Ammonia and nitrite oxidation rates in the inverse turbulent bed reactor determined by respirometric assay

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Abstract Respirometry, widely used to study kinetics of activated sludge, has been used to measure the oxygen uptake rate (OUR) in a nitrification biofilm process - the inverse turbulent bed reactor. The OUR has been measured at different initial concentrations of ammonia (10-100 mg $N\text{-}NH_4^+$ L^{-1}) and nitrite (3-60 mg $N\text{-}NO_2^-$ L^{-1}), as well as those of ammonia-nitrite mixtures. The OUR was independent of the initial concentration of ammonia (zero order reaction), but decreased with time. This result can be explained by a decrease in biomass concentration within the reactor during the experiments. Between each experiment, the reactor was washed with a buffer solution at a very high flow rate, that resulted in the washing out of suspended biomass but not of fixed biomass. The loss of biomass was estimated at about 50%, indicating that half the active nitrifiers in the reactor were in the form of suspended biomass. The biomass concentration within the reactor was estimated using a mathematical model and the values obtained were in agreement with the hypothesis.

Keyword Biofilm, nitrification, respirometry, ammonium oxidation, nitrite oxidation.

Introduction

Several kinetic studies of the nitrification process using respirometric measurements have been carried out in suspended growth reactors, to determine ammonia and nitrite removal rates by ammonia and nitrite oxidizing bacteria, respectively (Drtil et al., 1993; Wiesmann, 1994; Chandran and Smets, 2000). Mass balance based models considering diffusion and reaction are generally formulated for biofilm modeling in these studies, providing intrinsic kinetic models, very useful for predicting the performance of nitrifying biofilm reactors. In order to solve these models, it is required to include an intrinsic reaction rate model for both ammonia and nitrite oxidation (Denac et al., 1983; Picioreanu et al., 1998).

Respirometric assay can also be used to estimate kinetic parameters in biofilm reactors (Riefler et al., 1998). However, due to the possible formation of a thick biofilm, mass transfer inside the biofilm can limit substrate conversion. Therefore, measured conversion rates may not correspond to the intrinsic reaction rates. Respirometric assays provide several advantages over kinetic experiments based on measuring ammonia and nitrite concentration over time since DO concentration can be measured automatically on-line and with less error by a probe with a low response time and without disturbing the reactor.

The objective of the present work was to measure the oxygen consumption rate at different ammonia and/or nitrite oxidation concentrations by using the respirometric method in a laboratory inverse turbulent bed reactor (ITBR). In this ITBR, the nitrification process was previously established. From the measured transient oxygen profiles, kinetic parameter for ammonia and nitrite oxidation were estimated, and a calibrated model was used to calculate the active biomass responsible of ammonia and nitrite oxidation.

Materials and methods

Experimental set-up

The biofilm reactor used was an inverse turbulent bed reactor with an active volume of 1.35 L. The inverse turbulent bed reactor is a three-phase process recently applied at laboratory scale to the anaerobic treatment of wine vinasses (Buffière et al., 2000). In this system, an upward gas flow expands the bed of small floating particles (d=147 µm, ρ=0.69 kg L^{-3}) of a natural residue (i.e., Extendosphere™ provided by PQ Hollowsphere Ltd). This mineral granular material is composed of silica and alumina. The shape of the particles is spherical and the support concentration was 67 g L^{-1}.

Reactors operation before respirometric assays

Before the first respirometric experiment was carried out, operating conditions were kept constant for three months. A synthetic medium containing per liter 250 mg $N\text{-}NH_4^+$ as ammonium sulfate, 40.7 mg KH_2PO_4, 4.0 g $KHCO_3$ and 0.25 ml of a trace element solution was continuously fed into the reactor at a flow rate of 0.3 L h^{-1}. pH in the reactor was controlled at 7.5. Water at a controlled temperature of 30°C flowed continuously through the jacket column. A peristaltic pump was used to inject air at a flow rate of 30 L h^{-1}.

Respirometric assays

Air was injected continuously into the reactor at a flow rate of 30 L h^{-1} during each respirometric assay. Initially, dissolved oxygen (DO) concentration in the liquid increased until the saturation value (7.5 mg O_2 L^{-1}) was attained. After a period of 25-30 min of stable DO concentration, a small volume (1-5 mL) of a concentrated ammonium or/and nitrite solution was injected into the reactor. DO concentration was measured on-line and recorded by a data acquisition system. Each experiment was ended when a new stable DO concentration was obtained.
After each respirometric experiment was finished, a liquid medium without N substrate was pumped into the reactor for one hour at a flow-rate of 6 L h^{-1} to wash out the N-compounds from the reactor.

