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Determination of the intrinsic kinetic parameters of ammonia-oxidizing and nitrite-oxidizing bacteria in granular and flocculent sludge

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ABSTRACT

The different oxygen affinities of ammonia-oxidizing (AOB) and nitrite-oxidizing bacteria (NOB) are often used to define the operational strategy to achieve partial nitritation (PN) required before the anammox (AMX) process. For this purpose, apparent kinetic parameters are mainly used in the case of granular sludge, which can lead to errors when defining the operational conditions to obtain only nitritation (avoiding nitratation). In the present study, a mathematical methodology is proposed to determine the intrinsic kinetic parameters of AOB and NOB in granular sludge based on data obtained by respirometric assays. Additionally, the oxygen affinity constant (K_{O2}) and maximum specific rate (r_{max}) of flocculent and granular sludge sample, produced under mainstream and sidestream conditions were determined at various temperatures (15, 20 and 30 °C). The results show that for granules, the intrinsic K_{O2} and r_{max} values were lower and higher, respectively, than the apparent values. Furthermore, the K_{O2} values for flocs and granules at all of the tested temperatures were lower for NOB than for AOB. The values obtained for the kinetic parameters indicated that it is impossible to maintain partial nitritation by only controlling the dissolved oxygen concentration.

1. Introduction

Currently, many efforts are being performed to minimize energy consumption for wastewater treatment and the concept of water resource recovery facilities (WRRF) is emerging. These efforts are mainly focused on two different strategies: (a) optimizing the existing facilities by controlling the operating conditions; and (b) changing the current processes to other processes that are more energy efficient [1]. In this regard, the application of partial nitritation and anammox (PN/AMX) processes, instead of the conventional nitrification and denitrification processes, to remove autotrophically nitrogenous compounds is one of the most promising options [2,3]. These processes are already applied at full scale to treat the supernatants of anaerobic sludge digesters, which allows for the reduction of the total electrical consumption of wastewater treatment plants (WWTPs) down to 40–50% [4]. This reduction is due to the extra biogas produced in the anaerobic digester because organic matter is not consumed in the denitrification process and because the partial nitritation consumes less energy in aeration than complete nitrification. PN/AMX processes can occur in either two-units or single-unit systems. Despite the potential benefits of their implementation in the mainstream of WWTPs, until now its successful application have been limited [5–8].

An analysis of the data reported by such studies indicates two possible bottlenecks for the application of PN/AMX under mainstream conditions: (a) the relatively low nitrogen loading removal rates treated, which imply long hydraulic retention times; (b) the relatively high concentrations of nitrogenous compounds in the effluents, which make meeting the disposal requirements difficult. The first bottleneck is especially relevant in a single stage system that is based on granular biomass and can be related to the use of the low dissolved oxygen (DO) levels needed to maintain the balance between the ammonium oxidizing bacteria (AOB) and the anammox bacteria activities and, thus, the process stability. To overcome this drawback, the application of two stage systems was recently proposed to maintain suitable DO levels during the partial nitritation [9]. The second bottleneck is attributed to the presence of nitrate due to the proliferation of nitrite oxidizing bacteria (NOB). Therefore, the development of NOB seems to be a main factor that affects the effluent quality [2,10]. To this end, several

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strategies have been developed to maintain the stability of the partial nitritation process using NOB suppression [11–13].

The use of models is of great interest to determining and predicting the operational conditions that avoid the growth of NOB. For example, some years ago, to operate at low DO concentrations was a strategy proposed to accumulate nitrite during nitrification. This strategy was based on the fact that the values of the oxygen affinity constant (K_{O2}) of NOB are higher than those of AOB, as found by Balckburne et al. [14] at 19–23 °C for flocs, with values of 0.430 ± 0.080 mg O_2/L for NOB and 0.033 ± 0.003 mg O_2/L for AOB. The use of intermittent aeration was also proposed to suppress the NOB as these population exhibited a lag phase under the anoxic to aerobic transitionary conditions [15]. However, other studies show that NOB with very low oxygen affinity constants can grow under oxygen limiting conditions and cause the loss of the partial nitritation stability. For example, Wett et al. [2] reported K_{O2} values of 0.16 and 0.37 mg O_2/L and Regmi et al. [16] reported K_{O2} values of 0.16 and 1.14 mg O_2/L for NOB and AOB, respectively. However, a recent literature review of the K_{O2} values for NOB and AOB shows a wide range of variation, with minimums of 0.040 and 0.033 mg O_2/L, maximums of 0.97 and 1.16 mg O_2/L and averages of 0.19 ± 0.15 and 0.36 ± 0.4 mg O_2/L for NOB and AOB, respectively [17]. Regarding the effect of the temperature, specific studies are very scarce; for example, Wett et al. [2] showed that the K_{O2} values at 30 °C were higher than those at 20 °C for both NOB and AOB. The aggregate stage of the biomass (flocculent or biofilm) is also not very well addressed in the published research, and different values were found in the literature review performed by Arnaldos et al. [18], with respective values for NOB and AOB of 0.54 and 0.18 mg O_2/L in biofilms, of 3.00 and 1.00 mg O_2/L in granules and of 0.28–1.75 and 0.03–1.16 mg O_2/L in suspended biomass.

