Using Parameter Sensitivity Analysis of the CANON Biofilm Process: What To Measure, Where To Measure and Under Which Conditions?

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Abstract Steady state sensitivity analysis revealed that only the measurement of ammonium and nitrite yields information concerning the maximum specific growth rate and the affinity constants of ammonium oxidizers and Anammox organisms in the CANON process. However practically no information concerning the Anammox nitrite inhibition constant could be derived using steady state sensitivity functions. Therefore, dynamic experiments are proposed where nitrite is injected to a continuously operated CANON reactor. Based on experimental design calculations and practical considerations it was concluded that injecting 30 mgN L^{-1} of nitrite gave the most information concerning the nitrite inhibition constant of the Anammox process. The use of (bio)sensors to measure the nitrite and ammonium concentrations during the dynamic experiments is advised because of the high quality and frequency of such data.

Keywords Anammox, biofilm modelling, CANON, sensitivity analysis, optimal experimental design

Introduction

Recently innovative processes for nitrogen removal have been developed, for example the CANON process (Hao *et al.*, 2002a&b). These processes significantly improve nitrogen removal and are based on the combination of partial nitritation, where ammonium is partially oxidized to nitrite, and anaerobic ammonium oxidation (Anammox), a process in which ammonium and nitrite are combined to form nitrogen gas.

This combined completely autotrophic process has great potential since there is no need for external carbon addition, sludge production is very low, and oxygen input and aeration energy requirements are largely reduced (Jetten *et al.*, 1997). Characteristics of the Anammox organisms are the low growth rate, low biomass yield and the inhibition by oxygen and nitrite. Because of this low growth rate and biomass yield, start-up is lengthy and the use of a reactor with high retention capacity essential. Anammox was already cultivated in trickling filters, packed bed reactors, moving bed reactors, fluidized bed reactors, UASB reactors, SBR reactors, gas lift reactors and MBR reactors (Strous *et al.*, 2002; Wyffels *et al.*, 2003b). In the first four types of reactors, the bacteria grow as biofilms on the support material, while in the last four they form free-floating aggregates. In this contribution the focus will be on biofilm systems.

A modelling and simulation environment such as WEST® (www.hemmis.be, Vanhooren *et al.*, 2003) is a very suitable tool for further optimization of these completely autotrophic nitrogen removal as experimental work is very time consuming. In literature few studies towards modelling and simulation of the Anammox organisms are presented. Hao *et al.*

(2002 a&b) performed a thorough simulation study on the behaviour of a CANON system under different process conditions, such as varying temperature and dissolved oxygen concentration. However no verification with real data was performed and no start-up dynamics were included in the study. Koch *et al.* (2001) performed simulations with a similar system, but also did not include any start-up or long-term dynamic effects. In both studies different parameters for the CANON process are used. In future simulation studies it is therefore considered important to calibrate the parameters for the system under study. Since this is a time consuming task it is useful to follow a structured approach based on optimal experimental design for parameter estimation (OED-PE) (Dochain and Vanrolleghem, 2001).

With this OED-PE experiments can be designed that will produce high quality data required for an accurate model calibration. It is a solution to the complex problem of constrained choices resulting in an optimal experiment (De Pauw & Vanrolleghem, 2003). The basis of OED-PE is the Fisher Information Matrix (FIM) that summarizes the information content of the data (to be) collected in a certain experiment. Essential to calculate this FIM are the sensitivity functions (De Pauw and Vanrolleghem, 2003). These functions determine how much a parameter influences the variables of the process. Hence, if the sensitivity is large, the influence of the parameter is large and the measurement of the variable gives much information about the parameter, so that the parameter can be estimated with high accuracy. Care should also be taken that parameters are not correlated. All this can be evaluated through the FIM (Dochain and Vanrolleghem, 2001).

Materials and methods

Extension of ASM1: ASM1.e

For modelling purposes the Activated Sludge Model nr. 1 was extended with the Anammox process and 2-step nitrification and denitrification: ASM1.e (Dapena et al., 2003). In previous simulation studies endogenous respiration was used to describe decay (Hao et al., 2002 a&b; Koch et al., 2000). However, in this study the death-regeneration concept was preferred, because the behaviour under substrate limiting conditions of autotrophs is not yet clearly documented.