Calculations

Data from each respirometric experiment was used to calculate the initial oxygen uptake rate (r_o) corresponding to the ammonia and/or nitrite concentration assayed:

$$r_o = K_L a \cdot \left(C_o^* - C_o^i\right) - \left.\frac{dC_o}{dt}\right|_{t=ti} \qquad (1)$$

where C_o^i (mg L^{-1}) is the initial DO concentration in the reactor , C_o (mg L^{-1}) is the DO concentration in the reactor, C_o* is the DO saturation concentration, K_La is the oxygen transfer coefficient.

Mathematical model

A mathematical model was developed to estimate kinetic parameters and active biomass concentration from the transient profile of DO concentration. Model formulation was based on the following assumptions:

- Kinetics of ammonia and nitrite oxidation can be represented by a two term Monod- type equation:

$$r_{AO} = r_{AOMAX} \frac{C_{N-NH_4^+}}{K_{SAO} + C_{N-NH_4^+}} \cdot \frac{C_O}{K_{OAO} + C_O} \qquad (2)$$

with r_{AO} : ammonium oxidation rate (mg $N\text{-}NH_4^+$ g^{-1} h^{-1}), r_{AOMAX} : maximum ammonium oxidation rate (mg $N\text{-}NH_4^+$ g^{-1} h^{-1}), K_{SAO} : half-saturation constant for ammonium (mg $N\text{-}NH_4^+$ L^{-1}), K_{OAO} : half-saturation constant for oxygen of ammonium-oxidizing bacteria (mg O_2 L^{-1}).

$$r_{NO} = r_{NOMAX} \frac{C_{N-NO_2^-}}{K_{SNO} + C_{N-NO_2^-}} \cdot \frac{C_O}{K_{ONO} + C_O} \quad (3)$$

with r_{NO} : nitrite oxidation rate (mg $N\text{-}NO_2^-$ g^{-1} h^{-1}), r_{NOMAX} : maximum nitrite oxidation rate (mg $N\text{-}NO_2^-$ g^{-1} h^{-1}), K_{SNO} : half-saturation constant for nitrite (mg $N\text{-}NH_4^+$ L^{-1}), K_{ONO} : half-saturation constant for oxygen of nitrite-oxidizing bacteria (mg O_2 L^{-1}).
- Substrate conversion within the biofilm is not limited by the rate of mass transfer inside the biofilm.

The following set of equation (4-7) was obtained by combining mass balance for ammonia, nitrite, nitrate and oxygen:

$$\frac{dC_{N-NH_4^+}}{dt} = -\frac{1}{\varepsilon} \cdot r_{AO} \cdot f_{AO} \cdot C_B \cdot C_S \quad (4)$$

$$\frac{dC_{N-NO_2^-}}{dt} = \frac{1}{\varepsilon} \cdot \left(r_{AO} \cdot f_{AO} \cdot C_B \cdot C_S - r_{NO} \cdot f_{NO} \cdot C_B \cdot C_S \right) \quad (5)$$

$$\frac{dC_{N-NO_3^-}}{dt} = \frac{1}{\varepsilon} \cdot r_{NO} \cdot f_{NO} \cdot C_B \cdot C_S \quad (6)$$

$$\frac{dC_O}{dt} = \frac{1}{\varepsilon} \cdot \left(- r_{AO} \cdot f_{AO} \cdot C_B \cdot C_S \cdot Y_{AO} - r_{NO} \cdot f_{NO} \cdot C_B \cdot C_S \cdot Y_{NO} - r_e \cdot C_B \cdot C_S + K_L a \cdot \left(C_O^* - C_O \right) \right) \quad (7)$$

with ε: bed porosity, f_{AO}: fraction of ammonium-oxidizing bacteria, C_B: biomass concentration (mg g_{sup}^{-1}), C_S: support concentration (g L^{-1}), Y_{AO}: ammonium-oxidizer growth yield, f_{NO}: fraction of nitrite-oxidizing bacteria, Y_{NO}: nitrite-oxidizer growth yield, r_e: endogenous respiration rate (mg O_2 g^{-1} h^{-1}).

