Nitrite Oxidation Kinetics for a Better Understanding of the Processes in the CAFOs Effluents Treatment

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Abstract

Nitrite is an essential substance in the nitrification process; its oxidation in nature is carried out by the so-called nitrite oxidation bacteria (NOB). Understanding nitrite dynamics in natural waters or manure-contaminated waters (wastewaters) is fundamental to adjust treatment systems used in concentrated animal feeding operations (CAFOs). The aim of this work was to study the nitrite oxidation dynamics considering the effects of the DO concentrations and temperature variations and define a model capable of describing this process. For these purposes, the nitrite oxidation kinetics is characterized, analyzing the effect of relevant factors (microbial growth, pH, temperature and DO) under forced aeration (A) and anoxic conditions (NA) at 5, 10 and 20ºC.

Results showed marked effects of the temperature and OD in NOB growth rate and therefore, nitrite oxidation rate. At 20ºC, there were significantly higher rates of nitrite oxidation under NA condition than in A condition whereas at 5ºC this relation was the opposite. This suggests that at 20°C OD deficiency does not inhibit the growth of NOB, while at low temperatures (≤ 10°C), the OD is a conditioning factor for NOB growth. In addition, these results show that competition with other microorganisms strongly depends on temperature and oxygen concentration.

Keywords: Nitrite accumulation, competitive relationships, bacteria growth, effluents, feedlot, environmental modeling.

Introduction

Nitrite is a key component within the different transformations that nitrogen undergoes in aqueous systems, both natural and artificial. As an intermediate substance between ammonium and nitrate in nitrification, understanding its oxidation kinetics is essential to predict its dynamics both in surface water bodies and in treatment systems. Nitrite oxidation is accomplished by the so-called nitrite-oxidizing bacteria (NOB), which use nitrite as an electron donor in the breathing process[2]. Under nitrifying conditions, the NOB growth is directly linked to the nitrite production rate and the kinetics of nitrite oxidation.[20]

Aerobic nitrite oxidation is the second microbial mediated part of nitrification and, although it is commonly assumed that it is fast enough to be neglected against the first nitrification step[23], there are cases where nitrite concentration can be high[22]. The accumulation might appear especially when conditions suddenly change or when adverse conditions like alkaline pH values impair NOB activity[14]. This means that the first nitrification step (i.e. ammonia oxidation) is not always a limiting stage and it highlights the importance of understanding the factors affecting nitrite oxidation rate.

Nitrification is affected by several factors such as pH, dissolved oxygen (DO), temperature, substrate concentration and nitrifying microbial population[5,13]. These factors have been studied by many authors[9,26]. The concentration of DO has a significant effect on the rates of nitrifier growth and the nitrification efficiency. Although many researchers reported that lower DO inhibits the nitrifier growth, the critical values of DO recorded in the literature available were different. According to Picioreanu et al[19], the dissolved oxygen half-saturation coefficient of NOB is 1.2-1.5 mg/L. Mai et al[15] suggested maintaining 2 mg/L as the minimum oxygen level in aquaculture nitrification biofilters.

However, Richardson et al[21] suggested that the nitrifying bacteria cannot grow if the oxygen content falls below 0.5 mg/L in stream waters while Stenstrom and Song [24] proposed 0,1 mg/L. Also, Ikumi et al[10] demonstrated the dependence of nitrifiers growth time with temperature, which for NOB has an optimum rate at around 30-35°C. They also highlighted that the process is substrate limited (i.e. first order) and that for nitrifying bacteria the optimum temperature of carbon assimilation (growth) differs from that of nitrification (energy production).

In addition, they inferred that such slow growing autotrophic bacteria serve as mechanism to broaden the environmental conditions for survival, enabling better competition with heterotrophic bacteria. Therefore, it is necessary to study these factors in a more specific way, considering the nitrite oxidation separated from the ammonium oxidation and the nitrifying bacteria population of the CAFOs effluent.