The maximum specific growth rate (μ^{max}_{AN}, 0.019d^{-1} at 20°C) of the Anammox biomass was derived from Strous *et al*. (1999). The decay coefficient was set to 0.0025 d^{-1}, which is an order of magnitude below the maximum growth rate. The affinity constants for ammonium (K_{NH4}^{AN}) and nitrite (K_{NO2}^{AN}) were both set to 0.3 mgN L^{-1}. Monod kinetics were used to describe the dependency of the growth rate of Anammox on ammonium. Nitrite is not only a substrate, but is also inhibiting the Anammox process (Strous *et al.*, 1999). Therefore Haldane kinetics are more appropriate than Monod kinetics. An inhibition constant ($K_{I,NO2}^{AN}$) of 20 mgNO_2^--N L^{-1} was selected based on the experiments performed by Strous et al (1999). The maximum specific growth rate (μ^{max}_{NH}) of the ammonium oxidizers was set to 0.8 d^{-1} at 20°C. The affinity constants for ammonium (K_{NH4}^{NH}) and oxygen (K_{O2}^{NH}) were set to 2.4 mgNH_4^+-N L^{-1} and 0.6 mgO_2 L^{-1} respectively. Kinetics of heterotrophs and nitrite oxidizers were derived from other studies (Hao *et al.*, 2002a; Dapena *et al.*, 2004). Kinetics were made dependent of temperature by Arrhenius type of equations.

Simulation, sensitivity analysis and OED-PE

Simulation, sensitivity analysis and OED-PE were performed in the modelling and simulation environment WEST® (www.hemmis.be, Vanhooren *et al.*, 2003). The biofilm model and simulation approach of Van Hulle *et al.* (2003) were used for simulations with the CANON biofilm reactor. The sensitivity functions were calculated both in the bulk phase and along the biofilm depth. Influent ammonium concentration was chosen to be 100 mgNH_4^+-N L^{-1}. The hydraulic retention time and temperature were set to 6 h and 35°C respectively. The aeration coefficient ($K_l a$) was set to 110 d^{-1}. This value results from the simple rule that the flux of ammonium that should be oxidised is equal to the flux of oxygen to the reactor.

Results and discussion

Steady state concentration profiles

Table 1 presents steady state effluent concentrations of the CANON system when the total influent nitrogen concentration is 100 mgNH_4^+-N L^{-1}. Biomass concentration profiles in the biofilm are depicted in Figure 1. These concentration profiles are similar to the ones calculated by Hao *et al.* (2002a&b). In the CANON biofilm system the top of the biofilm is dominated by ammonium oxidizers, while Anammox is predominant in the inner layers, although also inert and slowly biodegradable biomass is present. The oxygen concentration in the biofilm decreases rapidly because of its consumption by ammonium oxidizers.

Table 1 Steady state bulk concentrations of a CANON biofilm system with a total influent nitrogen concentration of 100 mgNH_4^+-N L^{-1}.

	Concentration [mg L^{-1}]
NO_2^-	3.5
NO_3^-	9
NH_4^+	5.5
N_2	82
O_2	0.8

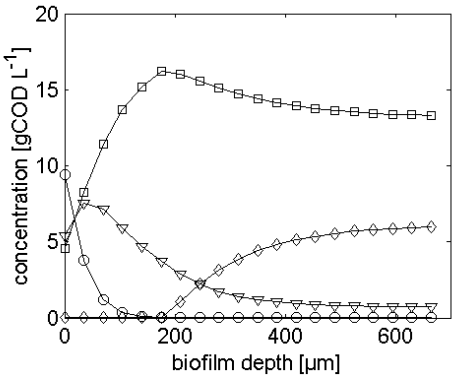

Figure 1 Biomass concentration profiles in a CANON biofilm. Ammonium oxidizers (O), Anammox (□), inert (▽) and slowly biodegradable biomass (◊) are present.