Kinetic parameters (r_{AOMAX}, K_{SAO}, r_{NOMAX}, K_{SNO}) were estimated by fitting the DO profile calculated by the model (equations 4-7) to the DO concentration profiles obtained at initial ammonia concentrations of 50 mg $N\text{-}NH_4^+$ L^{-1} and 100 mg $N\text{-}NH_4^+$ L^{-1}. K_{OAO}, K_{ONO}, Y_{AO} and Y_{NO} were set at values previously reported (Wiesmann, 1994), 0.63 mg O_2 L^{-1}, 1.32 mg O_2 L^{-1}, 3.16 mg O_2 mg^{-1} $N\text{-}NH_4^+$, 1.1 mg O_2 mg^{-1} $N\text{-}NO_2^-$ respectively. K_La was measured experimentally, 13 h^{-1}.

Values of ε, 0.9 and C_S, 67 g L^{-1} were constant for all experiments. Fraction of ammonia oxidizing (f_{AO}) and nitrite oxidizing (f_{NO}) bacteria in the mixed culture were not experimentally measured, and were set as 0.77 and 0.23 respectively, based on the results from Chandran and Smets (2000). Biofilm mass per mass of support (C_B) was measured, but the former mass includes cell biomass and extracellular polymeric material. The relative amount of cell biomass in the biofilm can vary between 10-90 % of the organic matter (Christensen and Characklis, 1990).

When estimation of the kinetics parameters was attempted, the fraction of cell biomass in the biofilm that best matched the experimental DO profile was 0.2. After the estimation of the kinetic parameters was completed, the values of the kinetic parameters in the model were set equal to the estimated ones. This calibrated model was then fit to the DO profile corresponding to the respirometric experiments not used for parameters estimation, to estimate the cell

biomass in the reactor. The estimates for kinetic parameters and biomass estimations were performed with a code written in MATLAB v 5.3 that implement a method for solving ordinary differential equations and a method for minimizing functions. This code minimize the residual sum of squares (RSS):

$$RSS = \sum_{i=1}^{n} \left(C_{OE}^{i} - C_{OM}^{i} \right)^2 \qquad (8)$$

where C_{OE} is the measured DO concentration, C_{OM} is the DO concentration calculated with the model and n is the number of data points in the DO concentration profile.

Results and discussion

Ammonia oxidation

Dissolved oxygen concentration during a respirometric experiment in which initial ammonia concentration was 50 mg $N\text{-}NH_4^+$ L^{-1} is shown in Figure 1. The observed rapid decrease of the DO concentration after substrate injection is a clear indication that the oxygen consumption rate was greater than the oxygen transfer rate. When a certain minimal DO concentration value was reached, a period of stable DO concentration began, depending on the mass of $(NH_4)_2SO_4$ injected. A substrate oxidation lag phase was not observed, since oxygen consumption, and then substrate oxidation, began immediately after substrate injection.