In concentrated animal feeding operations (CAFOs), the treatment system must operate in batch, since wastewaters are generated only after rains (from the manure-contaminated runoff water)[8]. Therefore, the involved reactions kinetics should be studied in a non-steady state system. Within the effluent treatment systems applicable in the rural environment, the anaerobic lagoon is the most widespread and used[18,28], both for its effectiveness and for

its low operating and maintenance costs. However, in areas with abundant rainfall it is not feasible to make use of foreign developments because frequent disturbances due to rainfalls prevent the proper performance of anaerobic lagoons. This is the case of the humid pampas region of Argentina, with annual average rainfall levels exceeding 1000 mm and a high concentration of CAFOs.

Thus, the best alternative would be the so called facultative systems, which involve an aerobic layer close to the surface and anoxic or even anaerobic ones in depth. To design, optimize and improve the facultative lagoons functioning, it is necessary to know the processes involved in nutrient removal in more detail. The nitrite oxidation process is crucial, as it is directly involved in the removal of nitrogen through nitrification/denitrification processes.

In this context and in order to simulate the nitrite oxidation process in detail and follow its behavior in natural waters and wastewaters, the purpose of this study is to characterize the nitrite oxidation kinetics by analyzing the effect of certain relevant factors (microbial growth competition, temperature, pH and dissolved oxygen) and to define a model capable of describing this process.

Material and Methods

Trial description and variables measured: For this study incubation tests were performed in a batch laboratory assembly. They were carried out in a forced-air incubator in darkness at three temperatures (5, 10 and 20°C) with or without forced aeration, named A or NA respectively. In this way, six treatments were formed with four replications each: A5, A10, A20, NA5, NA10 and NA20. For instance, A20 indicates a treatment with forced aeration at 20 °C. The incubation periods were dependent on the reaction kinetics resulting in 8 days for the lowest temperatures and 4 days for 20ºC.

In order to discriminate the nitrification reaction for the different treatments, a known matrix solution inoculated with feedlot effluent was used. This matrix solution consisted of: (1) $NaNO_2$ solution (final concentration of 5 mg/L), (2) nutrient solution according to the one used for BOD determinations by APHA[1] (omitting ammonia nitrogen addition) with glucose and glutamic acid as carbon sources, 1.5 g/L of each, (3) fresh bovine CAFO effluent as inoculum, 0.5 mL/L, with a COD of 1500 mg/L. Culture medium was formulated with a theoretical total BOD of 1290 mg/L O_2 and pH 7.

The following variables were daily quantified in each replica of the different treatments using standard methods[1]: pH and OD (potentiometric method), turbidity (T) (nephelometric method) and nitrite concentration [NO_2^-] (colorimetric method by diazotization-copulation). Turbidity (T) was considered as a measure of the general microbial growth[4,6]. Since nitrification is characterized by low biomass production[30], turbidity increase is mostly an indicator of general population growth while [NO_2^-] decrease can be related to the NOB growth rate due to its constant growth[16].

Statistical analysis: The data obtained in the field were analyzed statistically with the Infostat program. Descriptive statistics were used (mean and standard deviation) to quantify the analyzed variables. A nested Analysis of Variance (ANOVA) was performed for the variables measured to assess treatment effects. DGC-test was used to compare the mean values. Likewise, we used Pearson correlation analysis to study the factors and process dynamics.

Kinetic model: The kinetic model used to account for microbial growth was represented by Gompertz equation. This was modified by Zwietering et al[31] (equation (1) to explicitly incorporate a latency time related parameter. Such equation is often useful to plot the logarithm of the relative population size [y=ln(N1/N0)] against time 7 which allows for the calculation of: maximum microbial growth rate (μT), latency time (λT) and system carrying capacity (AT). T is the turbidity of a sample at time t and T0 is the initial turbidity. As already stated earlier, T was considered an indicator of the general microbial growth.

$$\ln\left(\frac{T}{T_0}\right) = A_T \cdot e^{-e^{\frac{\mu_T \cdot e}{A_T}\cdot(\lambda_T - t)+1}} \quad (1)$$

Nitrite oxidation rate was estimated from the amount of nitrite consumed. As mentioned before, nitrite depletion is considered as an indicator of the increase in NOB population [16,12]. Nitrite disappearance correlates inversely with the formation of cellular biomass and therefore, with the growth rate as previously stated. Then, the Gompertz model is used again to describe NOB growth rate (equation (2) where [NO_2^-] and [NO_2^-]$_0$ are the nitrite concentrations in t and t_0 (initial) time respectively, μN is the maximum nitrite consumption rate or its maximum NOB growth rate, λN is the latency time and AN is the system carrying capacity. The last parameter could contain information on the NOB vs. nitrite yield.

