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Pilot-scale ELAN® process applied to treat primary settled urban wastewater at low temperature via partial nitritation-anammox processes

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ABSTRACT

A single stage partial nitritation and anammox granular pilot scale reactor (600 L) was operated to treat primary settled sewage in an urban wastewater treatment plant. The fed wastewater contained low total nitrogen concentrations of 6–25 mg TN/L and the system operated without temperature control ranging from 18 to 12 °C. A control strategy, based on the pH value, was applied to stop the aeration supply. The pH set-point was fixed at 6.0 and allowed obtaining a total nitrogen removal efficiency approximately of 50% treating a load of 67 mg TN/(L·d) without the addition of any chemicals. Although nitrite oxidizing bacteria were present in the inoculated sludge, when the pH-based control was implemented (day 30) the ammonium oxidation was favored compared to the nitrite oxidation activity. Then, the system operated stable the rest of the operational period (days 30–94) despite the presence of organic matter in the wastewater and the high variability of nitrogen load and temperature during the operation. Nitrogen was autotrophically removed accomplishing the stringent discharge limits (10 mg TN/L) and nitrate concentrations in the effluent lower than 3 mg NO_3^--N/L. Both biomass concentration and granules size increased during the operational period indicating the growth of the biomass inside the reactor and therefore the potential treatment capacity.

1. Introduction

The application of the combined partial nitritation and anammox (PN/AMX) processes, constitutes a promising alternative to remove nitrogen from wastewater. Their implementation is expected to improve the energy efficiency of the wastewater treatment plants (WWTPs) [1]. Initially, in the partial nitration (PN) process, part of the ammonium contained in the wastewater is oxidized to nitrite by the ammonium oxidizing bacteria (AOB). Then, the anammox bacteria oxidize the remaining ammonium using the produced nitrite as electron acceptor and producing nitrogen gas and residual quantities of nitrate [2]. Compared to the conventional nitrification-denitrification processes, the PN/AMX processes represent an important reduction in the energy and resources consumption during the wastewater treatment. As both processes are autotrophic, no organic matter is required and therefore can be valorized for methane production, for example. Furthermore, aeration costs are reduced in 40%, the emissions of the greenhouse gases (CO_2, N_2O…) may be decreased by 83% and the sludge production is reduced approximately in 90% [3].

Different PN/AMX based technologies have been successfully implemented at full-scale to treat high strength wastewater (> 200 mg N/L) at mesophilic temperatures (~30 °C), known as sidestream conditions [4]. The most widely applied configuration is the one-stage system (about 90% of the total full-scale plants), where the PN/AMX processes take place in the same unit [4]. This configuration has as advantages the feasibility to treat high loads, its simple operation and control systems, and low investment costs [5]. Moreover, the implementation of the PN/AMX processes takes place mostly in biofilm systems which allow accumulating large biomass concentrations and dealing with high volumetric loads. Particularly, the granular systems are those that treat larger nitrogen loads [4].

In the last decade, the development of a process based on the autotrophic PN/AMX process to treat the nitrogen present in the mainstream of the WWTP has gained great interest. This stream contains approximately the 80% of the total nitrogen load entering the WWTP and is characterized by its low nitrogen concentrations (< 100 mg N/L)

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Table 1
Review of studies performed in one-stage systems where the PN/AMX processes took place to treat municipal wastewater at low temperature (≤25 °C).

Volume (L)	T (°C)	COD_T fed (mg/L)	COD_S fed (mg/L)	Nitrogen fed (mg N/L)	A-Stage	NLR (mg N/(L·d))	NRE (%)	Reference
1.35	20–24	50 ± 12	–	28 ± 5 TN	HRAS	80	75	[9]
200	19–31 (uncontrolled)	41 ± 10	32 ± 7	26 ± 4 TN	CEPT + HRAS	140	70	[10]
12	15	69 ± 18	46 ± 7	21 ± 5 NH_4^+-N	HRAS	40	63	[11]
200	15	44–71	–	46 NH_4^+-N	UASB	40	40	[12]
7000	20	120	–	34–41 TN	Aerobic	100	51	[13]
10	10–20	53 ± 27	29 ± 7	49 ± 11 NH_4^+-N	HRAS	61	75	[14]
200	25	49–106	44–88	35–50 NH_4^+-N	UASB	100	52	[15]
4000	19	–	62 ± 17	27 ± 5 NH_4^+-N	Aerobic	400	39	[16]
1	10–20	–	105–365	43 ± 7 TN	UASB	172	46–73	[17]
600	12–18 (uncontrolled)	16–197	–	6–25 TN	None	67	50	This study