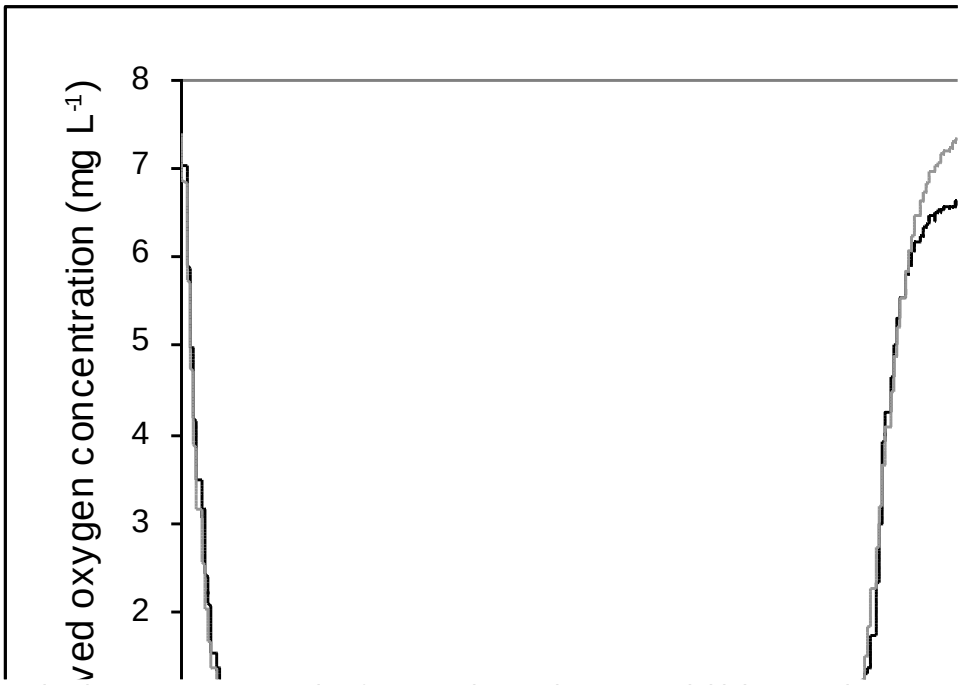

Figure 1. Dissolved oxygen concentration for a respirometric assay at initial ammonia concentration of 50 mg $N\text{-}NH_4^+$/L (e: experimental value; m: model prediction).

Ammonia oxidation rate was measured at increasing initial ammonia concentrations varying between 10 and 100 mg $N\text{-}NH_4^+$ L^{-1}. These results allowed for ruling out a possible trend between initial ammonia concentration and r_o (results not shown), but surprisingly, r_o decreased with time as Figure 2 illustrates. The time value represents the time elapsed since the beginning of the respirometric experiment series.

This observation could be explained from the analysis of the expression for oxygen consumption rate in a biofilm reactor, considering the initial conditions as not being substrate limiting (Grady et al., 1999) :

$$r_o = -r_{AOMAX} \cdot (f_{AO} \cdot C_B \cdot C_S) \cdot Y_{AO} \cdot \eta_o \quad (9)$$

The term between parentheses represents the concentration of biomass responsible for ammonia oxidation in the ITBR. The decrease of r_o may be attributed to a decrease in the concentration of ammonia oxidizing biomass in the reactor, since the effectiveness factor could not have changed in only 100 hours. Therefore, it may be assumed that about 50% of biomass was lost during this period. No increase of shear stress or detachment forces could explain the loss of attached biomass. Indeed, these high liquid flow rates have been shown to be without any effect on hydrodynamic conditions and cannot be responsible for biofilm detachment. Therefore, it is highly plausible that the biomass lost was suspended biomass. This suspended biomass was present inside the particle bed and was washed out during washing.. This result suggests that in our initial operating conditions, the ITBR was a hybrid reactor, with 50% of attached biomass and 50% of suspended biomass.

