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Limits of the anammox process in granular systems to remove nitrogen at low temperature and nitrogen concentration

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Abstract

When partial nitritation-anammox (PN-AMX) processes are applied to treat the mainstream in wastewater treatment plants (WWTPs), it is difficult to fulfil the total nitrogen (TN) quality requirements established by the European Union (<10 g TN/m$^3$). The operation of the anammox process was evaluated here in a continuous stirred tank reactor operated at 15 °C and fed with concentrations of 50 g TN/m$^3$ (1.30 ± 0.23 g NO$_2^-$-N/g NH$_4^+$-N). Two different aspects were identified as crucial, limiting nitrogen removal efficiency. On the one hand, the oxygen transferred from the air in contact with the mixed liquor surface favoured the nitrite oxidation to nitrate (up to 75%) and this nitrate, in addition to the amount produced from the anammox reaction itself, worsened the effluent quality. On the other hand, the mass transfer of ammonium and nitrite to be converted inside the anammox granules involves relatively large values of apparent affinity constants ($k_{NH_4^+\text{app}}$: 0.50 g NH$_4^+$-N/m$^3$; $k_{NO_2^-\text{app}}$: 0.17 g NO$_2^-$-N/m$^3$) that favour the presence of these nitrogen compounds in the produced effluent. The careful isolation of the reactor from air seeping and the fixation of right hydraulic and solids retention times are expected to help the maintenance of stability and effluent quality.

Keywords: Anammox; dissolved oxygen; granular biomass; nitrogen; SRT; temperature.
1. Introduction

The implementation of partial nitritation-anammox (PN-AMX) processes to remove nitrogen from the mainstream of the wastewater treatment plants (WWTPs) arises as one of the most promising options to increase the energy efficiency of these facilities (Morales et al., 2015). So far, several studies have been carried out operating PN-AMX systems at mainstream conditions, low ammonia concentration and low temperature using a one-stage configuration (Jiang et al., 2018; Lotti et al., 2014). These research works revealed two main drawbacks. On the one hand, the achieved volumetric nitrogen removal rates were below 10 - 30 g N/(m$^3$·d) at 15 °C (Akaboci et al., 2018; Laureni et al., 2016; Pedrouso et al., 2018) mainly due to the oxygen limiting conditions imposed in order to maintain the balance between the activities of both ammonia-oxidizing (AOB) and anammox bacteria. On the other hand, the operational stability lost as a consequence of the development of the nitrite-oxidizing bacteria (NOB) activity (Han et al., 2016; Li et al., 2019). Both factors are responsible for the deterioration of the effluent quality, which does not meet the European discharge requirements, in sensitive areas, of 10 g/m$^3$ of total nitrogen (TN) (European Directive 91/271/EEC).

Since the bottleneck to apply the PN-AMX process at mainstream conditions is the stability of the PN process, efforts have been recently focused on the evaluation of the two-stage configuration systems to carry out the PN and anammox processes separately (Jin et al., 2019; Kowalski et al., 2019). Using this approach, stable long-term PN was achieved by inhibiting NOB activity due to the presence of free nitrous acid (HNO$_2$) (Cui et al., 2019; Pedrouso et al., 2017. At this point, the performance of the subsequent anammox process needs to be further optimized to produce an effluent suitable for discharge.

The anammox process stability at mainstream conditions was demonstrated using enriched anammox biomass as inoculum and starting-up the reactors at temperatures close to 30 °C that were, then, stepwise decreased to 15 °C in a long-term acclimation
Another used alternative is the enrichment of anammox bacteria from activated sludge directly at low temperature (Hendrickx et al., 2014). However, although both strategies were successful, they required long start-up periods that are not useful for their implementation at full-scale WWTPs.

In the present study, a completely stirred tank reactor (CSTR), inoculated with anammox-enriched granular biomass coming from a reactor operated at 30 °C, was started-up directly at mainstream conditions (50 g TN/m³ and 15 °C). The objective was to evaluate the process stability and produced effluent quality in order to assess the anammox process feasibility at the operational conditions. Biomass mass transfer limitations and oxygen transfer from air were also considered to identify the operational considerations regarding those aspects limiting its full-scale application.

