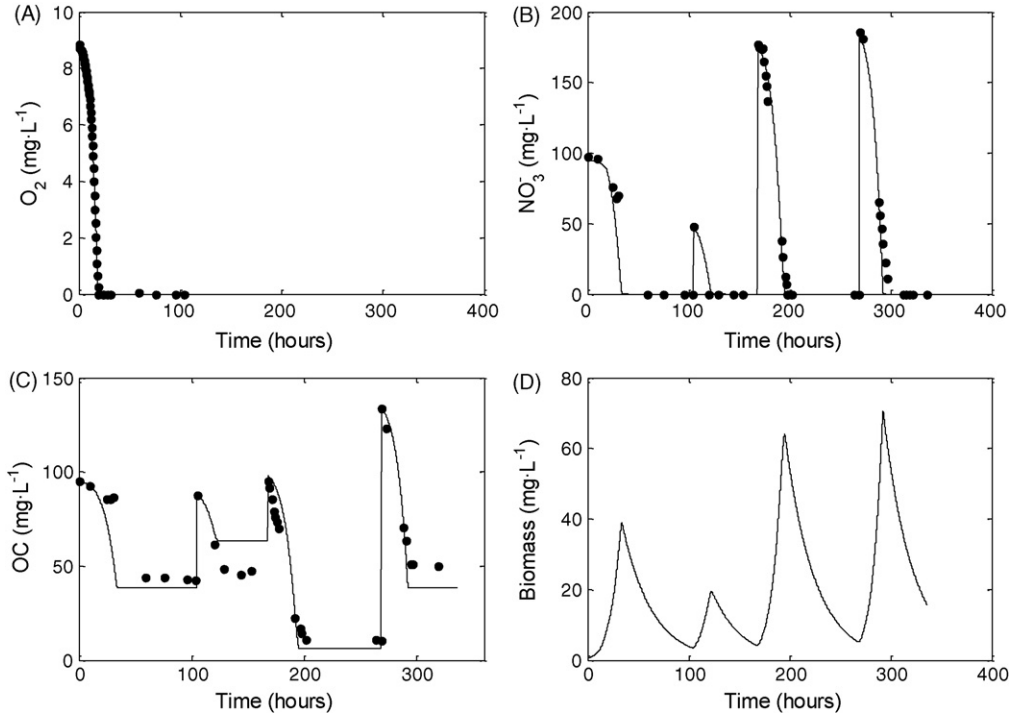

Fig. 3. Multiple pulse test. Experimental results (●) and model prediction with the estimated parameters (—): (A) dissolved oxygen, (B) nitrate, (C) organic carbon and (D) biomass (only model prediction).

are appropriate when simulating the stimulation of indigenous bacteria from the aquifer. Moreover, the calibrated initial biomass concentration of 0.47 mg L^{-1}, is consistent with, for example, the initial heterotrophic biomass concentration of 0.1 mg L^{-1} assumed by MacQuarrie et al. [23] in a model simulating the aerobic oxidation and denitrification of a wastewater plume in shallow aquifers.

Model predictions using the optimised parameters together with the experimental results are depicted in Fig. 3. It can be observed that the model described accurately oxygen and nitrate removal by heterotrophic bacteria, corroborating that it can suitably be used to simulate enhanced denitrification in microcosms with aquifer material. However, the model could not properly fit the DOC concentration profile, especially in the second pulse (Fig. 3(C)). The main differences were due to the disagreement between the theoretically considered stoichiometric ratios (i.e. Z, W and R) and the ratios obtained experimentally. These differences could be explained primarily in two reasons. On the one hand, it should be remembered that the stoichiometric ratios considered were obtained following a theoretical development, which reasonably might not agree with environmental processes. In addition, f_s was considered as a constant value throughout the experiment, but it is known that it may change with numerous environmental factors, such as substrate availability [24]. On the other hand, experimental characteristics such as the entrance of low quantities of oxyen in the microcosm or the presence of small amounts of other organic carbon substances in the aquifer soil could have influenced the experimental results. Finally, concerning the biomass, it should be mentioned that the model predicted an important mortality each time oxygen and nitrate were depleted (Fig. 3(D)).

4.2.2. Evaluation of estimated parameters quality

The objective function was calculated with different parameter values around the optimum as in the first experimental design. Fig. 4 shows the optimal values of the parameters and the contour plots for the same pairs of parameters. Compared with Fig. 2, tighter contours can be observed, demonstrating that the identifiability of estimated parameters was improved.

Parameter estimation results and their confidence intervals are shown in Table 4. As previously reported by Dochain and Vanrol-

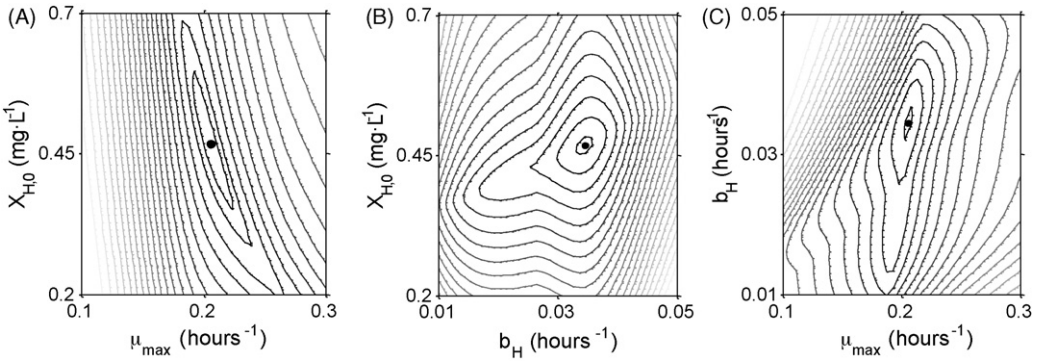

Fig. 4. Contour plot of the objective function for pairs of parameters: (A) μ_{max}–$X_{H,0}$ (B) b_H–$X_{H,0}$ (C) μ_{max}–b_H and optimised parameter values (●) with the multiple pulse test.