CEPT: Chemically enhanced primary treatment; HRAS: High rate activated sludge; UASB: Upflow anaerobic sludge blanket; NLR: Nitrogen loading rate; NRE: Nitrogen reomval efficiency.

and low temperature (< 25 °C). Moreover, the mainstream presents high variability, in terms of fluctuating temperatures, nitrogen and organic matter loads and flows, due to daily and seasonal variations and rainfall periods. Promising results regarding the operation of PN/AMX systems are reported in laboratory scale reactors fed with synthetic media where low total nitrogen loading rates (NLR) are generally treated [6–8]. To date, scarce information about the operation of PN/AMX process treating municipal wastewater at psychrophilic temperature is available (Table 1).

The lower temperature of the mainstream, compared with that at sidestream, causes a decrease of the microbial activities and consequently decreases the potential loads to be treated. The anammox process is more sensitive to temperature changes than the nitritation process [1]. However, only few studies evaluated the system performance at mainstream conditions with realistic variable temperature. Lackner et al. [14] studied the influence of seasonal temperature fluctuations in two PN/AMX laboratory systems, with suspended biomass and biofilm, by gradually decreasing the temperature 0.5 °C per week from 20 °C to 10 °C. They achieved relatively high total nitrogen removal efficiencies (NRE), up to 75%, treating municipal wastewater (~1.7 g COD_S/g N) at temperatures over 12 °C. Later, at lower temperatures, TN removal decreased and both nitrite and nitrate accumulated in the reactor media. The suspend biomass based system did not recover its capacity when the temperature was reestablished to 20 °C whereas this phenomenon was transient in the biofilm system.

Another issue to consider is the presence of organic matter in excess in the wastewater that drives the heterotrophic denitrifiers development, which compete with the anammox bacteria for the nitrite [18]. For this reason, a prior stage for organic matter removal, known as A-stage, is required to favor the autotrophic route and to improve the energy efficiency of the WWTP [19].

In addition, the suppression of the nitrite oxidizing bacteria (NOB) is one of the most challenging aspects for the implementation of the PN/AMX systems at mainstream conditions [20]. The overgrowth of NOB is difficult to control as these bacteria, at low temperatures, have higher growth rates and lower oxygen affinity than AOB [1]. Laureni et al. [11] operated two moving bed reactors (12 L), to treat aerobically pretreated wastewater (~2.2 g COD_S/g N), at limited dissolved oxygen (DO) concentrations and temperatures decreasing from 29 to 15 °C. These authors achieved the effective suppression of NOB inside the reactor but the treated load (approximately 40 mg N/(L·d) at 15 °C) was low due to the use of microaerobic conditions. At pilot scale, Lotti et al. [16] evaluated the PN/AMX process in a one-stage plug flow granular system operated at 19 °C treating aerobically pretreated wastewater (~2.3 g COD_S/g N) and obtained NRE of approximately 39 ± 8% at NLR higher than 400 mg TN/(L·d). These authors observed difficulties to maintain the appropriated balance of microbial populations and detected the development of NOB whereas heterotrophic denitrifiers did not outcompete the anammox bacteria. Malovanyy et al. [15] obtained similar results at pilot scale treating a NLR of 100 mg N/(L·d). They achieved NRE of 52% operating an integrated fixed bed activated sludge system (IFAS) at 25 °C fed with anaerobically pretreated wastewater. Besides, Seuntjens et al. [13] operated a one-stage PN/AMX pilot plant at 20 °C treating the effluent of an aerobic A-stage characterized by 34–16 mg NH_4^+-N/L and 120 mg COD/L with NLR of 90–100 mg N/(L·d). These authors achieved nitrogen removal efficiencies of 51 ± 23% when the treated wastewater presented an organic matter to nitrogen consumption ratio of approximately 3 g COD/g N, and with the nitrite nitrogen removal ranging from 62% to 49%.

All these research works indicate that the long-term stability of the process fed with urban wastewater at pilot and full scale is still uncertain and several challenges need to be addressed before the PN/AMX processes are applied at mainstream conditions.

The objective of this study is to evaluate the operation of a granular sludge system where the partial nitritation and anammox processes take place simultaneously at mainstream conditions. A one-stage PN/AMX system operated without temperature control (varying from 12 to 18 °C) and treating municipal wastewater containing 6–25 mg TN/L and 16–197 mg COD/L. The effects of the high variability of both composition and temperature were assessed.