2. Materials and methods

2.1. Set-up

The anammox process was carried out in a CSTR jacketed cylinder reactor with a useful volume of 1 L and a height to diameter (H/D) ratio of 1. The reactor temperature was maintained at 15 °C using a cryostat bath. A mechanical stirrer (58 rpm) provided a complete mixture inside the system. pH was not controlled and remained at 7.8 ± 0.2. A continuous peristaltic pump supplied the feeding while the effluent discharge took place by overflow. The reactor was inoculated with 2.8 kg VSS/m³ of anammox granular biomass (average particle radius: 0.7 mm) taken from an ELAN® full-scale reactor operated at 30 °C (Morales et al., 2015). Its maximum specific anammox activity was 252 g N₂-N/kg VSS·d at 30 °C. In order to retain the granular biomass inside the reactor, a plastic mesh was installed just below the liquid outlet port. At the beginning of the operation, the upper part of the reactor was covered by an expanded polystyrene plate that was in direct contact
with the liquid surface to avoid oxygen diffusion from air into the bulk liquid. Nevertheless,
this configuration was not successful, and the polystyrene plate was replaced on day 11 by
a methacrylate lid that allowed for the existence of a gaseous phase in the headspace of
the reactor, which was flushed out with N₂ gas.

The CSTR feeding was the effluent from the Mapocho-El Trebal urban WWTP (Santiago,
Chile), which contained chemical oxygen demand (COD) concentrations lower than 25
g/m³ (non-biodegradable COD) and ammonium concentrations between 32 and 48 g NH₄⁺-
N/m³. Sodium nitrite was added to obtain a nitrite/ammonium molar ratio of 1.30 ± 0.23
(close to the stoichiometric one needed for the anammox process). Moreover, wastewater
was diluted 1:2.3 with tap water in order to maintain the initial value of nitrogen
concentration while mimicking the effluent of a nitritation unit. A flux of N₂ gas was used to
purge the reactor feeding of oxygen and to prevent its entrance to the reactor. The
operational period was divided into 4 stages according to the established hydraulic
retention time (HRT) (Table 1). The nitrogen loading rates (NLR) during the operational
period ranged from 29.4 to 77.4 g TN/(m³·d).

Table 1

2.2. Analytical methods
pH, dissolved oxygen, ammonium, nitrite, nitrate, and volatile suspended solids (VSS)
concentrations were determined according to the Standard Methods (APHA, 2015). The
soluble chemical oxygen demand (COD₅) was determined by a semi-micro method from
the liquid samples filtered through 0.45 μm pore size filters (Soto et al., 1989). Granule
diameters were measured by means of image analysis (ImageJ software). The maximum
specific anammox activity (SAA) of the biomass (as g N/kg VSS·d) was determined
according to the guidelines proposed by Dapena-Mora et al. (2007).
2.3. Calculations

2.3.1. Minimum solids retention time (SRT) to fulfil the effluent quality

The minimum SRT (SRT\text{\textsubscript{min}} d) required to obtain the desired effluent quality (less than 10 g TN/m\textsuperscript{3}) was estimated from nitrogen mass balances performed in the bioreactor during its operation under steady-state conditions (Eq. (1), (2) and (3)) and considering the restriction of Eq. (4). For these calculations, feeding composition was fixed taking into account the following considerations: nitrite/ammonium concentrations ratio of 1.32 g NO\textsubscript{2}^-/N/g NH\textsubscript{4}^+\textsubscript{-N} and the nitrate generated to nitrogen consumed ratio of 0.11 g NO\textsubscript{3}^-/N\textsubscript{\text{produced}}/g N\textsubscript{\text{removed}}, according to the anammox stoichiometry (Strous et al., 1999). Eq. (1) is only valid for TN concentrations in the influent below 90.91 g TN/m\textsuperscript{3}. Otherwise, the TN concentration in the effluent will be larger than 10 g/m\textsuperscript{3} according to the anammox stoichiometry.

\[
(NH_4^+ - N)_{\text{eff}} = \frac{10 - 0.11 \cdot TN}{2.06} \quad (g \frac{N}{m^3}) \quad (1)
\]

\[
(NO_2^- - N)_{\text{eff}} = 1.32 \cdot (NH_4^+ - N)_{\text{eff}} \quad (g \frac{N}{m^3}) \quad (2)
\]

\[
(NO_3^- - N)_{\text{eff}} = 0.11 \cdot (TN_{\text{inf}} - (NH_4^+ - N)_{\text{eff}} - (NO_2^- - N)_{\text{eff}}) \quad (g \frac{N}{m^3}) \quad (3)
\]

\[
10 = (NH_4^+ - N)_{\text{eff}} + (NO_2^- - N)_{\text{eff}} + (NO_3^- - N)_{\text{eff}} \quad (g \frac{N}{m^3}) \quad (4)
\]