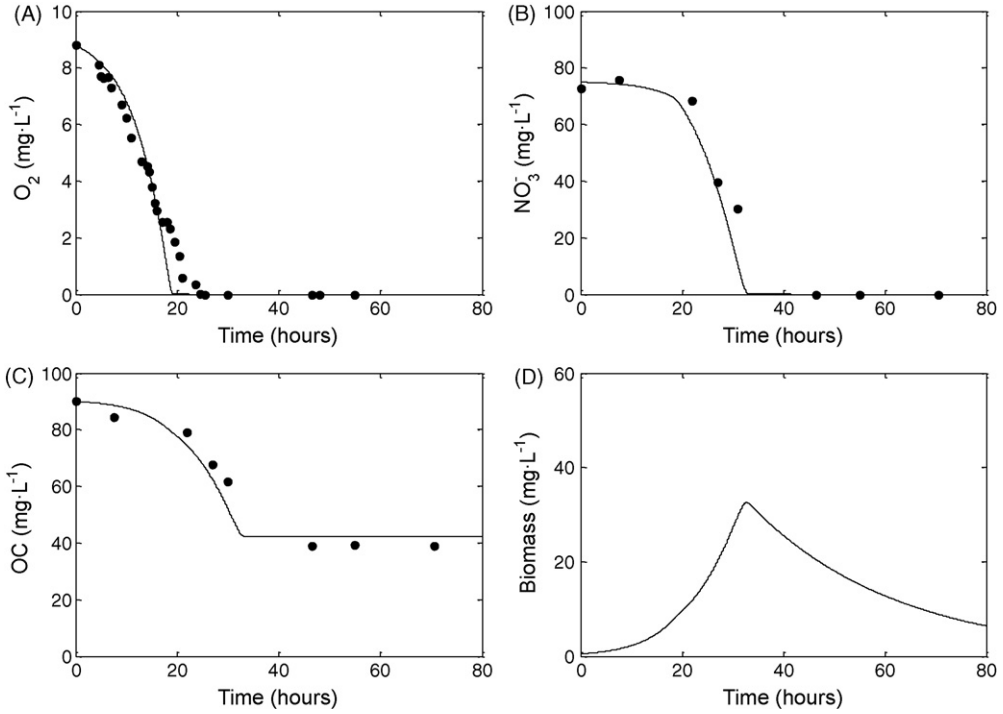

Fig. 5. Validation. Experimental results (●) of the single pulse test and model prediction (—) with the calibrated parameters from the multiple pulse test: (A) dissolved oxygen, (B) nitrate, (C) organic carbon and (D) biomass (only model prediction).

leghem [11], it should be noted that the confidence intervals are very small since they do not consider modelling errors and only the measurement errors are included in the matrix Q_i. Although the method applied might underestimate the confidence intervals, it can be concluded that the use of the multiple pulse test resulted in a better calibration of the kinetic parameters with respect to the use of the one pulse test, as also indicated by the contour plots analyses (Fig. 4).

4.3. Model evaluation and perspectives

The parameters calibrated in the multiple pulse experiment were tested against the single pulse experiment and it was proved that there was reasonable agreement between model predictions and the measured data (Fig. 5). Hence, it was clearly demonstrated that the new experiment design, consisting of four consecutive pulses of nitrate and glucose, was useful to calibrate the goal parameters and that the model could successfully explain the main processes involved in the microcosm tests.

Modelling the transport and fate of nitrate in the subsurface environment is a great deal for the comprehensive implementation of enhanced denitrification in aquifers. However, before having complete denitrification models in the subsurface environment, the development of accurate models describing the microbial processes is required. In this work, a first approach in order to model the implementation of an *in situ* enhanced denitrification process in a nitrate-contaminated aquifer was performed. However, future work should be addressed on the extension of this model to explain enhanced denitrification under dynamic conditions and on testing it with specially designed dynamic experiments. The final goal should be the simulation of chemical species and biomass behaviour in a real case of *in situ* bioremediation.

5. Conclusions

A mathematical model to describe enhanced denitrification in aquifer systems, considering the aerobic oxidation of organic matter and denitrification, could successfully explain the experimental data obtained in the microcosm batch tests.

A single denitrification test was not appropriate to calibrate the most sensitive parameters. Practical identifiability analysis revealed that $\mu_{max,H}$ and $X_{H,0}$ were parameters that were somewhat correlated and b_H could not be calibrated. In addition, calculation of confidence intervals demonstrated the large uncertainty of the calibrated values.

A second experimental design consisting of four consecutive denitrification tests could successfully be used to calibrate the most sensitive parameters. The estimated parameters were $\mu_{max,H} = 4.93\ d^{-1}$, $b_H = 0.83\ d^{-1}$ and $X_{H,0} = 0.47\ mg\ L^{-1}$. These values were consistent with published values. The contour plots of the objective function and confidence intervals of the calibrated parameters showed that the four-pulse experiment design improved parameter identifiability.

The proposed model and the kinetic parameter values estimated in this paper constitute a first approach for modelling the implementation of enhanced denitrification in nitrate-contaminated aquifer systems. Future work should be addressed on the extension of this model to explain enhanced denitrification under dynamic conditions and on testing it with specially designed dynamic experiments.

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