To obtain a realistic approach with the models, reliable values of the kinetic constants, such as the maximum specific rate (r_{max}) and the oxygen affinity constant (K_{O2}), are needed. In this regard, the use of respirometric analysis can be very helpful [19]. In the case of biomass comprising small flocs, the kinetics can be directly fitted to a Monod-type equation, while the mass transfer resistance should be taken into account for granular biomass [18]. The direct application of a Monod-type equation to data obtained from assays performed with granular biomass leads to the determination of apparent kinetic parameters, which can be used to predict the behaviour of the assayed biomass but are not useful to model systems with other types of biomass [20].

The main objective of this study is to estimate the intrinsic kinetic parameters (r_{max} and K_{O2}) of AOB and NOB of flocculent and granular biomass samples from data obtained using respirometric assays and evaluate the utility of the obtained parameters to determine the stability of the partial nitritation process.

2. Materials and methods

2.1. Biomass samples

The sludge samples used to perform the respirometric experiments were collected from two different sequencing batch reactors (SBRs) performing the PN/AMX processes in a single unit. The first biomass sample (S1) was taken from a 200 L pilot plant (ELAN® process) that was operated under sidestream conditions to treat the supernatant of an anaerobic sludge digester located in an urban WWTP [21]. This pilot plant operated at approximately 30 °C and treated nitrogen concentrations of ammonium that were between 500 and 1000 mg NH_4^+-N/L. The aeration in the ELAN® pilot plant was continuous during the reaction phase and immediately after the feeding phase of the SBR cycle. The S1 sample consisted mainly of granular sludge with a small fraction of flocculent sludge. Both fractions were separated with a sieve with a 0.3 mm pore size; the separated fractions were analysed separately. The granules of S1 had an average diameter of 1.40 mm and a density of 60 g VSS/$L_{granule}$. The second biomass sample (S2) was flocculent sludge from a laboratory SBR (4 L) that treated simulated urban wastewater and operated under conditions of 15 °C and 50 mg NH_4^+-N/L [22]. The aeration in this laboratory SBR was continuous and simultaneous to the feeding phase of the operational cycle.

2.2. Batch AOB and NOB activity tests by respirometric assays

The AOB and NOB kinetic parameters were determined using respirometric assays (in triplicate) performed using a Bench model Oxygen Meter 5300 (YSI, Yellow Springs, Ohio, USA) with oxygen selective probes (YSI 5331). The apparatus was a discontinuous respirometer provided with 15 mL vials (useful volume of 10 mL). The experimental setup was equipped with a computer data acquisition system and a thermostatic bath to control the temperature. The tested temperatures were 15, 20 and 30 °C for S1 and only 15 °C for S2, due to the low availability of this type of biomass from the laboratory reactor.

The protocol that was followed to determine the nitrifying activities was the one described by López-Fiuza et al. [23]. The sludge samples (S1 and S2) were washed with a phosphate buffer to avoid the presence of any type of substrate and/or inhibitory compounds. Then, a certain amount of biomass was added to the vials. Before starting the measurements, the samples were aerated until the saturated conditions were reached and were conditioned to the desired temperature over 30 min. Then, the oxygen probes were introduced inside the vials to start the registration of the DO concentration data. After an initial period of approximately 2 min the slope of the curve describing the endogenous oxygen consumption was determined. Then, 0.1 mL of the required substrate (ammonium or nitrite) was injected using a microsyringe to have a concentration inside the vial of 35 mg N/L, which caused faster oxygen depletion, as reflected by a steeper slope. With this procedure, the registered data gave a representation of the DO concentration variation throughout time. For the determination of AOB activities sodium azide was added (24 μM) to inhibit possible NOB activity [24].

2.3. Calculation of kinetic parameters

Since the ammonia and nitrite concentrations used during the respirometric assays are higher than those of the half-saturation constant reported for both AOB and NOB in the literature [25], the ammonia and nitrite oxidation rates can be expressed only as a function of the DO concentration.