Eq. (5) defines the SRT (equal to HRT) in a CSTR as the inverse of the biomass specific growth rate (\(\mu\), d\textsuperscript{-1}). In the case of the anammox granular biomass, its specific growth rate can be described by a double kinetic Monod model based on both substrates, ammonium and nitrite (Eq. (6)). Nitrogen mass transfer rate limitations inside the granules, at the low...
substrate concentrations imposed at mainstream conditions, were included in this model by means of their respective apparent affinity constants. These apparent affinity constants were estimated from the true affinity constants by means of Eq. (7) and using the coefficients from Table 2.

\[
SRT_{\text{min}} = \frac{1}{\mu} \cdot (d)
\]

\[
\mu = \mu_{\text{max}} \cdot \frac{(NH_4^+ - N)_{\text{eff}}}{k_{NH_4^{app}} + (NH_4^+ - N)_{\text{eff}}} \cdot \frac{(NO_2^- - N)_{\text{eff}}}{k_{NO_2^{app}} + (NO_2^- - N)_{\text{eff}}} - k_d \cdot (d^{-1})
\]

\[
k_{S_{app}} = R_p \cdot \frac{\sqrt{\frac{\mu_{\text{max}} \cdot \rho_{\text{biomass}} \cdot k_S}{Y_X/S-N}}}{D_S} (S = NH_4^+ \text{ or } NO_2^-, \text{ respectively})
\]

\( \mu_{\text{max}} \) represents the maximum specific growth rate of anammox bacteria \((d^{-1})\); \((NH_4^+ - N)_{\text{eff}}\) and \((NO_2^- - N)_{\text{eff}}\) ammonium and nitrite concentrations in the effluent, respectively \((g \text{ N/m}^3)\); \(k_{NH_4^{app}}\) and \(k_{NO_2^{app}}\) the apparent affinity constants for ammonia and nitrite, respectively \((g \text{ N/m}^3)\); \(k_d\) the anammox biomass decay coefficient \((d^{-1})\); \(R_p\) the particle radius \((m)\); \(Y_X/S-N\) the biomass yield coefficient \((g \text{ VSS/g N})\); \(\rho_{\text{biomass}}\) the biomass density \((g \text{ VSS/m}^3\text{granules})\); \(k_S\) the true value of the affinity constant \((g \text{ N/m}^3)\); \(D_S\) the substrate diffusion coefficient \((m^2/d)\).

Eq. (5) was solved by means of the Excel Solver tool, considering Eq. (1) to (4), (6) and (7), for different inlet nitrogen concentrations \((30-70 \text{ g TN/m}^3)\) and anammox biomass granule sizes \((R_p: 0.5-3.0 \text{ mm})\).

Table 2
2.3.2. Effect of air input on the effluent quality

In order to predict the effect of the air entrance to the anammox bioreactor over the effluent composition, a model based on stoichiometric considerations and mass balances was used. The feeding composition of the anammox reactor was defined by the characteristics of the effluent from a partial nitritation unit containing: 50 g TN/m³, a nitrite/ammonium ratio of 1.32 g NO₂⁻-N/g NH₄⁺- and a dissolved oxygen concentration of 1 g O₂/m³. Thus, the maximum oxygen transfer rate (OTR) from the atmosphere to the anammox reactor (g O₂/(m³·d)) would be given by Eq. (8):

\[ OTR_{max} = k \cdot \frac{1}{5} \cdot C_{O_2}^{sat} \left( \frac{g O_2}{m^3 \cdot d} \right) \]  

Equation (8)

\[ k = 0.24 \cdot (37 + 1.77 \cdot u) \]  

Equation (9)

where \(O_{TR_{max}}\) is the maximum oxygen transfer rate (g O₂/(m³·d)); \(k\) the mass transfer coefficient (m/d), which can be calculated by adapting the empirical equation reported by Ro and Hunt (2007) (Eq. (9)); \(\frac{1}{5}\) is the specific area of a rectangular tank (m²/m³) considering a height of 5 m typically used in urban wastewater treatments systems; \(C_{O_2}^{sat}\) is the maximum dissolved oxygen concentration (g O₂/m³), which was chosen as 10 g O₂/m³ for an effluent of an urban WWTP at 15 °C.