2. Materials and methods

2.1. Pilot plant operation

A pilot scale one-stage PN/AMX reactor was operated with the ELAN® (from the Spanish autotrophic nitrogen removal) process [21]. It had a volume of 600 L (with a height to diameter H/D ratio of 3.5). A set of two peristaltic pumps were used to fed (from the top) and discharge the reactor, respectively. A mechanical stirrer was used to guarantee the complete mixing, and oxygen was supplied using air diffusers placed at the bottom of the reactor. Moreover, the pilot plant was provided with online probes to measure pH (pHD connected to SC100) and DO (Hach LDO) concentrations. The SCADA software was used to monitor the pilot plant process and operation.

The pilot plant operated as a sequencing batch reactor (SBR) in cycles distributed among five different phases: feeding (10 min), anoxic reaction and mixing (15 min), aerobic reaction (variable length), settling (15 min) and effluent discharge (20 min). The imposed minimum settling velocity was of 2 m/h to enhance the biomass retention and to maintain long sludge retention times (SRT). The volume exchange ratio (VER) was fixed at 25%.

The pilot plant operational period (94 days) comprised three stages according to the aerobic phase duration (Table 2) and defined to avoid the proliferation of NOB activity. During Stage I (0–36 days) the aerobic phase had a fixed duration of 70 min and the air flow applied was of 6 L/min. In Stages II and III the aeration phase had a variable duration (from a minimum fixed time) based on the pH set point of 6.0. In this way, once the pH value decreased under 6.0 the aeration was stopped. In Stage II (37–61 days) the minimum time imposed for the aeration

Table 2
Operational stages of the PN/AMX pilot plant and operational cycle distribution.

Stage	Duration (days)	Set point to end up the aerobic phase	Aeration time (min)	Air flow (L/min)
I	0–36	Fixed time	70	6
II	37–61	pH ≤ 6.0	> 15[a]	6
III	62–94	pH ≤ 6.0	> 5[a]	4

[a] Minimum aeration time imposed before applying the control based on pH ≤ 6.

phase was of 15 min while the applied airflow was, as in the previous stage, 6 L/min. In Stage III (62–94 days), the minimum aeration time and the airflow were decreased to 5 min and 4 L/min, respectively.

The SBR was inoculated with granular biomass (1.5 g VSS/L) collected from an ELAN® pilot plant [21] located at a municipal WWTP in Tui-Guillarei (Pontevedra, NW of Spain). It operated at 30 °C to remove the nitrogen present in the supernatant from the sludge anaerobic digester (500–1000 mg N/L) of the plant. The main microbial populations present in the inoculum were anammox bacteria (*Brocadia fulgida)*, AOB (*Nitrosomonas*), and NOB (*Nitrospira*) [21].

2.2. Wastewater characteristics

The wastewater from the previous municipal WWTP was fed to the pilot plant after being primary settled, with the characteristics indicated in Table 3 for the different operational stages. The pilot plant operated during wintertime (characterized by frequent rain events) and, thus, the wastewater stream experienced high variable compositions.

2.3. Analytical methods

Influent and effluent streams were periodically sampled to follow the pilot plant performance and to determine the nitrogen removal efficiency and the effluent quality. Single operational cycles were monitored to evaluate the evolution of the concentrations of the different compounds in the liquid phase and the biomass. The concentration of chemical oxygen demand (COD), total nitrogen (TN), ammonium, nitrite and nitrate were determined spectrophotometrically with test kits (Hach Lange, Germany). Prior to the analysis, each sample was filtered using 0.45 µm pore size filters. Both pH and conductivity were measured using a Crison Multimeter MM41 with the corresponding probes.

The concentration of total suspended solids (TSS) and volatile suspended solids (VSS) as well as the sludge volume index (SVI) were determined according to Standards Methods [22]. The average diameter of the granules and size distribution were determined using a digital camera (Moticam 5) for image acquisition and then these images were processed using the Image ProPlus® software.

Table 3
Average wastewater composition fed to the PN/AMX pilot plant during the different operational stages.

