Modeling the decay of nitrite oxidizing bacteria under different reduction potential conditions

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Declarations of interest: none

Highlights:

A respirometric methodology was validated to measure NOB activity as OUR.
The NOB decay rate for anoxic conditions was 85% lower than for aerobic conditions.
The NOB decay rate for anaerobic conditions was 92% lower than for aerobic conditions.
Simulating with proposed reduction factor renders up to 86% less effluent soluble N.
Abstract

Autotrophic growth and decay rates of ammonium and nitrite oxidizing bacteria (AOB and NOB, respectively) have a significant impact on the design and on the process performance of wastewater treatment systems where nitrification occurs. Literature data on the separate decay rates of AOB and NOB is scarce and inconsistent. In this study, batch experiments based on respirometric techniques were conducted to determine the NOB decay rates under different oxidation-reduction potential conditions, in order to widen the understanding of nitrite dynamics.

The decay rate measured under anoxic conditions was 85% lower than under aerobic conditions, whereas under anaerobic conditions the decay rate reduction was 92%. A design and simulation tool was used to assess the impact of applying these results in differentiated areas of an activated sludge system. Simulations show a greater impact for systems with a sludge retention time under 10 days, for which up to a 16-fold increase in NOB biomass concentration and up to 86% and 80% reductions in ammonium and nitrite concentrations in the effluent were calculated.

Therefore, this work demonstrates that considering different decay rates for autotrophic biomass under different ORP conditions avoids underestimating system performance and over dimensioning new activated sludge schemes.

Keywords: ammonia oxidizing bacteria (AOB), decay rate, nitrite oxidizing bacteria (NOB), OUR, respirometry.
1. Introduction

The most used nitrogen removal strategy in wastewaters is the nitrification-denitrification process, through which ammonium is converted into nitrate (nitrification) and nitrate is converted to nitrogen gas (denitrification). Nitrification is a two-step process where two different groups of autotrophic bacteria take part: in a first step, ammonium oxidizing bacteria (AOB) convert ammonium into nitrite (eq. 1); in a second step, nitrite oxidizing bacteria (NOB) convert nitrite into nitrate (eq. 2). Denitrification is carried out by heterotrophic bacteria, which reduce nitrate and nitrite to nitrogen gas.

Nitritation: \[ \text{NH}_4^+ + 1.5 \text{O}_2 \rightarrow \text{NO}_2^- + 2 \text{H}^+ + \text{H}_2\text{O} \] (eq. 1)

Nitratation: \[ \text{NO}_2^- + 0.5 \text{O}_2 \rightarrow \text{NO}_3^- \] (eq. 2)

For most municipal wastewater treatment systems under normal operating conditions, nitritation is the limiting step and there is practically no nitrite accumulation. Therefore, this component can be disregarded: traditionally, most activated sludge models include one-step nitrification in their structure (e.g. 1]). This simplification renders acceptable results for conventional operation of nitrification processes [2].

However, under certain conditions, nitrite peaks can be detected in activated sludge systems. They usually indicate a disturbance in the microbiological processes, which normally happens under unstable operation caused by a number of reasons: insufficient oxygen, low temperature, high temperature, low sludge retention time or presence of inhibitory compounds. Nitrite concentration in these cases needs to be controlled, since it can rise to toxic levels. One-step nitrification models cannot predict nor analyze these problems.
Besides the abovementioned cases, in the treatment of side streams and of industrial wastewaters, processes where nitrite formation is specifically promoted are increasingly used. In these processes (e.g. partial nitrification and anaerobic ammonium oxidation processes) nitrite plays a fundamental role, and it becomes a key component to be measured and controlled. For this task, models which account for the two-step nitrification process have been developed or adapted in the last two decades [2-7]. These mathematical structures proposed by different authors to model nitrite show discrepancies about some of the aspects involved, such as how denitrification occurs or which nitrogen species are the active substrates. At the same time, significant variability of model parameter values among different studies can be found. In those studies, some of the adopted parameters were measured by the authors whereas some others were assumed from different literature sources. A review on nitrite modeling in wastewater treatment systems can be found in [2].