2.3.1. Flocculent sludge

In the case of the flocculent biomass, one can consider the mass transfer resistance to be negligible, the biomass to be totally active, and the dependence of the nitrification kinetics on the DO concentration to be generally described using the Monod expression (Eq. (1)). If this equation is integrated (Eq. (2)), Eq. (3) results and then can be rearranged into Eq. (4) by adjusting the terms.

$$-\frac{dC_{O_2}}{dt} = r_{max}\cdot\frac{C_{O_2}}{K_{O_2}+C_{O_2}}\cdot X \tag{1}$$

$$\int_{t_0}^{t} r_{max}\cdot X\cdot dt = -\int_{O_{2_0}}^{O_2}\frac{K_{O_2}+C_{O_2}}{C_{O_2}}\cdot dC_{O_2} \tag{2}$$

$$r_{max}\cdot X\cdot(t-t_o) = K_{O_2}\cdot ln\frac{C_{O_{2_0}}}{C_{O_2}} + \left(C_{O_{2_0}} - C_{O_2}\right) \tag{3}$$

$$t = \frac{K_{O_2}\cdot ln\left(\frac{C_{O_{2_0}}}{C_{O_2}}\right) + \left(C_{O_{2_0}} - C_{O_2}\right)}{r_{max}\cdot X} + t_o \tag{4}$$

where r_{max} is the maximum oxygen consumption rate (mg O_2/(g VSS·d)); K_{O2} is the oxygen half-saturation constant (mg O_2/L); C_{O20} and C_{O2} are the dissolved oxygen concentrations (g O_2/L) at time zero and at a certain moment of the experiment (t, in days), respectively; and X is the concentration of biomass in the vial (g VSS/L).

2.3.2. Granular sludge

For the granular biomass, the kinetic parameters were determined according to the diffusion-reaction model, which takes into account the intrinsic kinetics and the mass transfer resistance inside the granule. Since the differential equation of the diffusion-reaction model does not have an analytical solution for the Monod kinetic model, to obtain the intrinsic kinetic parameters, the curve describing the DO concentration evolution throughout time was divided into three regions corresponding to the three different observed kinetic behaviours [26,27]:

(a) Initially, the DO concentrations are higher than the half-saturation constant (K_{O2}) value, and the active zone of the granule is fully penetrated by oxygen (no diffusional limitations). Therefore, the reaction can be considered to be of zero order (n = 0) (Eq. (5) and Eq. (6)). If Eq. (5) is integrated, a correlation between the DO concentration and time is obtained (Eq. (7)). K_0 is the constant corresponding to the zero-order reaction.

$$-\frac{d(C_{O_2})}{dt} = K_0 \tag{5}$$

$$K_0 = r_{max} \cdot X \tag{6}$$

$$C_{O_2} = C_{O_{20}} - K_0 \cdot t \tag{7}$$

(b) In a second section of the curve, the DO concentrations are higher than the half-saturation constant (K_{O2}) value, but they are insufficient to fully penetrate the active zone of the granule (diffusional limitations). In this case, the intrinsic kinetics are still of zero order, but the observed oxygen consumption corresponds to half order kinetics ($n_{apparent} = 1/2$) (Eq. (8)), which has a constant $K_{1/2}$ given by Eq. (9). The integration of Eq. (8) results in Eq. (10).

$$-\frac{d(C_{O_2})}{dt} = K_{1/2} \cdot C_{O_2}^{0.5} \tag{8}$$

$$K_{1/2} = \frac{3}{R_p} \cdot \sqrt{\frac{2 \cdot r_{max} \cdot X \cdot D_{O_2}}{\rho_b}} \tag{9}$$

$$C_{O_2} = \left(\sqrt{C_{O_{20}}} - \frac{K_{1/2}}{2} \cdot (t - t_0)\right)^2 \tag{10}$$

where R_P is the radius of the granular particle (dm), ρ_b is the density of the granular biomass (g VSS/$L_{granule}$), and D_{O2} is the oxygen diffusivity in water at the experimental temperature (dm^2/d).

(c) In the final section of the curve, the DO concentrations are similar to the half-saturation constant (K_{O2}) values, and the intrinsic kinetic is of first order. At these DO concentrations, the diffusion resistance also limited the oxygen consumption rate and the observed kinetic has an apparent order of 1 (Eq. (11)), the kinetic constant being K_1 (Eq. (12)). Integration of Eq. (11) results in Eq. (13).

$$-\frac{d(C_{O_2})}{dt} = K_1 \cdot C_{O_2} \tag{11}$$

$$K_1 = \frac{3}{R_p} \cdot \sqrt{\frac{r_{max} \cdot X \cdot D_{O_2}}{K_{O_2} \cdot \rho_b}} \tag{12}$$

$$C_{O_2} = C_{O_{20}} \cdot e^{-K_1 \cdot (t - t_0)} \tag{13}$$

By combining Eq. (9) and Eq. (12), the value of K_{O2} can be calculated as a function of the K_1 and $K_{1/2}$ values (Eq. (14)), while combining Eq. (6) and Eq. (9) obtains the value of r_{max} as a function of the K_0 and $K_{1/2}$ values (Eq. (15)). In this way, the concentration of the active biomass is not required to estimate the K_{O2} and r_{max} values.