$$-\ln\left(\frac{\left[NO_2^-\right]}{\left[NO_2^-\right]_0}\right) = A_N \cdot e^{-e^{\frac{\mu_N \cdot e}{A_N}\cdot(\lambda_N - t)+1}} \quad (2)$$

By comparing NOB growth rate (μN) with the general microbial population growth rate (μT), the relative degree of competition between NOB growth and the general population can be estimated.

Results and Discussion

Nitrification process analysis: Temporal variations of the nitrite concentration ([NO_2^-]) and turbidity (T) for all the

examined conditions are shown in fig. 1A and fig. 1B respectively. As already mentioned, the NOB population growth is related to the consumed or decrease [NO_2^-] while the general microbial population can be inferred from variations in turbidity (T). For both variables ([NO_2^-] and T) and for each temperature analyzed, significant differences between A and NA treatments ($p<0.005$ (20°) and $p<0.01$ (5° y 10°) were found after the latency time.

At 5°C, NOB growth was limited due to low temperature (Fig. 1A). After eight incubation days, 35% and 80% of N-NO_2^- initial concentration were still retained for A5 and NA5 treatments respectively. Also, at 20°C, after 72 hours of incubation, very little N-NO_2^- is retained and only for treatment A20 (Fig. 1A). These results suggest an important dependency of nitrification on temperature and REDOX conditions which implies that at temperatures above 10°C, nitrification occurs more rapidly in non-aeration than under aeration conditions. This behaviour is reversed at very low temperatures.

Fig. 1B shows that trials with forced aeration (A) reach turbidity or larger biomass concentrations before 8 days of trial than those without aeration (NA), even at 5ºC indicating that the aeration conditions favour the increase of the general microbial population at any temperature. This is consistent with general microbial growth optimum conditions, as aerobic metabolism is known to be more energetically efficient.

The modified Gompertz model satisfactorily represents the time variation of microbial general population growth and NOB population growth. The parity plot between measured and predicted values is shown in fig. 2.

Table 1 shows the parameters obtained after fitting the experimental data to the model proposed (equations 1 and (2). The maximum rates of NOB growth (μ_N) and the general microbial population growth (μ_T) as well as their respective latency times (λ_N and λ_T) are listed. The μ_N rates found in this work are much higher than those previously reported in literature[3,30]. This difference is likely to occur due to the fact that the system studied here exhibited competition effects which may vary and be very different from others, such as ocean waters, rivers and continuous wastewater treatment systems.

In treatments A, as well as NA, μ_N values differ significantly with the temperatures analysed ($p<0.001$), reaching the highest values at higher temperature, confirming strong dependence with this variable (Table 1). In addition, at above 10°C, μ_N of NA treatments are significantly higher ($p<0.01$) than those of A. In contrast, at 5°C the trend is reversed (Table 1).

Although nitrification is limited in an anoxic environment, it is known that this process does not require large amount of oxygen, since the minimum DO required by NOB is about 0.1 mg/L[24]. Fig. 1A shows that nitrite concentration reaches negligible values (<0.02 mg/L) only in treatments NA20 and NA10 indicating that nitrification can efficiently proceed in conditions of poor oxygenation and temperatures equal or greater than 10°C, which justifies the μN greater valour of NA with respect to A treatment at 20°C. Differences found between μN and μT at these temperatures, for both treatments (A y NA) support this conclusion (Table 1).

The less intense competition with general biomass is likely to be responsible for these results, given the lower amount of oxygen. Such behaviour agrees with Wang et al[28,29] who reported that under low oxygen conditions, general heterotrophic microorganisms have a much less competitive effect over NOB, evidencing oxygen as a NOB growth controller.