Parameter	Stage I (0–36 days)	Stage II (37–61 days)	Stage III (62–94 days)
pH	7.46 ± 0.13	7.22 ± 0.28	7.06 ± 0.12
Temperature (°C)	15.97 ± 1.30	14.83 ± 1.03	14.02 ± 0.66
COD_T (mg/L)	158.58 ± 28.37	67.93 ± 26.45	44.50 ± 16.79
TN (mg N/L)	22.38 ± 3.03	17.70 ± 5.95	11.54 ± 3.15
NH_4^+-N (mg N/L)	13.32 ± 2.35	9.03 ± 4.06	6.41 ± 2.98
NO_3^--N (mg N/L)	0.88 ± 0.42	1.27 ± 0.86	1.65 ± 0.58
TSS (mg/L)	47.29 ± 14.24	38.20 ± 15.09	20.60 ± 4.58
COD_S/TN (g/g)	4.40 ± 0.84	2.74 ± 0.62	1.52 ± 0.77

2.4. Ex situ batch microbial activity tests

The maximum specific nitrifying activities (both for AOB and NOB) were determined by respirometric tests following the oxygen uptake rate according to López-Fiuza et al. [23]. Experiments were carried out in a 1.5 L vessel at room temperature. A portable oximeter (Hach HQ30d) with a Hach LDO probe was used for the data acquisition. The headspace of the vessel was flushed with argon gas to avoid oxygen transfer in the liquid media.

The maximum specific anammox activity (SAA) was determined using the methodology described by Dapena-Mora et al. [24]. The batch tests were performed in 135 mL flasks hermetically closed, and the headspace was flushed with Argon gas. The flaks were incubated at 30 °C, temperature used as reference, and once the substrates were added (70 mg N/L of ammonium and nitrite, respectively), the nitrogen gas pressure was recorded online.

2.5. Calculations

The contribution of the activity of the heterotrophic denitrifying bacteria to the total nitrogen removal was estimated via mass balances. Considering that only the total COD (COD_T) concentration was analyzed in the influent, and no information about the soluble COD (COD_S) was available, the incoming soluble organic matter was determined by subtracting the COD associated to the total solids (estimated by 0.75 g TSS/g COD according to ASM2 model) to the COD_T. Then, the maximum concentration of organic matter possibly removed by the heterotrophic denitrifying bacteria (HDN) was estimated by using the ratio of 0.2 g N_2-N produced/g COD consumed [25], and calculated according to Eq. (1):

$$HDN=0.2\cdot\left(COD_{T,inf}-\frac{TSS_{inf}}{0.\ 75\ gCOD}-COD_{S,ef}\right) \tag{1}$$

Note that the organic matter can be used for growth and/or aerobically oxidized, which means that the previous calculation provides only a theoretical value of the maximum concentration of COD possibly removed by hetrotrophic denitrification in the process.

3. Results and discussion

3.1. Reactor performance

The pilot reactor operated for 94 days. During Stage I (0–36 days), the aerated phase length was fixed and equal to 70 min (Table 2). The applied NLR in this stage was on average 62 ± 9 mg N/(L·d) and the achieved total nitrogen removal efficiency of 54 ± 14% (Fig. 1A). The TN concentration in the effluent was higher than the legal discharge limits in the area (10 mg N/L) in almost the whole Stage I. The average composition in the effluent was 8.24 ± 4.47 mg NH_4^+-N/L, 0.54 ± 0.32 mg NO_2^--N/L and 2.48 ± 2.64 mg NO_3^--N/L (Fig. 1B). Moreover, from day 30 onwards, with the decrease of the incoming nitrogen concentration, the nitrate concentration in the effluent rose up from 1 to 9 mg NO_3^--N/L indicating the presence of NOB activity.

Later from day 37 onwards (Stages II and III), due to the high variable wastewater composition, the SBR cycle configuration was optimized and changed to one with variable duration (Table 2). The endpoint of the aeration phase (pH ≤ 6) was imposed to guarantee a minimum ammonium concentration inside the reactor, and to avoid the availability of oxygen in excess that could lead to the proliferation of NOB. Furthermore, the pH value was set at 6.0, to avoid the inhibition of anammox bacteria occurring in acid conditions [26,27]. This strategy was based on the good correlation between the ammonium concentration (influent and effluent) and pH value in the reactor (Fig. 2). This correlation was previously described, and used by Malovanyy et al. [15] to control the load applied to a PN/AMX system to maintain a