Steady state sensitivities of variables measured in the bulk phase

Calculation of the steady state sensitivities of variables measured in the bulk phase (Figure 2) revealed that the maximum specific growth rate influences the process variables most, while affinity constants are less influential, as was also observed by Hao *et al.* (2002b). In steady state operation information concerning the maximum specific growth rate and the affinity constants can be derived. However practically no information concerning the inhibition constant can be derived using steady state sensitivity functions.
It can also be seen that measuring nitrogen gas or nitrate yields hardly any information concerning the parameters, while nitrite and ammonium concentrations are most influenced by the parameters. The oxygen concentration is obviously most influenced by the kinetics of the ammonium oxidizers, but ammonia and nitrite data are more sensitive. Further experimental efforts should therefore consider the measurement of ammonium and nitrite.

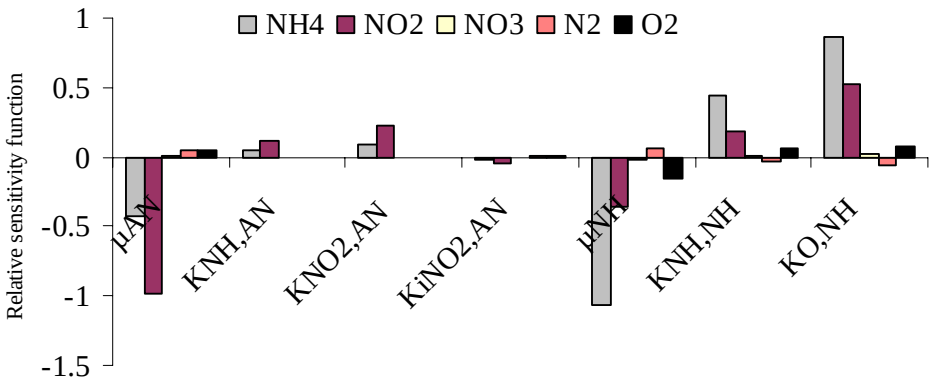

Figure 2 Steady state relative sensitivity functions in the bulk phase of the biofilm reactor

Steady state sensitivities of variables measured in the biofilm

The sensitivity functions (e.g. of ammonium and nitrite to the maximum specific growth rates of ammonium oxidizers and Anammox) tend to increase towards the inside of the biofilm (Figure 3). This means that in order to have the optimal amount of information, nitrite and ammonium should be measured in the inner parts of the biofilm. This seems logical for the kinetic parameters of the Anammox process, since Anammox is active in the inner parts of the biofilm. Noteworthy is that the sensitivities related to ammonium oxidizers show the same trend.

Such experimental design has some obvious drawbacks, because the determination of concentrations inside the biofilm is a tedious task, especially in full-scale installations.

Sensitivities under dynamic conditions with square wave influent

Sensitivity analysis under dynamic conditions was also conducted. For example in Figure 4 the average relative sensitivity functions related to the bulk concentrations of ammonium and nitrite are depicted when a square wave with 10 % noise with a twice a day frequency is added to the influent flowrate and concentrations. The values of the relative sensitivity

functions are similar to the ones in Figure 2, except for the functions related to the nitrite inhibition constant, which are somewhat higher. Application of dynamic conditions will therefore lead to more information concerning this inhibition constant, even though the Anammox process is very vulnerable to these dynamic conditions.