Growth and decay coefficients of autotrophic bacteria directly affect the performance of nitrification, since they determine the amount of bacteria in the system and, as a result overload and bacteria wash-out are phenomena that depend on them. Therefore, they are the most important parameters affecting the design and operation of activated sludge systems. Unlike growth, autotrophic decay is an uncertain process that has been seldom studied. Still, Koch et al. [8] identified it in the set of sensitive parameters for ASM3. The term decay represents the loss of bacterial activity, which includes maintenance, lysis and predation, and is proportional to biomass loss. Specifically differentiated AOB and NOB decay rates were not frequently measured in the studies referred above, although some authors have developed and calibrated specific models for nitratation, thus obtaining a decay coefficient for NOB [9].
It has been noted that NOB (and AOB) decay rates under anoxic conditions are smaller than under aerobic conditions [10-13], although the range of observed decay rate reduction ranges from 30 to 100% and therefore further research is needed to clarify this phenomenon. Expanding the knowledge of the activity kinetics of the NOB and their dependence on ORP conditions will allow for a better control of the nitrification process and will help adapting the design of wastewater treatment plants for nitrification.

The aim of this work is to determine the NOB decay rate ($b_{NOB}$, d$^{-1}$) in different reduction potential (ORP) conditions (aerobic, anoxic and anaerobic) by means of laboratory batch experiments using respirometric techniques, which are simple and reproducible. The studied biomass from which three different decay rates were obtained was obtained from a pilot scale wastewater treatment plant. On the other hand, simulations on an activated sludge system run with the software DESASS [14] were used to assess the extent to which the use of differentiated NOB decay rates influence the results (water quality) based on system parameters (mainly SRT and anoxic-anaerobic-aerobic volumes) as compared to using an unified decay rate.

2. Material and methods

2.1 Setup descriptions

2.1.1 Pilot plant

NOB used in this study were obtained from a pilot plant which was located within the full-scale WWTP "Conca del Carraixet" and treated its primary settler effluent. The pilot plant had a modified University of Cape Town (UCT) scheme for both organic matter and nutrient removal. Temperature was controlled, with a set point at 30 °C. The
average hydraulic retention time (HRT) and the SRT for the pilot plant were maintained at 9.6 h and 7 days, respectively. The blower frequency was controlled to keep the dissolved oxygen (DO) concentration in the aerated compartment around a desired set point (2-2.5 mgO₂·l⁻¹). Figure 1 shows the layout of this process.

2.1.2 Reactor 1

Reactor 1 consisted of an aerobic completely stirred tank reactor (CSTR) installed in the laboratory, with a total volume of 10 L. Temperature was kept at 30 °C with the aid of a thermostatic bath. Dissolved oxygen (DO) concentration in the tank was monitored with a Cellox 325 electrode (WTW, Germany) connected to an oximeter (Oxi 320, SET WTW, Germany). An air blower was switched on when DO went below 2 mg O₂/L, aerating the reactor through fine bubble diffusors installed at the bottom. The blower switched off when DO reached 5 mg O₂/L.

2.1.3 Reactors 2

Reactors 2Ae, 2Ax and 2An were used for achieving different ORP conditions in the laboratory. Each had a working volume of 3 L. Their temperatures were controlled at 30 °C and the DO concentrations were monitored with a Cellox 325 electrode (WTW, Germany) connected to an oximeter (Oxi 320, SET WTW, Germany). Reactor 2Ae had an aeration system analogous to that one described for Reactor 1. Nitrate was added to Reactor 2Ax to sustain a concentration between 7 and 10 mg NO₃-N/L.

2.1.4 Batch Reactor

The Batch Reactor, with a volume of 300 mL, was used to examine the activity of the NOB biomass present in the different reactors, by means of short specific respirometric
experiments. This reactor was water jacketed for keeping temperature at 30 °C. An air blower aerated the samples at the beginning of each specific respirometry. The DO concentration was monitored like previously explained.

2.2 Experimental procedure

2.2.1 Bringing biomass to endogenous conditions

In the first place, the biomass from the pilot plant was brought to endogenous conditions in Reactor 1. For this, the aeration control system described above was switched on and biomass was therefore given enough oxygen (and time) to consume all possible substrate. The periods when the blower was in off mode were used to determine the Oxygen Uptake Rates (OURs), calculated (in Excel 2011) as the slope of the recorded DO concentrations regression line. Endogenous conditions were achieved when the OUR values remained practically constant, which happened after approximately 20 h.