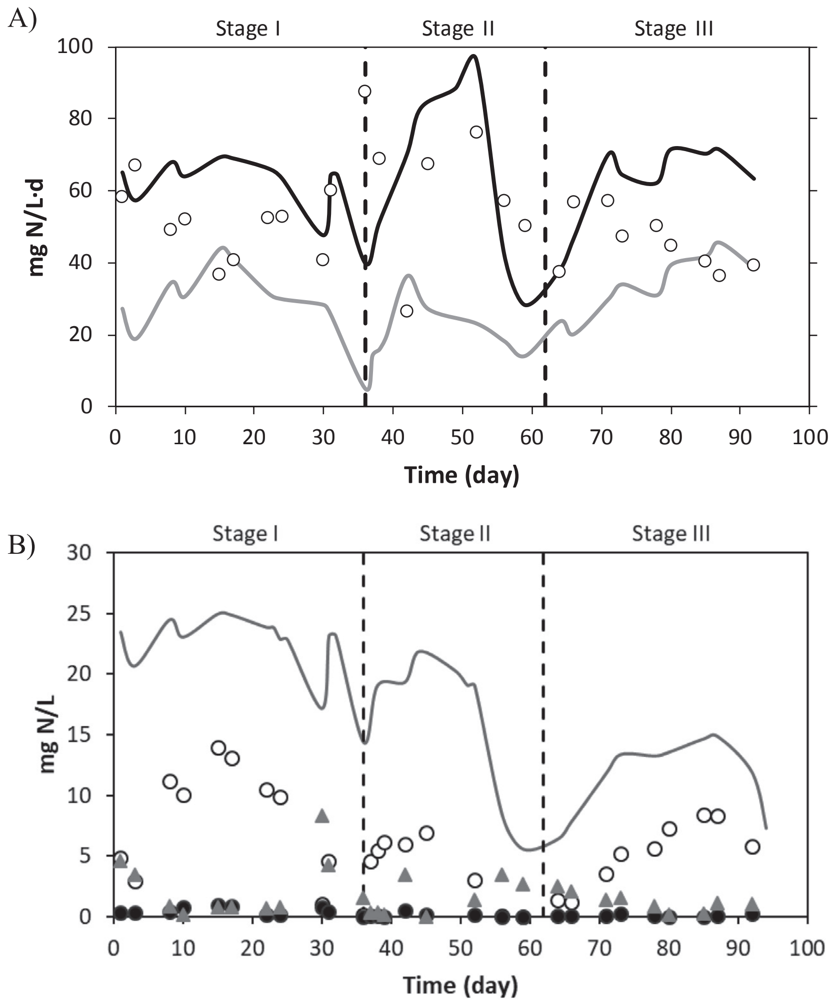

Fig. 1. (A) Evolution of the total nitrogen loading rate (mg N/(L·d)) in the influent (—) and effluent (—) and the percentage of total nitrogen removal efficiency (○). (B) Evolution of the nitrogen concentrations (mg N/L): total nitrogen in the influent (—) and ammonium (○), nitrite (●) and nitrate (▲) in the effluent.

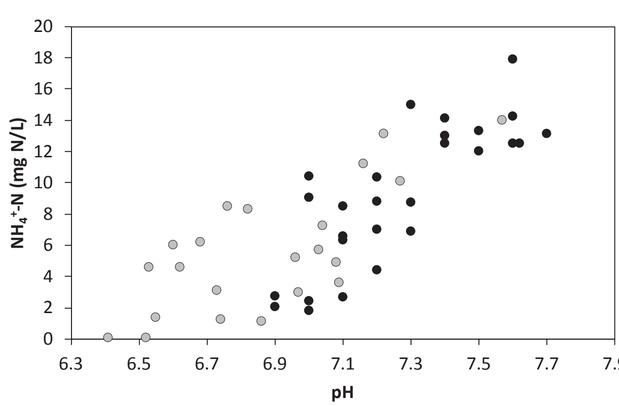

Fig. 2. Correlation between pH and ammonium concentration in the influent (●) and effluent (●) of the pilot SBR.

stable ammonium concentration in the effluent. Although, these authors showed that the deviation of the results was lower when an ammonium set point was used for the same purpose, a pH-based control was selected in the present study, as pH probes are more reliable and less expensive.

The pH value in the influent of the pilot plant was higher than 6.9 (Table 3 and Fig. 2), throughout the complete operational period, which facilitates the control of the process based on a set point of pH ≤ 6.0. Since the nitritation process consumes alkalinity, the addition of chemicals is not needed to control the pH value at 6.0, contrary to previous studies [13,16]. Most of the already published PN/AMX studies control the pH value inside the reactor to values higher than 7.0. Seuntjens et al. [13] treated in a PN-AMX unit the effluent of an aerobic A-stage supplemented with extra ammonium, and added Na_2CO_3 to compensate the alkalinity consumption and to maintain the pH value at 7.0–7.3. Lotti et al. [16] controlled the pH at 7.0–7.5 by addition of NaOH.