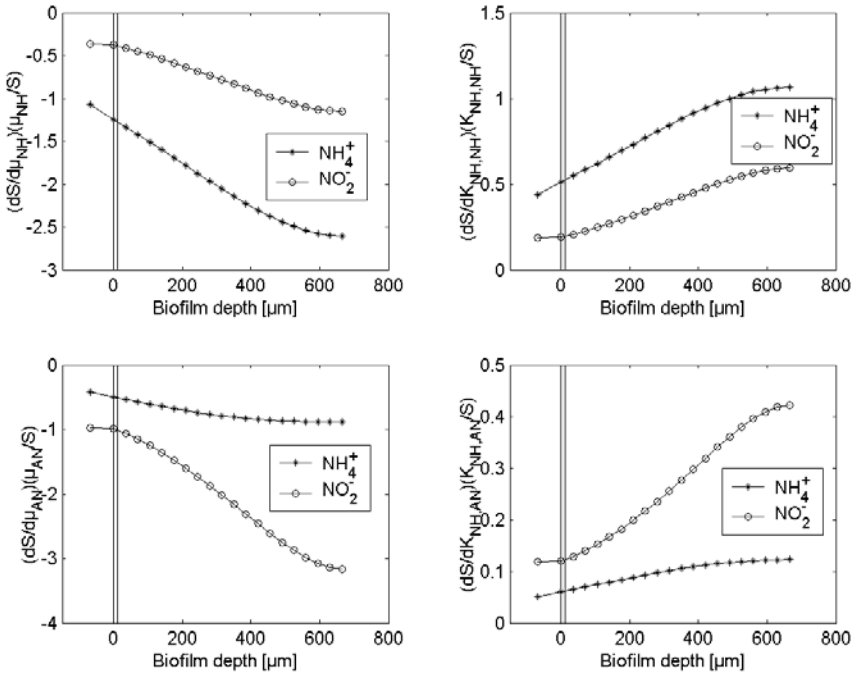

Figure 3 Steady state relative sensitivity functions of ammonium and nitrite measured in the biofilm to the maximum specific growth rate and ammonium affinity constant of ammonium oxidizers (top) and Anammox (bottom).

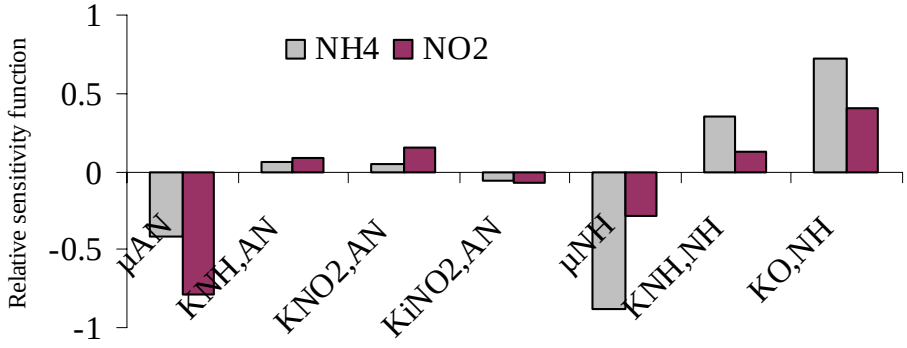

Figure 4 Average relative sensitivity functions of measurements in the bulk phase of the biofilm reactor when a square wave with 10 % noise with a twice a day frequency is added

Sensitivities under dynamic conditions with pulse substrate injection

The pulse injection of ammonium and/or nitrite to the process could also lead to more information. This experiment is easy to implement, but the question is how much of what should be injected?

Two types of experiments can be conducted: an injection of ammonium and an injection of nitrite. Injection of, for example, 25 $mgNH_4^+$-N L^{-1} ammonium to the reactor would lead to less information on the ammonium affinity constant of Anammox since the ammonium concentration would increase above its value, 0.3 $mgNH_4^+$-N L^{-1}. Similarly, the injection of nitrite would lead to less information on the nitrite affinity constant of Anammox. However, more information on the nitrite inhibition constant would be obtained. Two experiments will therefore be necessary: one with ammonium and one with nitrite injection.

Injecting too much nitrite would seriously endanger the operation of the Anammox reactor. On the other hand, injection of small amounts of nitrite or ammonium in the reactor would not lead to significant additional information. Therefore, the optimal amount of ammonium and nitrite injected would lie between 10 and 50 $mgNH_4^+$-N L^{-1} and 10 to 30 $mgNO_2^-$-N L^{-1} respectively. Since the information concerning maximum growth rate and affinity constant is already reasonable, the focus will be on the development of an optimal experiment for the determination of the nitrite inhibition constant.