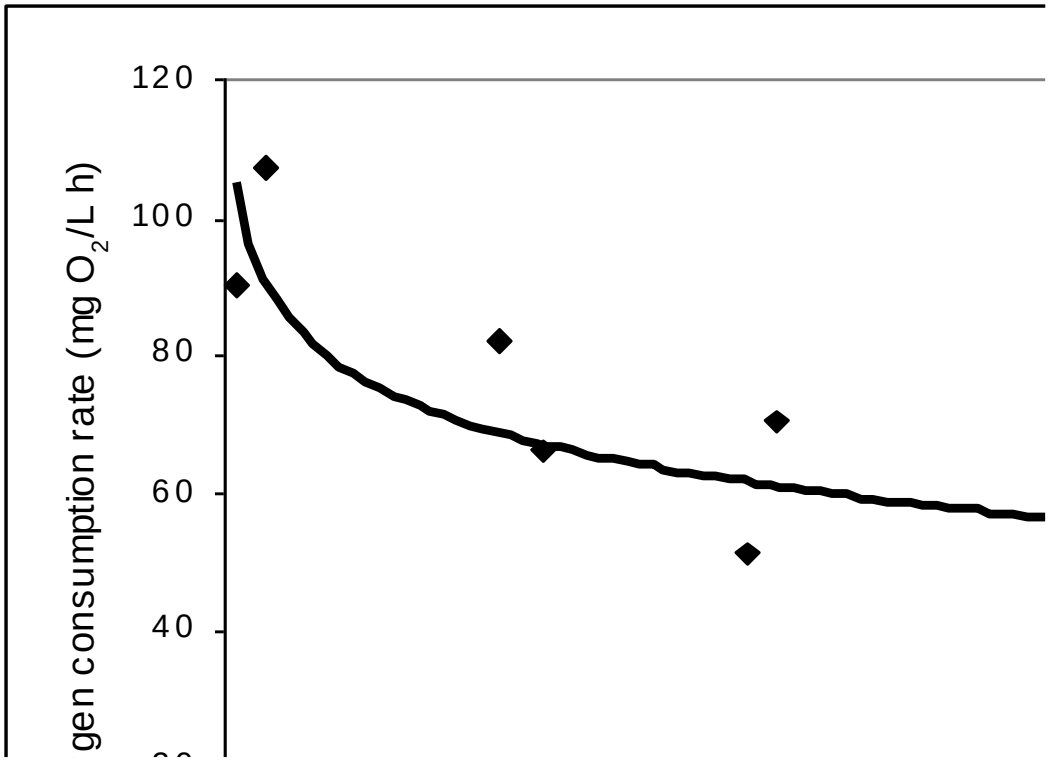

Figure 2. Oxygen consumption rates as a function of the time elapsed since the beginning of the respirometric series.

Nitrite oxidation

A respirometric assay corresponding to an initial nitrite concentration of 60 mg $N\text{-}NO_2^-$/L is presented in Figure 3. As observed for ammonia oxidation experiments, the DO concentration curve also forms a well. Since the amount of oxygen required for nitrite oxidation (1.32 mg O_2 mg^{-1} $N\text{-}NO_2^-$) is lower than that required for ammonia oxidation (3.16 mg O_2 mg^{-1} $N\text{-}NH_4^+$), the depth of the DO concentration curve well in Figure 1 is greater than that of the well in Figure 2, despite initial ammonia concentration being lower than initial nitrite concentration.

Oxygen consumption rates for nitrite concentrations in the range of 3 to 60 mg $N\text{-}NO_2^-$ L^{-1} were practically constant, Figure 3. Calculated mean r_o was 57.6±6.11 mg O_2 L^{-1} h^{-1}. This value is comparable to the mean r_o value obtained for the last four ammonia oxidation experiments (50.6 ±7.1 mg L^{-1} h^{-1}). This value is quite constant, which would indicate that the concentration of nitrite-oxidizing bacteria was constant during the experiments. Biomass concentration was not measured experimentally in the reactor but values obtained using the model were constant in the nitrite oxidation experiments (60.2 ± 5.4 mg g^{-1})

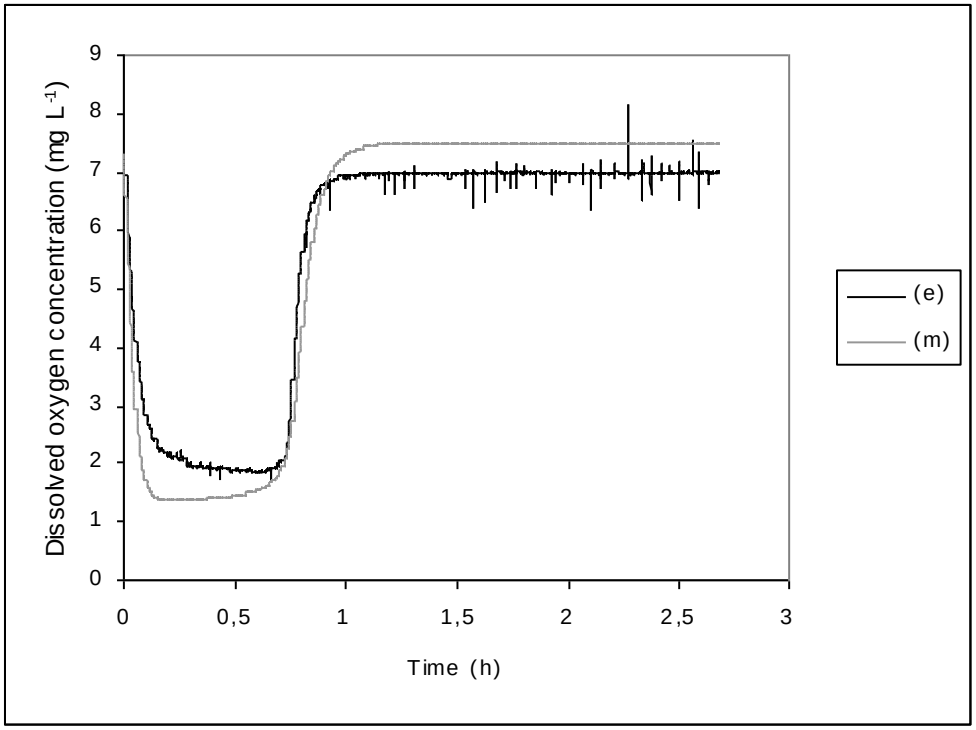

Figure 3. Dissolved oxygen concentration in a respirometric assay at initial nitrite concentration of 60 mg $N\text{-}NO_2^-$/L (e: experimental value; m: model prediction).