$$K_{O_2} = \frac{1}{2} \cdot \left(\frac{K_{1/2}}{K_1}\right)^2 \tag{14}$$

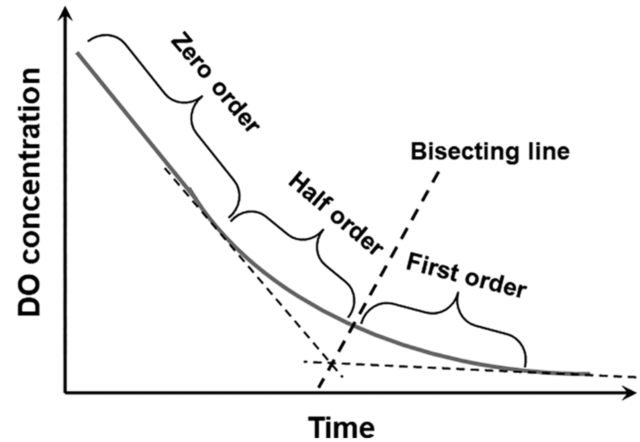

Fig. 1. Method used to determine the boundary between zones describing half-order and first-order reactions.

$$r_{max} = 2 \cdot \frac{D_{O_2}}{\rho_b} \cdot \left(\frac{3}{R_p} \cdot \frac{K_0}{K_{1/2}}\right)^2 \tag{15}$$

To determine the data regions corresponding to the different kinetic orders, a method analogous to that proposed by Talmadge and Fitch to define the different settling regimes was applied [28]. The region corresponding to the zero-order kinetic behaviour was defined using the linear trend between the DO concentration and time. To fix the boundaries of the regions belonging to the half-order and first-order behaviours, first, tangent lines are drawn at the beginning and the end of the curve. Then, the angle formed by the intersection of both tangent lines is bisected. The intersection between the angle bisector line and the curve provides the transition point from the half-order behaviour to the first-order behaviour (Fig. 1).

The determination of the kinetic parameters was performed by minimizing the sum of the squares of the residues for each of the integrated kinetic models (Eqs. (4), (7), (10) and (13)) [29]. These kinetic models were not linearized to generate less error propagation in the obtained parameters and to obtain a better description of the oxygen concentration evolution throughout time [30,31]. The determination of the parameters and their variance was performed using the MATLAB function "fitnlm". In the case of the intrinsic kinetic parameters of the granular systems, only the variance of the apparent kinetics is known, and therefore the variance of the parameters was estimated using the propagating the error [32] and assuming that there is no correlation between the apparent constants (Eqs. (16) and (17)).

$$s_{r_{max}}^2 = \left(\left(\frac{3}{R_p^2}\right)^2 \cdot \frac{D_{O_2}}{\rho_b \cdot K_{O_2}} \cdot 2 \cdot \frac{K_0}{K_{1/2}^2}\right)^2 \cdot s_{K_0}^2 + \left(\left(\frac{3}{R_p^2}\right)^2 \cdot \frac{D_{O_2}}{\rho_b \cdot K_{O_2}} - 2 \cdot \frac{K_0}{K_{1/2}^3}\right)^2 \cdot s_{K_{1/2}}^2 \tag{16}$$

$$s_{K_{O2}}^2 = \left(\frac{K_{1/2}}{K_1^2}\right)^2 \cdot s_{K_{1/2}}^2 + \left(\frac{K_{1/2}^2}{K_1^3}\right)^2 \cdot s_{K_{1/2}}^2 \tag{17}$$

The uncertainty of each parameter was calculated with a certainty of 95% (Eq. (18)):

$$\text{Error margin} = s_{parameter} \cdot 1.96 \tag{18}$$

3. Results and discussion

Normally the values of the kinetic parameters that are used for AOB and NOB in models are fixed independently of the operational conditions, such as the aggregate state of the biomass (flocs or biofilm) and temperature. As Arnaldos et al. [18] stated, these and other factors are crucial to determining adequate values of the kinetic parameters used

Table 1
Kinetic parameters estimated at various temperatures for the AOB and NOB present in the different evaluated sludge types.