The OED-PE algorithm in WEST was used to determine the optimal nitrite injection, assuming a measurement frequency of once every 15 minutes and an 8 hours experiment duration. The D-optimal criterion (determinant of the FIM) was used. It focuses on an overall reduction of parameter uncertainty. For the determination of the optimal amount of nitrite the sensitivity functions of ammonium and nitrite to the nitrite inhibition constant of the Anammox biomass were used in the FIM calculation.

From the OED-PE calculations it became clear that the maximum amount of nitrite injected gave most information concerning the nitrite inhibition constant. Corresponding relative sensitivity functions and ammonium and nitrite concentration profiles are depicted in Figure 5. When determining the inhibition constant, a trade-off between process stability and information content thus becomes evident. Also, measuring ammonium yields more information than measuring nitrite.

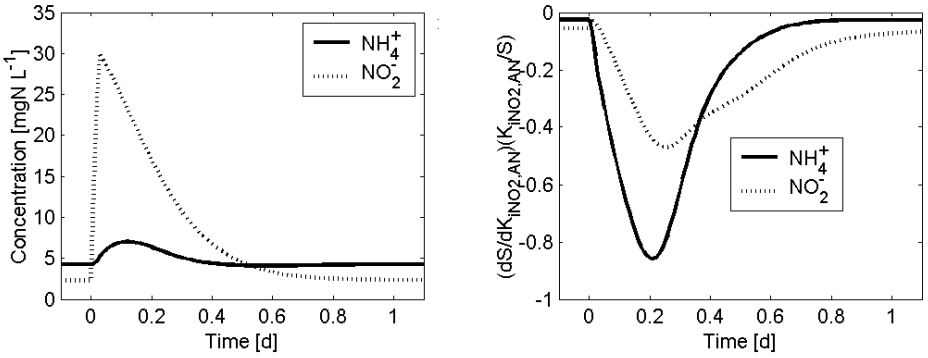

Figure 5 Ammonium and nitrite concentration profiles (left) and corresponding relative sensitivity functions (right) in the bulk phase of the biofilm reactor after injection of 30 mg NO_2^- - N L^{-1} at time 0.

Use of (bio)sensors

All the above mentioned reactor operations and experiments and the corresponding sensitivity functions assume accurate measurements and sensors with high resolution. For nitrite, a biosensor (Revsbech *et al.*, 2000, Sin *et al.*, 2004) is already described in literature and commercial application is expected soon. This nitrite biosensor contains bacteria that reduce nitrite, but not nitrate, to N_2O that is subsequently monitored by a built-in electrochemical sensor. Nitrite measurement will therefore not be interfered by the presence of nitrate. The biosensor has a linear calibration curve in a range of about 0-30 $mgNO_2$-N L^{-1} at 35°C (Nielsen et al., 2002; Sin *et al.*, 2004). It is in this range that the nitrite concentrations in the CANON reactor will typically evolve. For ammonium a sensor as described by Rieger *et al.* (2002) can be used. The operational principle is based on analyzing the potential difference between a reference electrode and a measuring electrode whose potential is sensitive to ammonium. The sensor has a linear response in a range of 0-30 $mgNH_4^+$-N L^{-1}. With such sensors high quality and high frequency data can be collected.

Conclusion

Information concerning the maximum specific growth rate and the affinity constants of the CANON process can be obtained by measuring the bulk ammonium and nitrite concentrations in a biofilm reactor. Measuring nitrate or nitrogen gas concentration yields hardly any information on the kinetic parameters.

In order to improve the information content concerning the nitrite inhibition constant a dynamic experiment is proposed where nitrite is injected to the Anammox reactor and the ammonium and nitrite evolution are measured. Based on experimental design calculations and practical considerations it was concluded that injecting 30 $mgNO_2^-$-N L^{-1} of nitrite gave the most information concerning the nitrite inhibition constant of the Anammox process.

The use of (bio)sensors to measure the nitrite and ammonium concentrations during the dynamic experiments is advised because of the high quality and frequency of their data.

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