Mixtures

Two respirometric experiments were carried out injecting a mixture of $(NH_4)_2SO_4$ and $NaNO_2$ into the reactor. Figure 4 shows the DO concentration profile when the initial ammonia and nitrite concentration in the reactor were 50 mg $N\text{-}NH_4^+$ L^{-1} and 15 mg $N\text{-}NO_2^-L^{-1}$, respectively. Calculated r_o value for this experiment was 63.7 mg O_2 L^{-1} h^{-1}. Initial concentrations in the other experiment were 30 mg $N\text{-}NH_4^+$ L^{-1} and 10 mg $N\text{-}NO_2^-$ L^{-1}, and the resultant r_o value 65.6 mg O_2 L^{-1} h^{-1}.

Kinetic parameters and biomass estimation

From the oxygen profiles obtained at initial ammonia concentrations of 50 mg $N\text{-}NH_4^+$ L^{-1} and 100 mg $N\text{-}NH_4^+$ L^{-1}, the kinetic parameters for ammonia and nitrite oxidation were estimated. Calculated values are the following : r_{AOMAX}=0.015 mg $N\text{-}NH_4^+$ h^{-1} g^{-1}, K_{SAO}=0.25 mg $N\text{-}NH_4^+$ L^{-1}, r_{NOMAX}=0.13 mg $N\text{-}NO_2^-$ h^{-1} g^{-1}, K_{SNO} =1.6 mg $N\text{-}NO_2^-$ L^{-1}. These parameters were used to model the DO evolution in the experiment at 50 mg $N\text{-}NH_4^+$ L^{-1}. They were also applied in a calibrated model to calculate cellular biomass concentration in the reactor.

The model predictions and the experimental data in an experiment at 60 mg $N\text{-}NO_2^-$ L^{-1} (Figure 3) and with a mixture of ammonia and nitrite (50 mg $N\text{-}NH_4^+$ L^{-1} and 15 mg $N\text{-}NO_2^-$ L^{-1}) (Figure 5) are presented. The biomass concentration was estimated in each case and was 64.55 mg g^{-1} in the experiment with nitrite and 25.7 mg g^{-1} in the experiment with the mixture. In the experiments with nitrite, the average biomass concentration was 60.2 ± 5.4 mg g^{-1}. The constant value in these experiments with nitrite supports the hypothesis of distribution of the biomass between suspended biomass and attached biomass.