Table 1
Kinetic nitrification model adjusted parameters

T (ºC)	# μ_N (1/day)		λ_N (day)		μ_T (1/day)		λ_T (day)	
	NA	**A**	**NA**	**A**	**NA**	**A**	**NA**	**A**
5	0.16 aaa	0.57 aba	6.21 aaa	2.77 aba	0.99 aaa	2.71 abb	1.12 aab	2.14 aba
10	1.71 baa	3.12 bba	2.65 baa	2.83 aaa	1.04 abb	2.98 aba	0.36 bab	0.97 bbb
20	30.5 caa	9.98 cba	1.04 baa	1.92 baa	6.91 bab	19.7 bbb	0.04 cab	0.74 bbb
T (ºC)	**AN**				**AT**			
	NA		**A**		**NA**		**A**	
5	2.34 aaa		1.20 aba		21.89 aab		293.38 abb	
10	5.15 baa		1.00 aba		345.53 bab		563.57 bbb	
20	5.26 baa		2.47bba		36.03 aab		311.50 abb	

#maximum NOB growth rate (μ_N), maximum general microbial growth rate (μ_T), its respective latency times (λ_N and λ_T) and its carrying capacity (AN and AT). All curves showed adjustments with $R^2>0.98$. Different letters indicate significant differences. First letter: differences between temperatures for values of the same column ($p<0.001$). Second letter: differences between NA and A for each parameter and for each temperature ($p<0.01$). Third letter: differences between μN and μT, as well as between λN and λT, for the same treatment ($p<0.05$)

At 5°C, μ_N of NA treatment is significantly lower than those of A ($p < 0.01$) resulting in greater nitrite retention in NA condition than in A (Fig. 1A). This behaviour could suggest that at low temperature the competition between general microbial population and NOB is tempered.

In fig. 1A and fig. 1B and for all the treatments, latency periods of more than one day are observed. These periods show the absence of microbial growth (Fig. 1B) and the permanence of nitrite concentrations (Fig. 1A), implying absence of chemical or biological nitrite oxidation. Latency time decreased significantly ($p<0.01$) from 5 to 20°C in all cases (Table 1). Likewise, a great difference was observed between λ_N and λ_T, being the first much greater than the second, both in A and NA and for each temperature. Such differences were more pronounced in NA conditions. These results indicated that the NOB is more sensitive to temperature effects when the oxygen is low.

Consequently, compared to general microbial population, under such conditions (Table 1), NOB is more sensitive to temperature effects but more tolerant to low oxygen levels.

Control and inhibition factors

Effects on pH: pH varied differently during A and NA treatments (Fig. 3). Under A conditions and for the different temperatures, pH rose markedly, reaching a steady value that varies between 7.5 (at 5°C) and an average value of 8.3±0.2 (for 10°C and 20°C). The pH value variation at different temperatures was possibly due to the time needed to reach the asymptotic value. The high pH at which it stabilizes could indicate the presence of bacteria with alkalinizing metabolism such as Azotobacter spp. (cosmopolitan). Such bacteria could bring alkalinity to water in greater proportion than that consumed by nitrification. This idea is supported by the correlation found between turbidity and pH ($r=0.67$, $p<0.001$).

The pH steady value is considered as optimal pH for NOB growth[27]. Likewise, Park et al[17], reported an optimal pH for NOB growth of 7.9 ±0.4, while Jimenez et al[11] reported that the NOB activity was nearly the same for the pH range 7.5–9.95. Therefore, NOB activity should not be inhibited at the pH recorded under conditions A. In contrast, NA treatments were subjected to significant acidification during the degradation process, reaching pH values of around 5.45±0.21 in agreement with a more active nitrification[25] or pre-eminence as dominant process [turbidity (T) vs. pH: $r= -0.44$; $p<0.001$].