		AOB		NOB	
Sludge type	T (°C)	$r_{max,AOB}$ (mg NH_4^+-N/(g VSS·d))	$K_{O2,AOB}$ (mg O_2/L)	$r_{max,NOB}$ (mg NO_2^--N/(g VSS·d))	$K_{O2,NOB}$ (mg O_2/L)
S1 Granules (Apparent)	15	32.67 ± 0.88	0.90 ± 0.20	86.63 ± 1.75	1.77 ± 0.07
	20	66.21 ± 0.29	0.87 ± 0.02	64.75 ± 0.88	0.48 ± 0.01
	30	105.58 ± 2.63	1.68 ± 0.07	161.00 ± 1.75	0.56 ± 0.02
S1 Granules (Intrinsic)	15	77.00 ± 0.29	0.75 ± 0.02	192.5 ± 0.88	0.33 ± 0.01
	20	73.21 ± 0.29	0.14 ± 0.01	159.25 ± 0.88	0.09 ± 0.01
	30	95.67 ± 0.58	0.28 ± 0.01	211.75 ± 1.75	0.09 ± 0.01
S1 Flocculent	15	129.21 ± 0.58	0.56 ± 0.02	82.20 ± 0.54	0.34 ± 0.01
	20	174.71 ± 4.08	0.72 ± 0.06	122.04 ± 4.76	0.21 ± 0.01
	30	392.29 ± 44.35	1.94 ± 0.18	175.61 ± 1.47	0.59 ± 0.03
S2 Flocculent	15	106.75 ± 1.75	0.72 ± 0.04	224.875 ± 3.50	0.63 ± 0.03

S1: Biomass sample from a pilot plat at sidestream conditions; S2: biomass sample from a laboratory reactor at mainstream conditions.

in the models. The results obtained in the present research work show that the values of kinetic parameters change with the temperature and the biomass aggregation state (flocs or granules).

3.1. Granular sludge: Comparison between the apparent and intrinsic kinetic parameters

The results gathered from the respirometric experiments that were performed at various temperatures are presented in Table 1. The values that were obtained by directly applying the Monod equation, without considering the mass transfer diffusion throughout the granule, were determined as “apparent” values. While the “intrinsic” kinetic parameters were determined using the methodology proposed in Section 2.3.2.

The apparent values for K_{O2} were higher than the intrinsic ones because the diffusion effect inside the granule was not considered [33,34]. Wu et al. [20] explained that due to the intra-particle diffusion resistance, the measured apparent K_{O2} is usually larger than the intrinsic value, as demonstrate by the results obtained in the present research work (Table 1). The error between the apparent and intrinsic values increases with the increase of the temperature for AOB, while for NOB no significant differences are observed with the variation of the temperature (Fig. 2a). In the case of the K_{O2} determined for AOB at a low temperature the error is low (approximately 20%), since those microorganisms are located in the outer layer of the granule (a lower mass transfer resistance) and their activity in these conditions is low; the biological activity rate is presumably the limiting step.

Interestingly, for the biomass sample S1, the K_{O2} values for the flocculent biomass are, in general, higher than the intrinsic values in the granular biomass (Table 1). However, similar or relatively lower values were obtained for the flocs in comparison to the apparent oxygen affinity values for the granular biomass fraction. Thus, the use of the apparent values would lead to the conclusion that the flocculent biomass has a higher oxygen affinity than the granular one; whereas, if the diffusion limitation is taken into account, the reality is the opposite.

The apparent r_{max} values were lower than the intrinsic ones. Since r_{max} refers to the concentration of the biomass, when the apparent value is determined, the entire biomass of the granule is considered; although, in practice, not all of the biomass is active, resulting in the lower apparent values. When the error between the apparent and intrinsic values is determined, the correlation with the temperature seems to indicate that at higher temperatures the difference is lower since the biomass is more active (Fig. 2b). Higher activities led to more similar apparent and intrinsic r_{max} values. Moreover, the ratio of apparent r_{max} for the AOB to the NOB varies with temperature, showing a high disparity (average value of 0.68 ± 0.32), whereas if the intrinsic values are considered, the ratio is maintained during the temperature variation. Thus, excluding the diffusion resistance in the granular biomass, the temperature effects on both the AOB and NOB r_{max} values are similar. Using the apparent maximum consumption rates would therefore lead to a systematic underestimation of the activities, with a special effect on the NOB located in deeper zones, which can lead to an overestimation of the AOB advantage over the NOB activity. Knowledge of the intrinsic values can presumably allow anticipation of the partial nitritation process instability.