At the defined operational conditions, if oxygen is available in the liquid media it is considered that it will be mainly used to oxidize nitrite to nitrate. In this case, nitrite becomes the limiting substrate for the anammox process. Oxygen can enter the reactor...
partly dissolved in the feeding and partly transferred from the atmosphere through the liquid surface. According to the stoichiometry of nitrite oxidation reaction, 0.9 g NO₃⁻-N are produced per g O₂ consumed. If complete removal of nitrite is considered by the anammox route, the nitrate generated during the anammox process and the remaining ammonium can be determined from the stoichiometric ratios of 0.26 g NO₃⁻-N removed/1.32 g NO₂⁻-N removed and 1 g NH₄⁺-N removed/1.32 g NO₂⁻-N removed.

Equation (10) allows the prediction of the effluent quality in terms of nitrogen concentration. It can be determined as the sum of: a) the nitrate generated from the oxidized nitrite (considering the dissolved oxygen (C₀₂) present in the influent (supposed as 1 g O₂/m³) and that transferred from the atmosphere); b) the nitrate produced from the anammox reaction and; c) the remaining ammonium.

\[
T_{N_{eff}} = \left( C_{O_2} + OTR_{max} \cdot HRT \right) \cdot 0.9 \, \frac{g \, NO_3^- - N}{g \, O_2} + \frac{0.26 \, g \, NO_3^- - N}{1.32 \, g \, NO_2^- - N} \cdot \left[ 29 \, \frac{g \, NO_3^- - N}{m^3} - \left( C_{O_2} + OTR_{max} \cdot HRT \right) \cdot 0.9 \, \frac{g \, NO_3^- - N}{g \, O_2} \right] + \\
\left( 21 \, \frac{g \, NH_4^+ - N}{m^3} - \frac{1}{1.32} \, \frac{g \, NH_4^+ - N}{g \, NO_2^- - N} \cdot \left[ 29 \, \frac{g \, NO_3^- - N}{m^3} - \left( C_{O_2} + OTR_{max} \cdot HRT \right) \cdot 0.9 \, \frac{g \, NO_3^- - N}{g \, O_2} \right] \right) \left( \frac{g \, N}{m^3} \right) \quad (10)
\]

3. Results

3.1. Reactor performance

During the start-up period (Stage I), the applied NLR was approximately 33 g N/(m³·d) (Table 1). On day 7, a sudden increase in the effluent nitrate concentration was measured (Fig. 1). However, this nitrate production was not justified by the anammox process activity since ammonium concentration did not decrease proportionally. Thus, this nitrate rise was due to nitrite oxidation. Although this effect was associated with air entry into the reactor, dissolved oxygen concentration measured in the bulk liquid was lower than 0.1 g O₂/m³ (detection limit value). N₂ gas was supplied through the headspace of the reactor to avoid the air entrance. Then, the anammox process stability was restored and the nitrogen removal efficiency reached 84%. Afterwards, a similar episode of nitrate accumulation
occurred between days 25 and 32 (Stage III) due to the failure of the N₂ gas supply to the reactor headspace. Once more, nitrite was preferentially oxidized while no ammonia consumption was observed. This fact can be explained by the different values of the oxygen affinity constant observed for NOB (0.09-0.33 g O₂/m³) and AOB (0.14-0.75 g O₂/m³) present in the biomass used as inoculum (Val del Río et al., 2019). In fact, previous studies carried out at low temperature and nitrogen concentrations, already reported nitrate concentrations higher than the ones expected from the anammox stoichiometry, associated to nitrite oxidation due to oxygen infiltrations inside the reactors (Li et al., 2018; Sánchez-Guillén et al., 2016; Zhang et al., 2019).