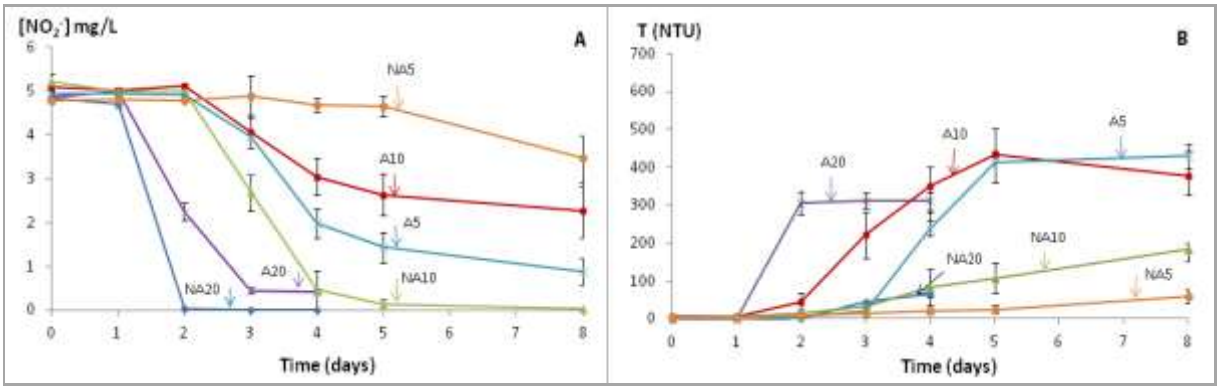

Figure 1: Variation of (1A) nitrite concentration [NO_2^-] and (1B) turbidity (T) with the reaction time in aerated (A) and non-aerated (NA) treatments, at different temperatures (5, 10 and 20°C). Error bars show the standard deviation for each mean. n=4

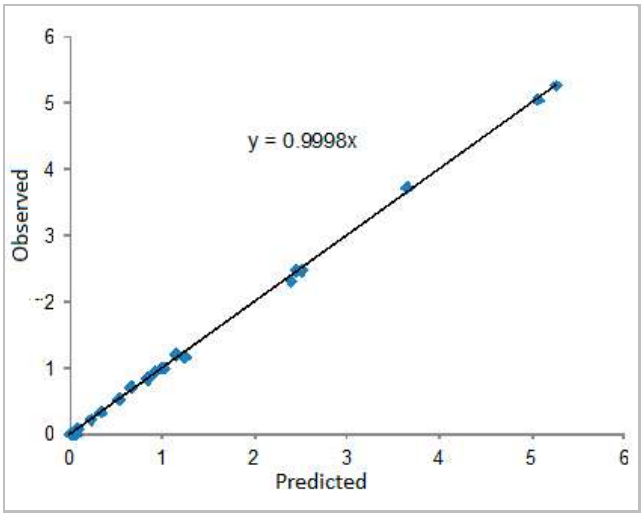

Figure 2: Parity plot between measured and predicted values for proposed model of NOB nitrification

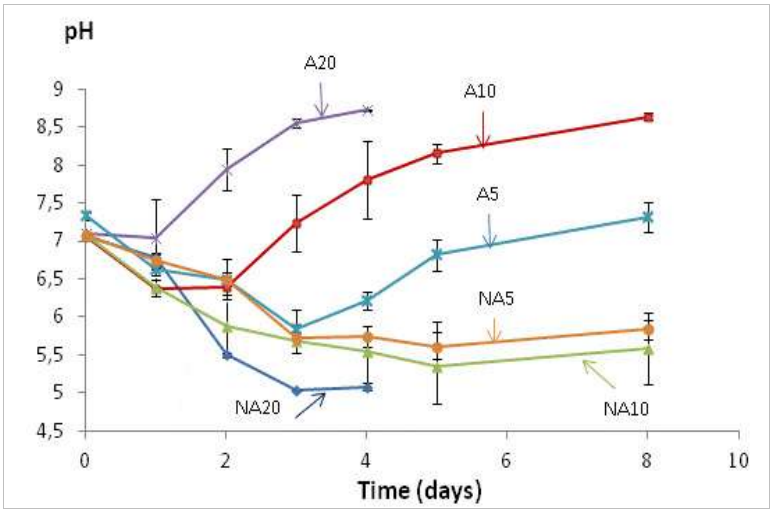

Figure 3: Variation of pH with the reaction time in aerated (A) and non-aerated (NA) treatments, at different temperatures (5, 10 and 20°C). Error bars show the standard deviation for each mean. n=4