3.2. Values of Ko_2: Differences between AOB and NOB

The K_{O2} values obtained in the present research work for both types of sludge, flocculent and granular, at all the temperatures tested were $K_{O2,NOB} < K_{O2,AOB}$, (without to consider the apparent values), indicating that NOB had a higher affinity for oxygen than AOB. The

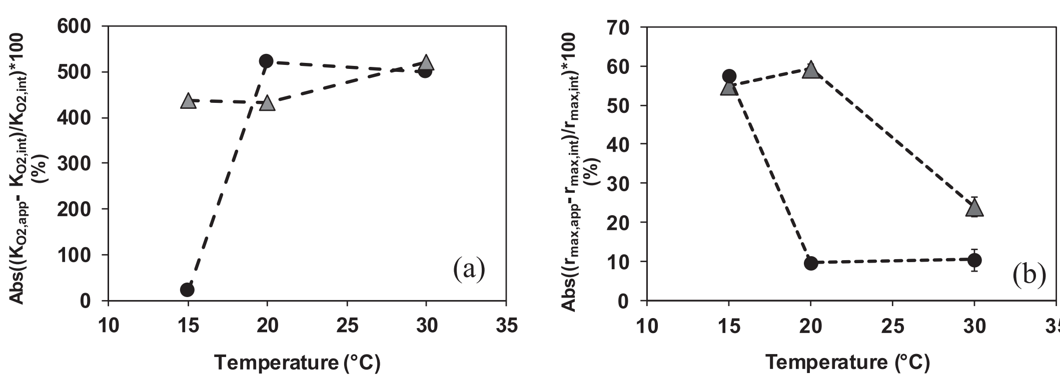

Fig. 2. Error between the apparent and intrinsic kinetic values of S1 granules at various temperatures for AOB (●) and NOB (△): (a) K_{O2} error and (b) r_{max} error.

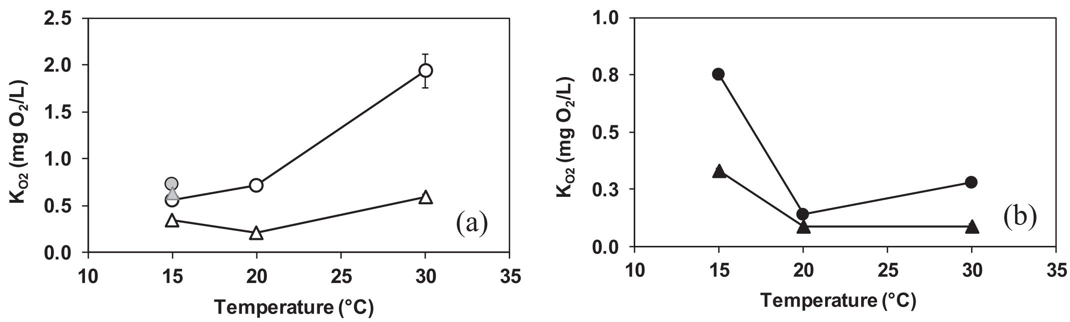

Fig. 3. Values of K_{O2} at various temperatures for: (a) flocculent sludge S1-AOB (○), S1-NOB (△), S2-AOB (◯) and S2-NOB (△); (b) granular sludge S1-AOB (●) and S1-NOB (▲).

values of K_{O2} between AOB and NOB are comparable only for the flocculent sludge from the mainstream (S2) at 15 °C and the granular sludge from the sidestream (S1) at 20 °C.

With the decrease of the temperature, the values of K_{O2} for the flocculent biomass decreased (Fig. 3a), with a higher effect for the case of AOB than for the case of NOB. However, no clear tendency was observed for the granular biomass (Fig. 3b); possibly, the decrease of the temperature from 30 °C (temperature inside the reactor origin of the biomass) to 15 °C (not acclimated) provoked this different tendency between the granules and flocs. As stated by Manser et al. [34], the K_{O2} values decrease with the decrease of the temperature due to the substantially reduced microbial activity.

A basic microbiological analysis using fluorescence *in situ* hybridisation (FISH) of S1 and S2 revealed positive results for *Nitrospira* spp. (probe Ntspa712) and negative for *Nitrobacter* spp. (probe Nit3) (see Supplementary Material). Blackburne et al. [33] studied the oxygen affinity constants for *Nitrobacter* and *Nitrospira.* and did not observe differences between the species; although more recent research work indicates that *Nitrospira.* have a higher oxygen affinity than *Nitrobacter* [35]. The research work of Ushiki et al. [35] studied the kinetics of two different pure strains of *Nitrospira* at 29 °C, and they obtained values of K_{O2} between 0.09 and 0.14 mg O_2/L, which are in the range of the values obtained in the present research work and validate the conclusion that NOB, similar to *Nitrospira*, have a high oxygen affinity. Additionally, *Nitrospira.* are the most common type of NOB in wastewater treatment systems, which indicates that due to the oxygen affinity, it is difficult to suppress them at low dissolved oxygen concentrations.