After achieving endogenous conditions, the sludge in Reactor 1 was split into reactors 2Ae, 2Ax and 2An, being the ORP the only difference among them, as previously explained: in Reactor 2Ae the oxygen concentration remained over 2 mg O2/L; in Reactor 2Ax nitrate concentration was kept over 7 mg NO3-N/L; Reactor 2An remained under anaerobic conditions. There was no detectable nitrite in the reactors, and therefore, in the absence of substrate, there was no NOB growth. NOB activity from all three reactors was examined along the whole study, which lasted 160 hours, by performing short respirometric studies as explained in section 2.2.3 below.

2.2.2 Determination of NO2 concentration required to achieve maximum growth rate
Prior to the respirometric studies, eight samples from the sludge under endogenous conditions in Reactor 1 were used for determination of the required nitrite concentration to increase the growth rate up to its maximum. Each of these eight samples was transferred to the Batch Reactor and kept under aeration before adding a certain nitrite concentration ranging from 0.5 to 10 mg NO₂⁻·L⁻¹. Each test was short enough (5 min) to assume that biomass concentration in the reactor remained constant. Since (different) substrate additions caused a (different) increase in the NOB activity, an increase in the OUR could be measured each time (calculated as the difference between the measured OUR before and after nitrite addition). The obtained values from the eight tests were represented along a substrate concentration axis and thus the nitrite concentration required to achieve the maximum growth rate could be determined. The affinity constant for nitrite could also be obtained by minimizing the sum of the squared errors between experimental and predicted data, which was done using the Solver function in Microsoft Excel 2011.

2.2.3 Respirometric studies

Each respirometric study consisted in measuring the OUR increase of a specific biomass sample due to nitrite oxidation by NOB. For this, the following steps were followed: i) the sample was transferred to the Batch Reactor, where the initial OUR (OUR₁) was first measured (corresponding to endogenous activity) as the linear decrease in DO; ii) Nitrite from a 10 g·L⁻¹ sodium nitrite solution was added manually to the reactor in order to reach a concentration of at least 3 mg NO₂-N·L⁻¹. The nitrite concentration required for reaching maximum growth had been determined in a previous experiment (see 2.2.2). The substrate pulse addition reactivated the NOB biomass and was enough to reach maximum growth rate. iii) OUR was measured three consecutive times after substrate addition, and the highest measured value was taken as
iv) The difference ($OUR_2 - OUR_1$) was calculated, which was, as expected, always positive and corresponded to the oxygen uptake rate due to nitrite oxidation by NOB.
v) The sample was disposed of.

In the case of Reactor 2Ax and Reactor 2An (see below) the sample had to be reaerated before adding the nitrite, so that oxygen was available for substrate consumption. In all cases pH was monitored to assure that it stayed between 7.5 and 8.5.

2.3 Analytical methods

Nitrate (NO3-N) and nitrate (NO3-N) were determined by applying Standard Methods [15] (4500-NH3-G, 4500-P-F, 4500-NO2-B, 4500-NO3-H, respectively) in a Smartchem 200 automatic analyzer (Westco Scientific Instruments, Westco).

2.4 Simulations

The software DESASS [14] was used to simulate a WWTP for biological nitrogen removal. The mathematical model implemented in this software is the Biological Nutrient Removal Model No.2 (BNRM2, [16]). In this study, the parameter values proposed by Henze et al., [1] were used for heterotrophic bacteria. For ammonia oxidizing and nitrite oxidizing bacteria the parameter values determined by Jimenez et al., [17-18] and Jimenez [7] were used.

3. Results

3.1 Determination of NO2 concentration required to achieve maximum growth rate

As explained before, each OUR was calculated with Excel 2011 as the slope of the DO concentration regression line. $R^2$ was always above 0.97. An example can be seen in
The different nitrite concentrations tested were plotted against the obtained OUR increase due to their oxidation (specifically, against the percentage of this increase over the maximum OUR measured). According to these results, the concentration needed in further experiments to assure maximum growth rate of NOB was set to 3 mg NO$_2$-N · L$^{-1}$, since in that case 99.4% of the maximum OUR was already achieved (figure 3).

Additionally, a Monod half saturation constant for nitrite ($k_{NO}$) of 0.378 mg NO$_2$-N · L$^{-1}$ was calculated which properly reproduced the experimental data (figure 3), albeit with a regression coefficient $r^2=0.67$. The decrease observed in the OUR when 10 mg NO$_2$-N were added suggests a NOB inhibition by free nitrous acid (FNA) accumulation.