Fig. 1

In order to determine the maximum removal capacity of the system, the applied NLR was increased up to approximately 80 g N/(m³·d) during Stage II. A nitrogen removal rate (NRR) close to 62 g N/(m³·d) was observed, but the concentration of TN in the effluent exceeded the desired value of 10 g TN/m³. In order to produce an effluent suitable for discharge, the HRT was increased from 0.6 up to 1.2 d (Stage III) and it was fixed at 1.0 d (Stage IV). Although operational conditions were supposed to favour the anammox process efficiency, on day 60 of operation, a new sudden increase of nitrate concentration in the effluent was observed. On this occasion, nitrite oxidation was due to the oxygen produced by algae growing on the reactor walls. This phenomenon can be considered only relevant in this small system since the volume where light is present for algae growth is large with respect to the total reactor volume. Later, on day 65, algae were removed from the system and the walls of the reactor were covered to protect them from light. After that, the anammox process recovered its stability, which confirmed that algae growth was hindering the anammox process stability since the oxygen that they produced favoured the
oxidation of nitrite by NOB. This problem is expected it to be negligible in full-scale
reactors where the surface exposed to sunlight and its penetration depth into the
wastewater takes place in a very low volume of the whole system (Vergara et al., 2016).

The maximum value of specific anammox biomass activity measured inside the reactor (22
g N/kg VSS-d) corresponded to Stage II, when the system was overloaded. However, this
value decreased to 10 g N/kg VSS-d when the system was operated under conditions that
allowed the obtention of an effluent with a TN concentration lower than 10 g TN/m³ (Fig.
2). These values are close to those of 7-39 g N/kg VSS-d reported in other research works
after a long-term acclimation period and under similar operating conditions (Hendrickx et
al., 2014; Sánchez-Guillén et al., 2016; Wu et al., 2018). During the operational period,
anammox granules maintained their integrity but decreased in size (Fig. 3) due to a
progressive decrease in biomass concentration. In this way, the solids concentration was
1.6 kg VSS/m³ at the end of the operational period. This biomass loss throughout the
operational period could be attributed to the shear stress caused by the mechanical stirrer
and/or the decay of part of the inoculated biomass. Since the inoculum comprised
anammox and nitrifying bacteria, AOB and NOB might have been removed selectively
from the system as anoxic conditions were imposed. Additionally, the reactor was started-
up with a biomass concentration higher than the one required to treat the applied NLR, fact
that correlated with previous experiences reporting that the surplus biomass fraction left
the system (De Cocker et al., 2018; Hao et al., 2015).

Fig. 2

Fig. 3
3.2. Main drawbacks to obtaining a suitable effluent quality

Most of the existing research work focused on the application of the anammox process at mainstream conditions evaluated the maximum nitrogen removal rates achieved but they paid no attention to the produced effluent quality (Wu et al., 2018; Zhang et al., 2019). Taken into account that nitrate is produced by the anammox process, to achieve a TN concentration below 10 g TN/m³ in the effluent, the reactor needs to operate at quite low substrate concentrations. The low TN concentrations required would limit the biomass kinetics and, therefore, the achievable NRR. On the other hand, eventual events of air entrance from the atmosphere into the anammox reactor media need to be considered in full-scale systems since, in general, the reactors implemented at mainstream are not covered. The effects on the effluent quality of both diffusional nitrogen mass limitation inside the granular biomass, and the air entering into the anammox reactor will be discussed in the following sections.

3.2.1. Diffusional mass resistance in anammox granular biomass

When the anammox process is operated at low nitrogen concentrations, the reaction rate is slow. In addition, due to the associated slow growth rate of the anammox biomass, it is frequently grown as granular biomass in order to favour its retention inside the reactor. This biofilm biomass, along with low substrate concentrations in the feeding, are expected to hinder the process efficiency due to diffusional nitrogen limitations. To evaluate substrate diffusion, ammonium and nitrite true affinity constants should be considered. Their reported values for anammox biomass grown in suspension are lower than 0.07 g NH₄⁺-N/m³ and 0.05 g NO₂⁻-N/m³, for kₐ₄ and kₐ₂ respectively (Hao et al., 2015). However, in biofilm systems, these affinity constants increase, indicating that the apparent ones should be used to describe diffusion limitations. In these conditions, mass transfer limitations need to be included in the expression of the process kinetic rate (Eq. (6)) by
using the apparent value of the substrate affinity constants. This effect is more relevant at high temperature as it was already observed several authors, who reported values of 25.0-96.4 g NH$_4^+$-N/m$^3$ and 0.66-56.4 g NO$_2^-$-N/m$^3$ for $k_{NH4\text{-app}}$ and $k_{NO2\text{-app}}$, respectively (Chen et al., 2011; De Prá et al., 2016; Tang et al., 2013). In the present study, the apparent substrate affinity constants were calculated by means of the Excel Solver tool using steady-state data obtained between days 20 and 60 of operation (Fig. 1). Results for $k_{NH4\text{-app}}$ and $k_{NO2\text{-app}}$ were of 0.50 g NH$_4^+$-N/m$^3$ and 0.17 g NO$_2^-$-N/m$^3$, respectively, were obtained which were higher than the true ones reported by Hao et al. (2015).