The difference between the K_{O2} values also serve to explain the distribution of different microbial populations in biofilms. Wu et al. [20] noted that the microorganisms that have an intrinsically higher oxygen affinity (lower K_{O2}) will be located in deeper zones inside the biofilm in comparison with other microorganisms with a lower affinity (higher K_{O2}). The biomass sample S1 used in the present research work, which is from a PN/AMX process operated under sidestream conditions, had K_{O2} values for AOB that were higher than those for NOB. This fact means that NOB have a higher affinity for oxygen, and consequently they probably will be located inside the granules in deeper zones than the AOB, which agrees with previous analyses of the microbial structure in PN/AMX granules [36,37].

Wu et al. [20] hypothesised that the literature discrepancies identifying if the AOB population or the NOB population has the higher oxygen affinity can be attributed to the fact that, usually, the apparent values are compared instead of the intrinsic values. Their results show that the intrinsic oxygen affinity for NOB is larger than for AOB. However, when the particle diameter is greater than 100 µm, the apparent values have the opposite behaviour. According to those results, at a large flocs size, the apparent $K_{O2,AOB}$ value changes, whereas no significant differences was observed with the apparent value of $K_{O2,NOB}$, since NOB are in the outer layer of the aggregates.

As indicated by Picioreanu et al. [38] it was totally assumed until relatively recently that the AOB oxygen affinity was higher than that of NOB. However, the number of studies that show a contrary tendency, such as the present one, has grown in recent years, demonstrating that the flocs size, the enrichment degree of the obtained biomass, the operational conditions, the specific strain, etc. are variables that influence the kinetic parameters [18,38,39]. For example Lawson et al. [40] reviewed this fact based on the recent discovery of comammox *Nitrospira* and indicated that these bacteria have a higher oxygen affinity than AOB and that their presence is common in wastewater treatment systems, which highlights the necessity to determine the competition between different strains. It is generally stated that *Nitrospira*, the same as NOB, are k-strategist microorganisms (well adapted to low DO and nitrogen concentrations), whereas *Nitrobacter* are r-strategist microorganisms with a lower oxygen affinity and therefore are more predominant in nitrogen rich environments.

Finally, the highest K_{O2} values, corresponding to biomass sample S2, show the low adaptation to the temperature of the nitrifying sludge as this biomass comes from a reactor operated at 15 °C. This increase in the oxygen affinity is higher for NOB than for AOB, showing that low temperatures presumably favour NOB *Nitrospira* over AOB [2].

3.3. Suppression of NOB activity based on the control of the DO concentration

In view of the results obtained in the present study for the AOB and NOB kinetic parameters, understanding the implications of working at high or low DO concentrations, which can help one to know the competition between AOB and NOB, is of interest for the further application of anammox based processes.

3.3.1. Application of high DO concentrations

When high DO concentrations are applied, the Monod equation follows zero-order kinetics. This case of competition between AOB and NOB can be studied by the comparison of the r_{max} values (Eq. (6)). The results obtained for S1 (adapted to sidestream conditions) indicate that the growth of AOB is very favoured over NOB at 30 °C, while for the decrease of the temperature the difference between both is reduced (Fig. 4a). In the case of S2 (adapted to mainstream conditions) at 15 °C, it is clear that the growth of NOB is favoured over the growth of AOB. For the granular sludge (Fig. 4b), the growth of NOB is favoured over the growth of AOB at all the temperatures tested (from 15 to 30 °C).

3.3.2. Application of low DO concentrations

When low DO concentrations are applied, the Monod equation follows first-order kinetics. This case of competition between AOB and NOB can be studied by the comparison of the ratio r_{max}/K_{O2} (Eq. (12)). The results, which are plotted in Fig. 5, show that the growth of NOB is favoured over the growth of AOB at all the temperatures that were assayed and for all types of biomass (flocculent, granular, sidestream and mainstream), except for the S1 flocs at 15 °C, where the values for AOB and NOB are very similar. Notably, in the case of granular sludge

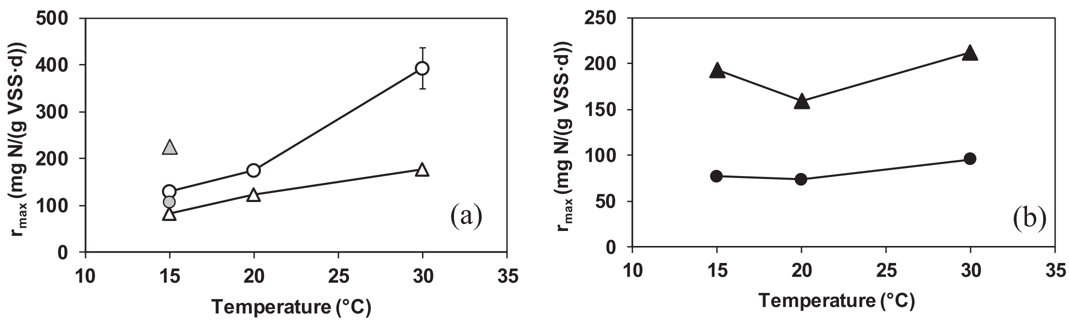

Fig. 4. Values of r_{max} at various temperatures for: (a) flocculent sludge S1-AOB (○), S1-NOB (△), S2-AOB (grey ○) and S2-NOB (grey △); (b) granular sludge S1-AOB (●) and S1-NOB (▲).