Values of $k_{NO}$ found in the literature range from 0.008 mg NO$_2$-N · L$^{-1}$ [19] to 3 mg NO$_2$-N · L$^{-1}$ [20]. Jiménez et al. [17] obtained a similar value of 0.26 NO$_2$-N · L$^{-1}$ when working with sludge obtained in the same pilot plant used in this study. Nitrite affinity, and in general, substrate affinity, can vary among bacterial cultures due to biomass floc morphology, nitrifier enrichment [21], conditioning processes and depending on the history of the sludge (process characteristics in origin, wastewater strength, etc).

### 3.2 Respirometric studies from biomass under aerobic, anoxic and anaerobic conditions

Reactor 2Ae maintained a culture of NOB under endogenous conditions and under constant oxygen supply. Seven samples were taken and underwent the respirometric test described in the Batch Reactor. The results, plotted in figure 4a, were seven points representing the OUR (that is, the increase in oxygen uptake due to the supplemented nitrite oxidation, calculated as the difference between the OUR before and after nitrite addition) along the 160 hours of the experiment. As expected, the measured OURs
decreased with time up to a 100% decrease. Regression coefficients were always above 0.96.

The same procedure was applied to data obtained from Reactor 2Ax and Reactor 2An. In Reactor 2Ax a reduction in the OUR of 40% was observed after 160h (figure 4b), whereas in Reactor 2An this decrease was only of 20% (figure 4c). In all cases, measured ammonium and phosphate concentrations increased with time during the operation of the reactors (due to hydrolysis processes), confirming the limitation of activity of nitrifiers due to the absence of DO.

Assuming that the measured OUR is directly proportional to the amount of NOB biomass in the culture (measured as mg COD·L⁻¹):

\[ \text{OUR} = k \cdot \text{NOB} \] (3)

And considering that the NOB decay function follows a first order kinetics with a decay constant \( b \) (d⁻¹), in agreement with general literature (elsewhere):

\[ \frac{d\text{NOB}}{dt} = -b \cdot \text{NOB} \] (4)

Then equation (5) can be obtained after integration of equation (4) and combination with equation (3):

\[ \text{OUR} = \text{OUR}_0 \cdot \exp(-b \cdot t) \] (5)

Equation 5 can be used to reproduce the obtained data. A decay constant was obtained for each case (using the Solver function of Microsoft Excel 2011) which minimized the error between experimental and predicted data (table 1).
The obtained aerobic decay rate is in the high end of the range found in literature (0.15 d$^{-1}$ in [12]; 0.22-0.28 d$^{-1}$ in [22]; 0.5 d$^{-1}$ in [23], which could be partially explained by the high temperature used in this study. On the other hand, it has been demonstrated that decay rates are different in different systems [24] and therefore the origin, history and degree of physiological adaptation of the culture can, at least partly, explain the differences in literature data as well. Martinage and Paul [25], for instance, reported increases of autotrophic decay rates from double to 4.5 times higher following changes in the wastewater fed to the analyzed activated sludge system. It also has to be taken into account that the design of the experiments used for the determination of the decay coefficient could also be an important factor affecting the reported decay rates.

Obtained data in the current work showed that nitrite oxidizing bacteria of the studied system present very low decay rates under anoxic conditions (85% smaller than aerobic) as well as under anaerobic conditions (92% smaller than aerobic). These reduction factors are among the highest values reported, in the range of those authors who found reductions between 50 and 100% [12, 22, 23].

The exact reason for this decrease is not well known. It could be hypothesized that maintenance or endogenous respiration, which involve consumption of external and cell-internal substrate respectively, cannot take place under anoxic and anaerobic conditions, since NOB are not able to utilize their own substrate due to the lack of oxygen. Therefore, when again available, more substrate (and therefore oxygen) is consumed (feast-famine phenomenon, [11]) and thus a higher activity than expected is observed. This would explain that the measured OURs along the experiment do not decay as fast as in aerobic conditions.

Another factor which could play a role in the decrease of the anoxic and anaerobic decay constants could be predation by other microorganisms, which causes bacterial
losses and therefore decay in biomass activity. Predators, mainly protozoa, which are present to a greater or lesser extent in activated sludge systems, are predominantly obligate aerobes and therefore their impact in biomass loss is stronger under aerobic conditions. Martinage and Paul [25] reported that 50% of their observed decrease in autotrophic anoxic decay rate with respect to aerobic decay rate was explained by grazers.