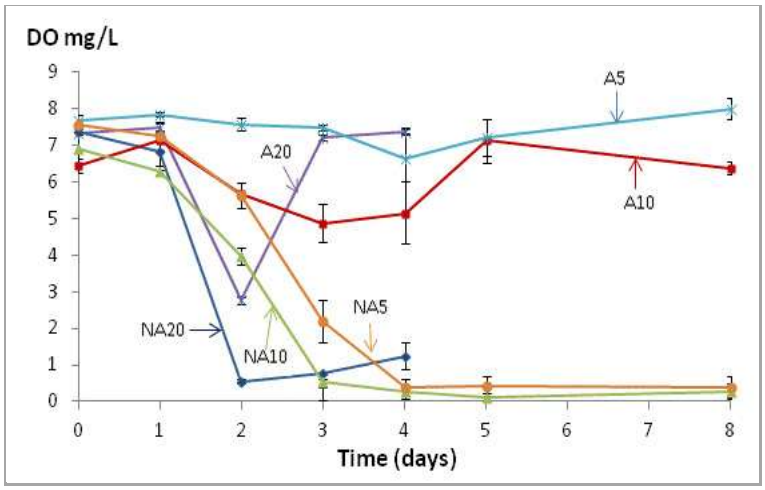

Figure 4: Variation of DO with the reaction time in aerated (A) and non-aerated (NA) treatments, at different temperatures (5, 10 and 20°C). Error bars show the standard deviation for each mean. n=4

Dissolved Oxygen (DO) and Temperature: In both cases (A and NA), DO showed a marked decrease as soon as the biological growth began, recovering afterwards only for the A treatment (Fig. 4). A significantly positive correlation (r=0.70) with [NO_2^-] was found in NA treatments, showing the disappearance of nitrite corresponding to the nitrification process.

The limitation of DO for microbial growth appears to be more important in the general microbial population than in NOB. This can be quantified by an inhibition factor according to equation (3)[23]:

$$fr = \frac{\mu_{NA}}{\mu_A} = \frac{[DO]}{K + [DO]} \qquad (3)$$

where μ_A is either the general population growth or the NOB growth rate in aerated environment (A), μ_{NA} is the same in non-aerated environment (NA), DO is the mean dissolved oxygen concentration for the NA treatments (without considering the initial concentration), K is the mean saturation constant (mg/L OD) and *fr* is the inhibition factor arising from the availability of oxygen. For the last two parameters, the subscripts T and N correspond to general microbial population and NOB respectively. Thus, the ratio between μ_{NA} and μ_A represents the inhibition factor due to oxygen deficit, assuming there is no oxygen inhibition due to be in excess.

For the general microbial population, the average values of fr_T and K_T are 0.356±0.009 and 0.56±0.020 mg/L OD respectively and showed no relation with temperature change. In contrast, for NOB, fr_N logarithm does depend linearly on temperature as expressed by equation (4):

$$\ln(fr_N) = a \cdot t + b \qquad (4)$$

where *a* is the proportionality constant between fr_N and temperature *(t)* (1/ºC) and *b* is a dimensionless parameter. Equation (4) satisfactorily represents the dependence of fr_N on temperature (t) (R^2=0.997; p<0.001), values being 0.162 1/°C and -2.144 estimated for *a* and *b* respectively while at

5 and 10 ºC limitation given by oxygen for NOB was of comparable magnitude as for general microbial population (K_N= 0.804±0.05 and 0.255±0.01 mg/L DO respectively; at 20ºC no inhibition was observed for NOB since rate (μ_N) in NA20 treatment was higher than in A20 (Table 1).

The latter could arise from a lower susceptibility to low oxygen concentrations of NOB than that of the general microbial population for temperatures near 20ºC. Consequently, the different competition effects depend on temperature and therefore the interaction between DO and temperature would modulate the competitive relationship between NOB and general microbial population, as it is shown in nitrification process analysis.

General Microbial Growth Competition: The ratio between the NOB growth rate (μ_N) and the general population growth rate (μ_T) indicated the relative inhibition arising from bacteria growth competition and followed a linear relationship with temperature (equation 5; R^2=0.9999) for treatments with no aeration (NA):

$$\frac{\mu_N}{\mu_T} = c \cdot t + d \qquad (5)$$

where *c* is the proportionality constant and *d* is a dimensionless parameter. Values obtained for *c* and *d* are 0.283±0.004 1/ºC and -1.23±0.06 respectively. Contrarily, for aerated treatments (A), there was no statistically significant relationship between the relative inhibition and temperature. These results would indicate that oxygen was the primary limiting factor and that competitive inhibition only happens when DO is in excess.