According to the microorganisms growth balance and stoichiometric considerations used for calculation, the minimum SRT to get an effluent with a concentration below 10 g TN/m$^3$ increases with the increasing TN concentrations in the inlet and with the anammox granules size (Fig. 4). Once the anammox process occurs at the highest efficiency, the nitrate concentration in the outlet is directly proportional to the inlet TN concentration. Therefore, to meet the nitrogen disposal limits, ammonium and nitrite concentrations should be low enough to compensate those of nitrate produced (26% of the TN in the influent). It is more difficult to reach the appropriate ammonium and nitrite conditions in the case of anammox granular biomass since apparent substrate affinity constants augment with the size of the granules. This low affinity for both substrates is then counteracted by maintaining long SRT (Fig. 4). Even for relatively small granules ($R_p$: 0.5 mm) and low inlet TN concentrations (30 g NH$_4^+$-N/m$^3$), SRT values higher than 70 days are required. Anammox granular biomass systems are suitable to maintain high SRT values (between 162 and 525 d) (De Cocker et al., 2018; Sánchez-Guillén et al., 2016) without negatively affecting the biomass activity. This is also the case of membrane systems (Hoekstra et al., 2018; van der Star et al., 2008) due to their selective biomass retention. In the present study, average SRT values reached were about 98 d, which could justify the good effluent quality obtained during the stable operational periods used for calculations.
When operating full-scale AMX systems in mainstream conditions, if this long SRT values cannot be achieved, a strategy based on generating anammox biomass in the sludge-line unit and supplying it to the mainstream reactor is a feasible option. In this way, the biomass concentration inside the reactor could be even higher than that supported by the NLR applied. This strategy could be feasible since the decay coefficient ($k_d$) of anammox biomass at 15 °C is very low ($0.0007 \text{ d}^{-1}$) (Hao et al., 2015), which implies that anammox biomass can survive for long periods in the presence of very low substrate concentrations. Besides, high biomass “buffer capacity” could be kept for long periods without the continuous addition of new biomass.

3.2.2. Oxygen input to the anammox reactor

Only a few years ago, researchers realized that at low temperatures the oxygen affinity constant of AOB was larger than that of NOB. Due to the NOB extremely low affinity constant, this population proliferates at mainstream conditions and makes the maintenance of the process stability difficult, in special when PN-AMX processes take place in a one-stage reactor (Laureni et al., 2016; Malovanyy et al., 2015; Morales et al., 2016). In the case of AMX reactors, AOB and NOB populations are developed at long-term in spite of the lack of intentional oxygen supply to the system (Li et al., 2018). This means that $\text{NO}_2^{-}\text{consumed/NO}_4^{+}\text{consumed}$ and $\text{NO}_3^{-}\text{generated/NO}_4^{+}\text{consumed}$ ratios suddenly increase after an accidental air entrance in the system (Li et al., 2018; Ma et al., 2013; Sánchez-Guillén et al., 2016; Zhang et al., 2019). These undesired air intakes can be minimized by the use of reactors with a lower specific surface area (higher height) in contact with the atmosphere. Nevertheless, this type of reactors is not suitable to treat high flowrates such as those
entering the WWTP. In general, secondary treatments are designed as flat reactors with a maximum height of 5 m, which involves a specific surface area of 0.2 m²/m³. This design facilitates the contact between air and wastewater and a consequent oxygen dissolution in the liquid media.