Salem et al. [24] performed respirometric studies which lasted between 3 and 14 days, and obtained, for NOB in a nitrifier-enriched culture, anoxic decay rates which were lower than the anaerobic rates -although the difference was very small (0.06 d$^{-1}$ versus 0.07 d$^{-1}$). These rates were only 25% and 12.5% lower than the aerobic decay rates. In an activated sludge system, the same authors found an aerobic decay rate of 0.21 d$^{-1}$, with anoxic decay rate 43% lower and anaerobic decay rate 71% lower. In view of the results obtained in this study and the previous works on the matter, it seems reasonable to conclude that: i) decay of NOB under aerobic conditions is confirmed to be higher than under anoxic and anaerobic conditions for the same culture and ii) the origin, state and history of the culture will influence both the aerobic decay rate and the decrease of decay rate under anoxic/anaerobic conditions. It can be noted that the results of fluorescence in situ hybridization (FISH) technique showed that NOB population in all reactors was mainly composed of *Nitrospira* (data not shown).

### 3.3 Simulations

Two sets of simulations have been carried out in order to numerically assess the effect of considering different lysis rates for different environmental conditions present in an activated sludge system. A Modified Ludzack Ettinger scheme with an anoxic reactor volume being one third of the total volume was simulated in the software DESASS,
using the mathematical model and parameters detailed in section 2.4, except for the
lysis rate of autotrophs under anoxic conditions, which was reduced by 85%. Although
the reduction factor of AOB decay rate under anoxic or anaerobic conditions has not
been determined, it has been assumed that the decay rate of AOB is affected by the
same reduction factor as for NOB since a decrease in the decay rate has also been
observed for AOB [24]. The influent wastewater pattern proposed in the Benchmark
Simulation Model n.1 [26] was used in this work. Therefore, the proposed MLE-scheme
WWTP was designed to handle an influent flow of 18,446 m³·d⁻¹. The simulated
scheme is shown in the supplementary material and the concentrations of the main
pollutants are shown in Table 2.

In the first two sets of simulations settling and biological processes taking place in the
secondary settler were not considered. Thus, the secondary settler was simulated as a
pure concentrator. In these simulations the performance of the MLE-scheme for
nitrogen removal at different sludge retention times (SRT) (ranging from 7 to 22 days)
was evaluated considering the same lysis rate in all the environmental conditions (1st
set) and considering a reduction factor of 85% for lysis rate under anoxic conditions (2nd
set).

In the last two sets of simulations settling and biological processes taking place in the
secondary settler were considered. The settling processes model [27] integrated in the
BNRM2 consists in a one-dimensional model based on both the solids flux concept and
the conservation of mass. The settling velocity is obtained by using the model proposed
by [28] for the flocculated and hindered settling. The settling velocity in the lower
layers is corrected by a compression function similar to the one proposed by [29]. This
model is linked to the biological model in order to consider the biological processes
taking place in the ten layers in which secondary settlers were divided. As it was done
previously, in these simulations the effect of sludge retention time on nitrogen removal was evaluated considering the same lysis rate in all the environmental conditions (3rd set) and considering a reduction factor of 85% for lysis rate under anoxic conditions (4th set). A summary of the simulation conditions for each set are shown in Table 3.

Considering the reduction of anoxic and anaerobic decay rates showed to have a greater impact on NOB than on AOB biomass concentration. This impact was more relevant when biological reactions were taken into account in the settler, since the settler acts as a non-aerated reactor. NOB washout was predicted at a SRT of 7 days when the same decay rate was used in all the ORP conditions. On the contrary, when a reduction factor for the lysis rate was considered, the simulation predicted NOB proliferation.

Figures 5a and 5b show the effect of considering a decay rate reduction factor on the effluent ammonium concentrations at different simulated SRT. When a decay rate reduction factor was introduced the ammonium levels in the effluent were lower than the respective values in the sets where the same decay rate was used for all ORP conditions. This impact is more relevant at lower SRT, and when considering biological processes in the secondary settler. The same trend can be observed for the nitrite levels in the effluent (figures 6a and 6b). For complete nitrification in the calculated example, a SRT of at least 10 days would be chosen based on simulations without a decay rate reduction factor. On the contrary, results obtained when a decay rate reduction factor is used indicate that a SRT of 8 days would suffice. Designing the system with an SRT of 10 days instead of 8 would lead to an excess reactor volume of 25% (for the same suspended solids concentration).