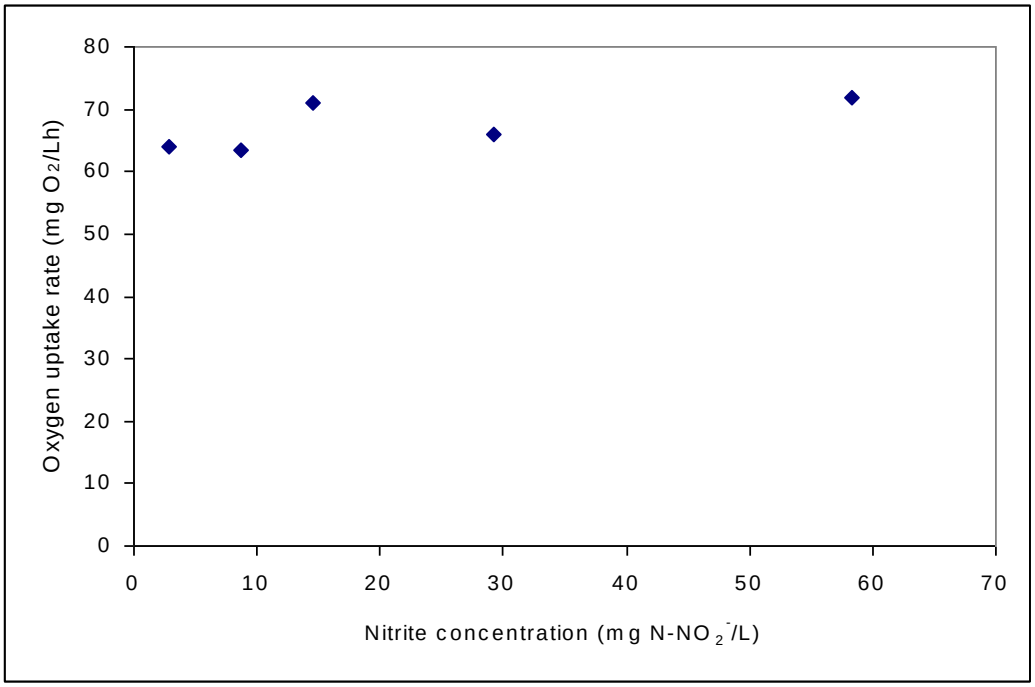

Figure 4. Oxygen consumption rates for nitrite oxidation experiments.

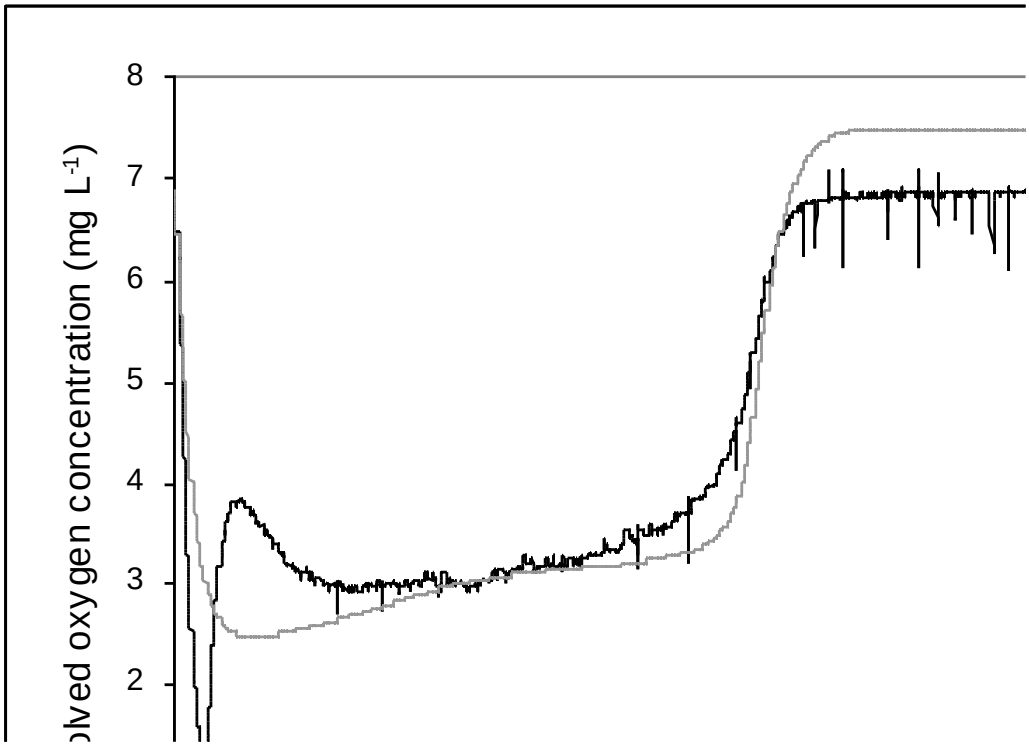

Figure 5. Dissolved oxygen concentration for a respirometric assay (initial ammonia concentration 50 mg $N\text{-}NH_4^+$/L, initial nitrite concentration 15 mg $N\text{-}NO_2^-$/L; e: experimental value, m: model prediction).

Conclusions

Respirometry was used to measure the oxygen uptake rate in ammonia and nitrite oxidation experiments in an inverse turbulent bed reactor. The results obtained show that the biomass in this kind of reactor is present not only as biofilm but that suspended biomass accounts for a large fraction. From the DO concentration profiles, it has been possible to estimate the kinetic parameters of ammonia and nitrite oxidation and to calculate values of biomass concentration in the reactor that support the experimental results obtained.

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