Besides the pH control, a minimum aeration time of 15 min in Stage II was fixed to guarantee the sufficient ammonium oxidation to nitrite. Thus, the registered average pH values at the end of the cycle were 5.65 ± 0.25. The NRE was maintained at 58 ± 18%. In this stage, the total NLR applied was increased, due to the decrease of hydraulic retention time (HRT) from 8.67 h (Stage I) to 6.21 ± 1.48 h (Stage II), reaching maximum values up to 96 mg N/(L·d). The TN concentration in the effluent was lower than 10 mg N/L. Moreover, due to a decrease in both organic matter and nitrogen concentrations (Table 3) in the incoming wastewater, the DO concentration inside the reactor increased from 1.25 ± 0.49 mg O_2/L in Stage I to 3.26 ± 1.79 mg O_2/L in Stage II. The average composition of the effluent was 4.57 ± 3.46 mg NH_4^+-N/L, 0.14 ± 0.18 mg NO_2^--N/L, 1.51 ± 1.51 mg NO_3^--N/L and 4.96 ± 5.10 mg COD/L. After a raining period on day 52, the total applied NLR sharply decreased from average values of 78 ± 17 mg N/(L·d) (days 37–52) to 28 mg N/L·d on day 60. At the end of Stage II, the incoming TN concentration in the reactor was lower than 10 mg N/L. Then, the ammonium was fully depleted, and the excess of aeration caused a nitrate build up and a drop of the pH to minimum values of 5.34. Such low pH values have been reported to be prejudicial for both AOB and anammox bacteria [26,28]. Indeed, it was proposed that, instead of the low pH value, the actual inhibitor of both bacterial groups, and NOB, is the free nitrous acid (FNA). However, in the present study, due to the observed negligible nitrite accumulation, the maximum FNA concentration reached was of 0.007 mg HNO_2-N/L, lower than the reported inhibitory values for AOB and NOB [29]. The sole pH decrease can be responsible for the inhibition of the nitrification process by provoking the deactivation of nitrifying enzymes and changes in the equilibrium carbonate-bicarbonate [28]. Actually, it is widely stated that the nitrification process does not occur at pH values lower than 6.0 [30]. Anammox bacteria are more sensitive to pH than the nitrifying bacteria being their optimal pH range between 6.7 and 8.3 [2,24].

Finally, in Stage III the airflow was reduced from 6 L/min to 4 L/min and the minimum aeration time from 15 min to 5 min (Table 2). The DO concentration decreased to average values of 1.57 ± 0.26 mg O_2/L. The HRT was further shortened from 6.21 ± 1.48 h (Stage II) to 4.62 ± 1.48 h (Stage III) according to the cycle duration. During this stage, the NLR was restored to average values of 62 ± 12 mg N/(L·d) but the NRE slightly decreased to 45 ± 8%. However, in this stage the nitrogen removal efficiency was not limited due to an increase of the effluent nitrate concentration (development of NOB activity) as in the Stage I, since the concentration measured in the effluent was approximately of 1.25 ± 0.83 mg NO_3^--N/L. This concentration was similar to the incoming nitrate concentration (1.65 ± 0.58 mg NO_3^--N/L), which indicates that the concentration of ammonia oxidized to nitrate was negligible. At the beginning of the Stage III, the ammonium was almost completely removed. Later on, its concentration in the effluent increased progressively from 1 to almost 9 mg NH_4^+-N/L (day 85) caused by the limited aeration supply, while the pH value reached an average value of 5.95 ± 0.04 at the end of the operational cycle. The average composition in the effluent during this stage was of 5.22 ± 2.69 mg NH_4^+-N/L, 0.12 ± 0.09 mg NO_2^--N/L, 1.25 ± 0.78 mg NO_3^--N/L and 9.89 ± 3.73 mg COD/L.