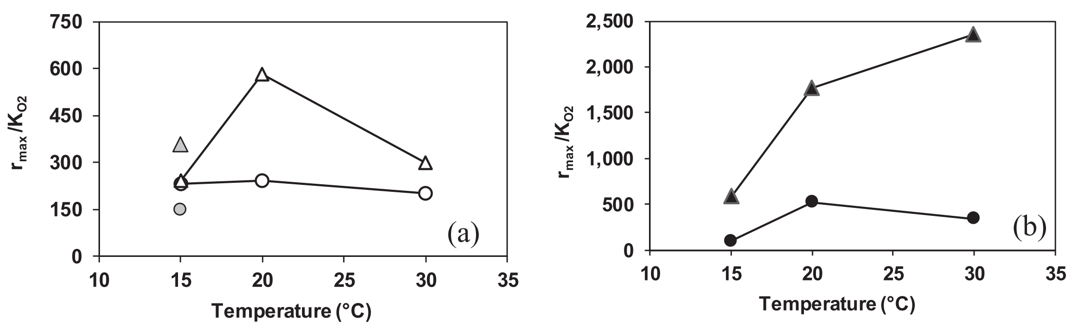

Fig. 5. Values of r_{max}/K_{O2} ratio at various temperatures for: (a) flocculent sludge S1-AOB (○), S1-NOB (△), S2-AOB (grey ○) and S2-NOB (grey △); (b) granular sludge S1-AOB (●) and S1-NOB (▲).

with the increase of the temperature, the r_{max}/K_{O2} ratio for NOB rose, and consequently the difference with AOB is higher.

3.3.3. Implications for autotrophic nitrogen removal by partial nitritation and the anammox processes

Suppression of the NOB activity based on the DO concentration limitation is therefore inadequate in view of the results obtained in this research work for S1 and S2 (Figs. 4 and 5). In the case of the application of PN/AMX processes in the sidestream of a municipal WWTP the high substrate concentration (ammonium) helps to suppress the NOB activity. Moreover, in those conditions the most widely distributed NOB specie is *Nitrobacter* which is characterize by higher K_{O2} values.

However, in the case of application in the mainstream of the WWTP the low substrate concentration and the low temperature make to suppress efficiently the NOB difficult, and the only control of DO concentration and/or aeration regime is insufficient. In the one stage configuration (PN/AMX in the same unit), anammox bacteria can consume nitrite to avoid the growth of NOB, but the low DO concentration that is necessary to maintain a certain anammox activity in the mainstream will ultimately lead to the development of NOB, as reported in previous research works [22,41]. Laureani et al. [6] achieved stable PN/AMX system operation under microaerobic conditions (DO concentration of 0.15–0.18 mg O_2/L), but the nitritation rate was slow, making the process difficult to implement in the mainstream of a WWTP. Combined control strategies are required to limit the NOB activity under mainstream conditions to achieve high enough removal rates of the effluent to meet the discharge limits. One example of combined control strategies is reported by Isanta et al. [42]; these authors proposed to use the dissolved oxygen concentration and the residual ammonium concentration (DO/NH_4^+-N ratio) to control the competition between AOB and NOB.

In recent years, the application of the two-stage system (partial nitritation and anammox processes in separate units) has gained attention, since more mechanisms for inhibiting the NOB can be used. As nitrite must be readily available in the system, the inhibition of NOB must be promoted using another type of inhibitors, such as free nitrous acid [12,13].

4. Conclusions

The methodology proposed in the present study to determine the intrinsic kinetic parameters of AOB and NOB in granular sludge may be a good alternative to avoid the use of apparent kinetic values and to better interpret the response of single PN/AMX systems. The obtained results confirm that the intrinsic values of K_{O2} and r_{max} are lower and higher, respectively, than the apparent values. This fact underlines the necessity of having adequate intrinsic kinetic parameters for AOB and NOB to exploit the possibility of partial nitritation under different conditions (flocs or granules, high or low temperatures). Analysis of the data obtained with the sludge samples (flocs from the mainstream, granules and flocs from the sidestream) indicates that it is impossible to stably maintain the partial nitritation process by only controlling the DO concentration.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2018.12.048.

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