In terms of reactor operation, when low N concentrations are treated at low temperatures, the applied NLR is low. Therefore the air transferred from the atmosphere with respect to the TN present in the liquid is higher than in the reactors treating high TN concentrations at high temperature. On the other hand, the N₂ gas produced from the anammox reaction is too low to hinder the air solubility. Moreover, at mainstream conditions, NOB are not expected to be inhibited by free ammonia (NH₃) as it occurs when treating wastewater streams with high nitrogen concentrations (Sun et al., 2015). In these conditions, eventual air transferred from the atmosphere negatively affects the effluent quality. Furthermore, the effluent quality worsens when wind speed and HRT increase (Fig. 5), thus they favour the mass transfer coefficient and increase the contact time, respectively, increasing the dissolved oxygen in the liquid media. Considering the commonly applied HRT in wastewater treatment systems (0.16-0.33 days), it is recommended the use of protective covers in anammox reactors in order to avoid oxygen transference and obtain the required effluent quality.

**Fig. 5**

### 4. Conclusions

When anammox biomass grows as biofilm, nitrogen mass transfer limitations reduce the reactor efficiency, which hinders compliance with discharge limits. Thus, on the one hand, the composition of the produced effluent in a PN-AMX system mainly depends on the
treated wastewater nitrogen content and on the other hand, the removal efficiency depends on the NOB activity.

To reach the required low TN concentrations in the produced effluent, it is crucial to minimize the presence of dissolved oxygen in the feeding media. The oxygen transferred from the air could lead to 75% of the nitrate produced. For this reason, it is recommended to cover full-scale reactors in order to isolate them.

The use of an external biomass source (e.g. biomass from an AMX reactor in operation in the sludge line) might be an alternative when it is not possible to avoid mass diffusional limitations by the maintenance of large SRT in full-scale operations.

If even after applying the previously commented strategies is still impossible to comply with the discharge limits, another alternative is to operate the anammox unit as an overloaded one placing a subsequent reactor to refine the final effluent. In this unit, the supply of low COD concentrations might be advisable to remove the previously formed nitrate via heterotrophic denitrification.

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5. References


https://doi.org/10.1016/j.biortech.2012.11.025

fixed film activated sludge (IFAS) reactor by partial nitritation/anammox process. 
Bioresource Technol. 198, 478-487. https://doi.org/10.1016/j.biortech.2015.08.123

Morales, N., Val del Rio, A., Vázquez-Padín, J.R., Gutiérrez, R., Fernández-González, R., 
of dissolved oxygen concentration on the start-up of the anammox-based process: 

Morales, N., Val del Río, A., Vázquez-Padín, J.R., Méndez, R., Campos, J.L., Mosquera-
Corral, A., 2016. The granular biomass properties and the acclimation period affect the 
partial nitritation/anammox process stability at a low temperature and ammonium 
https://doi.org/10.1016/j.procbio.2016.08.029

Pedrouso, A., Val del Río, A., Morales, N., Aiartza, I., Morales, N., Vázquez-Padín, J.R., 
ELAN® process applied to treat primary settled urban wastewater at low temperature 
https://doi.org/10.1016/j.seppur.2018.02.017

Pedrouso, A., Val del Río, A., Morales, N., Vázquez-Padín, Campos, J.L., Méndez, R., 
Mosquera-Corral, A. 2017. Nitrite oxidizing bacteria suppression based on in-situ free 
https://doi.org/10.1016/j.seppur.2017.05.043

37, 539-563. https://doi.org/10.1080/10643380601174749


https://doi.org/10.1002/bit.21891


Figure Captions

Fig. 1. Concentrations of the nitrogenous compounds throughout the operational period: inlet NO$_2^-$-N (●); inlet NH$_4^+$-N (■); outlet NO$_2^-$-N (○); outlet NH$_4^+$-N (□); outlet NO$_3^-$-N (Δ) as g N/m$^3$.

Fig. 2. TN concentration in the effluent of the CSTR (○); and maximum TN concentration limit for discharge of 10 g N/m$^3$ (continuous line).

Fig. 3. Images of the biomass collected from the reactor on days 7 (A) and 46 (B) of operation.

Fig. 4. Minimum SRT (SRT$_{\text{min}}$) required to obtain an effluent from the anammox reactor containing a TN concentration lower than 10 g N/m$^3$. Granule radius (R$_p$) used in the calculations were 0.5 mm (●); 1 mm (■); 2 mm (○); and 3 mm (△).

Fig. 5. Effect of HRT and wind speed on the effluent quality of an anammox reactor operated at mainstream conditions. Tested wind speeds (u) were: 0 m/s (●); 1 m/s (■); 4 m/s (○); and 10 m/s (△). The grey-shadowed area corresponds to the operational zone where the effluent does not meet the disposal requirements.