These results indicate that the optimization of the cycle configuration is required to avoid the observed ammonium removal limitation. A detailed analysis of the nitrogen species concentrations and pH value throughout an operational cycle with a prolonged aeration time was carried out on day 88 (Fig. 3). During the first 25 min (feeding and anoxic reaction) nitrate mass slightly decreased. Then, after the beginning of the aeration phase, a decrease of the ammonium mass was observed together with the increase of the amount of the nitrogen oxidized species. From the minute 70 onwards, nitrate production speed up and even small amounts of nitrite were accumulated (< 1 mg NO_2^--N/L). At this point, the registered pH value was of 5.9 and this value might be the next set point in the operation strategy to further exploit the AOB activity allowing to oxidize more ammonium (without

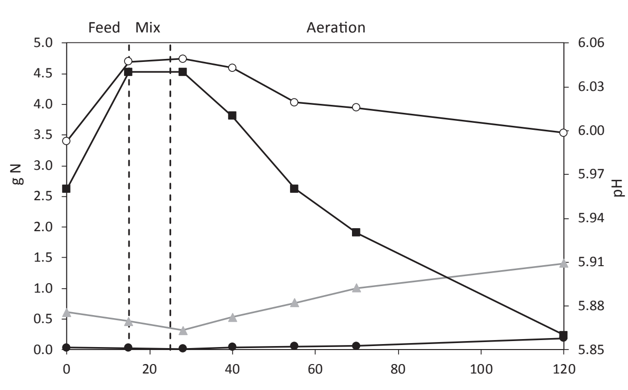

Fig. 3. Evolution of the nitrogen species during the SBR cycle with prolonged aeration (day 88): mass of ammonium (○), nitrite (●) and nitrate (▲) in g N, and pH (■).

extensively increase the nitrate production) and to reach higher nitrogen removal efficiencies.

During the whole experimental work period, average total nitrogen removal efficiencies of 52 ± 14% were achieved treating municipal wastewater characterized by high variable compositions. The TN concentrations in the influent varied widely from 25 to 6 mg N/L (on average 17 ± 6 mg N/L) and the organic matter from 16 to 197 mg COD/L. Similar NRE were achieved by Malovanyy et al. [15] treating anaerobic pretreated urban wastewater with relative high variability in both nitrogen and organic matter concentrations (Table 1). However, the temperature in the current study was on average 10 °C lower than the used in the cited study [15]. Similarly, Han et al. [10] achieved a NRE up to 70% treating higher NLR without temperature control ranging from 19 to 31 °C, higher than the one observed in the present study. Lotti et al. [16] treated much higher NLR (up to 570 mg N/(L·d)) with a maximum NRE of 46% limited by the high nitrate concentrations registered in the effluent (9 mg N/L), and approximately 18 mg TN/L present in the effluent. Regarding the previous studies the fact that many of them used municipal wastewater supplemented with ammonium must be considered [13,14], which is not the case of the present study. Laureni et al. [11] also treated low loaded wastewater achieving NRE up to 63% removing NLR of approximately 25.2 mg N/(L·d), similar to the 33.5 mg N/(L·d) removed in the present study (Table 1). As in Stage III of this study, the nitrogen removal rate achieved in Laureni et al. [11] was limited by the ammonium oxidizing rate due to the imposed microaerobic conditions. Despite the similar results obtained in both research works, Laureni et al. [11] used a previous A-stage for organic matter removal and they did not face the fluctuations of temperature and applied NLR that took place in the present study, which will be commonly happening in the full-scale systems.

Besides the uncontrolled temperature and the changes in terms of composition due to the rainfall, low pH set point allows for the operation without any chemical addition and the obtainment of an effluent that accomplishes the legal discharge limit of 10 mg N/L (Fig. 1B, days 30–94). However, the impact of the fluctuations of the inlet nitrogen concentrations need to be considered in the future as a possible factor that can compromise the fulfillment of the discharge limit, and further research is necessary to evaluate even larger fluctuations. Regarding the temperature, Daverey et al. [26] state that the negative effect of the low temperature over the anammox bacteria activity might be partially compensated by maintaining a high pH value. The optimal pH range presumably narrows along with the temperature decrease, registering anammox activity at 13 °C for pH values from 6.8 to 8.8, whereas at 10 °C activity was only observed from pH 7.3 to 8.3 [31]. However, at the present study, the pH value was maintained under these values and nitrogen was successfully removed.