Conceptual Model: The factors and data discussed and analysed above resulted in:

- Temperature proportionally influences nitrite oxidation. The higher is the temperature, the higher is the μN.
- DO concentration can favour or inhibit the oxidation of nitrite depending on the competitive relationship between NOB and the general microbial population.
- The effect of temperature changes the effect of DO concentration on populations.
- At high temperatures (20ºC), DO determines the growth of the general population much more strongly than that of the NOB, since both populations are in a competitive situation for the carbon substrate. At low DO, the growth of the general microbial population is inhibited, allowing the NOB (more tolerant at low oxygen levels) to displace them competitively.
- At 5 and 10ºC the NOB growth rate is reduced and NOB cannot sustain competition with the general population preventing the occurrence of competitive displacement at low DO levels.
- Under low oxygen conditions, general microbial population could have a much less competitive effect over NOB, evidencing oxygen as a NOB growth controller.
- NOB, compared to general microbial population, is more sensitive to temperature effects but more tolerant to low DO levels.
- There is an important dependency of nitrite oxidation with temperature and OD concentration. These two factors would modulate the competitive relationship between NOB and general microbial population, setting up the following scenarios:

1) At high OD and high temperature, growth of the general microbial population is favoured.
2) At low oxygen and high temperature: NOB growth is favoured.
3) At low oxygen and low temperature, growth of the general microbial population is favoured (the NOB inhibition is more)
4) At high OD and low temperature: competitive interaction is minimized; optimal condition of balance between populations is presented; system stability is favoured applicable to treatment systems such as facultative lagoons.

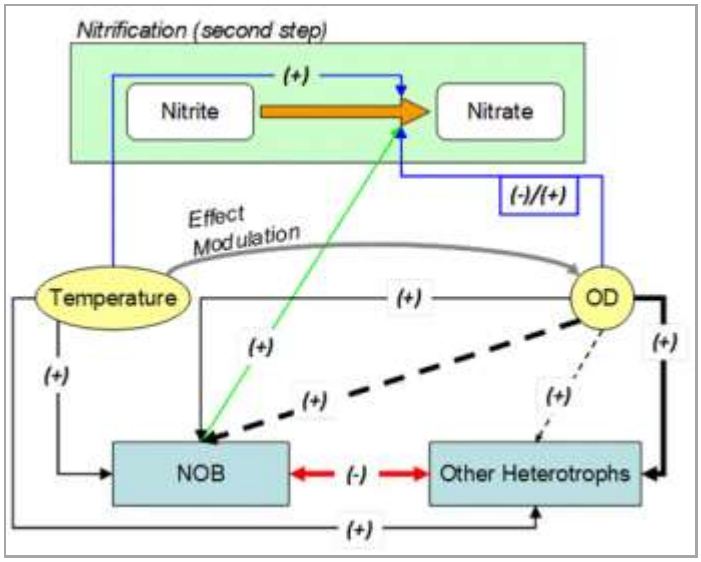

Figure 5: Conceptual model of nitrification process dynamics

Fig. 5 represents the conceptual model of nitrite oxidation dynamic in natural waters and wastewaters obtained from the analyzed results. In this model, the green area shows the global reaction while the rest of the graph shows the factors affecting it. The positive sign (+) implies that the changes between variables are directly proportional and the negative sign (-) varies inversely.

Blue arrows show the resulting influence on the overall reaction given by temperature and DO. Black arrows show the effect of oxygen and temperature over microbial population, being a solid line for temperatures higher than 10°C and dashed for same or lower 10°C temperatures. The red arrow represents the indirect interaction between general microbial population (inter-specific competition) and the green arrow, the effect of population size of NOB. For the black and red arrows, thickness indicates the relative degree of incidence of a control variable over process.

Conclusion

Understanding nitrite dynamics in natural waters or manure-contaminated waters (effluent) is fundamental to adjust effluent treatment systems used in concentrated animal feeding operations (CAFOs). Therefore, this conceptual model could contribute to improve CAFO management combining production efficiency and environmental quality.

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