These sets of simulations demonstrate that using the same decay rate under all ORP conditions can have as a consequence that the performance of an existing system is
underrated when its operational conditions are simulated with tools such as the one
presented here, or that an activated sludge system is greatly over-dimensional in its
design phase.

It has to be noted, however, that in a full-scale WWTP bacteria and predators change
their environmental conditions more frequently, and therefore biomass might not
behave exactly like in the laboratory reactors where anoxic and anaerobic conditions
were maintained for several days. Further research is needed to validate the calibration
methodology and the obtained results with data from full-scale WWTPs.

4. Conclusions

A fast and reproducible methodology was proposed to measure NOB activity in the
form of oxygen uptake rate. This respirometric method was applied to a UCT bacterial
sludge and an important reduction was observed for anoxic and anaerobic decay rates,
compared to those under aerobic conditions. Simulations of an Activated Sludge System
were performed taking this fact into account. Differences in the ammonium and nitrite
levels in the effluent obtained could translate in a 25% smaller designed reactor volume.
This procedure avoids underestimating the performance of an existing system or the
over dimensioning of a new Activated Sludge scheme.

E-supplementary data of this work can be found in online version of the paper.
References


**Figure legends**

Figure 1: Layout of the modified UCT pilot plant origin of the NOB culture.

Figure 2: OUR calculation as the slope of the DO concentration vs. t regression line.

Figure 3: Dots: experimental OUR values (% over maximum OUR) plotted versus concentration of added nitrite. Line: predicted values with $k_{NO} = 0.378 \, \text{mg NO}_2\text{-N} \cdot \text{L}^{-1}$.

Figure 4: Dots: experimental data. Line: model calculation. a: OUR evolution under aerobic conditions; b: OUR evolution under anoxic conditions; c: OUR evolution under anaerobic conditions.

Figure 5: Ammonium concentration in effluent in a) Sets 1 and 2 and b) Sets 3 and 4, using same and smaller decay rate and for each SRT simulated.

Figure 6: Nitrite concentration in effluent in a) Sets 1 and 2 and b) Sets 3 and 4, using same and smaller decay rate and for each SRT simulated.

Figure S1: Screenshot from the software interface showing the simulated scheme and settler effluent characteristics.
Table 1: obtained decay constants for NOB in different OPR conditions

<table>
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<th>Decay constant b (d^{-1})</th>
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<td>Aerobic conditions</td>
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Table 2: Concentrations of the main pollutants in the influent to the simulated WWTP

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<th>Value</th>
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<td>mg TSS · L⁻¹</td>
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<td>VSS</td>
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Table 3: Simulation conditions for each simulation set performed

<table>
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<th>Set</th>
<th>Biological processes considered in Settler</th>
<th>$b_{\text{anox}}/b_{\text{aer}}$</th>
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<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
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<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
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<td>0.15</td>
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<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
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<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>Yes</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Fig 1

Fig 2

DO before addition

DO after addition

Lineal (DO before addition)

Lineal (DO after addition)

\[ y = -257.14x + 199.7 \]
\[ R^2 = 0.9836 \]

\[ y = -527.62x + 403.17 \]
\[ R^2 = 0.9974 \]
Fig 3

NO₂-N (mg·L⁻¹)

OUR (%)
Evolution of NOB activity in Reactor Ae

Evolution of NOB activity in Reactor Ax
Evolution of NOB activity in Reactor An

![Graph showing the evolution of NOB activity with time. The y-axis represents OUR (mg O₂·L⁻¹·d⁻¹) and the x-axis represents time (h). The graph shows a decreasing trend.]
Settler without biological processes

- NH4
- NH4 with smaller anoxic b (mg N/l)

mg NH4-N/l

SRT (d)

Settler with biological processes

- NH4
- NH4 with smaller anoxic b (mg N/l)

mg NH4-N/l

SRT (d)

Fig 5a y 5b
Settler without biological processes

- [■] NO2-
- [□] NO2- with smaller anoxic b (mg N/l)

mg NO-N/l

SRT (d)

7 8 9 10 11 12 13 14

a)

Settler with biological processes

- [■] NO2-
- [□] NO2- with smaller anoxic b (mgN/l)

mg NO-N/l

SRT (d)

7 8 9 10 11 12 13 14

b)

Figs 6a y 6b
Fig S1

Graphical abstract