3.2. Biomass properties

The operation at mainstream conditions has been reported to cause PN/AMX granules disintegration [20] due to the low nitrogen concentration and low microbial specific activity at low temperature. This phenomenon did not occur in the present study since both biomass concentration and the average granular size increased (Table 4). The biomass concentration increased in Stage I from 1.5 (day 0) to 2.0 g VSS/L (day 36) and then this value remained almost stable. The average granular size remained invariable or slightly increased from 1.00 ± 0.06 mm to 1.22 ± 0.11 mm at the end of the operational period. This fact indicated that not only more granules formed but also, they became bigger. In Stage I the biggest granule size measured was of 2.5 mm, while in Stage III the 22% of the total volume corresponded to particles bigger than this size (Fig. 4). In good agreement with the present study, Lotti et al. [16] also observed an increase of the granule size in their system, despite the presence of COD and solids in the influent traditionally considered negatively affect the aggregate properties.

Table 4
Biomass properties during the different stages of the PN/AMX pilot plant operation.

Parameter	Stage I (0–36 days)	Stage II (37–61 days)	Stage III (62–94 days)
TSS reactor (g/L)	2.37 ± 0.48	2.93 ± 0.24	2.91 ± 0.33
VSS reactor (g/L)	1.51 − 1.98	2.26 ± 0.28	2.10 ± 0.19
TSS effluent (mg/L)	48.43 ± 41.70	19.60 ± 4.34	20.00 ± 8.22
SRT (d)	21.94 ± 5.37	–	26.58 ± 11.89
SVI (mL/g TSS)	83.62	58.06	66.59
Diameter (mm)	1.00 ± 0.06	1.15 ± 0.06	1.22 ± 0.11

The estimated SRT was long throughout the whole operational period (Table 4). Long enough SRT value is required to prevent the biomass washout caused by the low growth rates of the microorganisms involved in the PN/AMX process [20,3].

The improvement of the biomass settling capacity was also corroborated by the improvement of the sludge volume index (SVI) that was of 84 mL/g TSS in Stage I, considered a moderate compaction capacity. Then, in Stage II the SVI decreased to 58 mL/g TSS and in Stage III remained at a similar value (66 mL/g TSS). Therefore, the settling properties are considered optimal (< 80 mL/g TSS) [32]. The effluent quality in terms of total suspended solids accomplished the discharge limits in the region (35 mg TSS/L) being on average in Stages II and III of 19.87 ± 7.24 mg TSS/L (Table 4). This value was higher during Stage I, probably due to the higher solids concentration in the influent (Table 3), indicating that an optimization of the cycle was required.

The improvement of the settling properties will allow operating the system with sedimentation phases shorter than the one used in this research work, which involves the shortening of the HRT and consequently the increase of the total nitrogen load treated. The longest HRT assayed in the pilot plant was of 8.7 h, comparable to that used in the conventional treatments at WWTPs, and it was decreased even to minimum values of 4.1 h.

The maximum specific AOB activity of the biomass increased progressively throughout the operational period from values of 25 mg N/(g VSS·d) in Stage I to a maximum value of 91 mg N/(g VSS·d) in Stage II. As the biomass concentration in the reactor also increased, the ammonium oxidation capacity rose up to 182 mg N/(L·d). Taking into account the applied nitrogen load, the system was operated at 50–75% of its maximum ammonium oxidation capacity. As nitrite did not accumulate in the system (Fig. 3), the operation can be further optimized to enhance the AOB activity and increase the total nitrogen removal rate.

NOB activity was not detected by the respirometric assays performed with the biomass from the reactor. As the activity of the NOB consume less oxygen per unit of nitrogen oxidized than that of AOB, the sensitivity of this method might be not enough for the determination of the presumably low NOB activity. For this reason, in certain periods during the reactor operation, nitrate concentrations higher than those corresponding to the stoichiometry of the anammox process were obtained in the effluent corresponding to an estimated maximum nitrite oxidizing rate of 8 mg N/(g VSS·d). The average NOB activity measured in the reactor was of 3.5 ± 2.0 mg N/(g VSS·d) and it was negligible during Stage III.

The measured SAA of the biomass at 30 °C (as reference temperature, [24]) was up to 115 mg N/(g VSS·d). It was widely reported that the anammox activities sharply decrease with the temperature [33,34]. Considering the reported activation energy for anammox bacteria of 70 kJ/mol [2,33], the estimated maximum SAA at the operational temperature (15 ± 1 °C) would be 20–30 mg N/(g VSS·d). The obtained values were comparable to those found by other authors [20,33]. In the present study the measured SAA indicate that the system presumably had higher potential nitrogen removal capacity than the observed nitrogen removal rate, indicating that anammox activity was limited by the nitrite production by AOB (its concentration was negligible throughout all the operational period). It is also possible that the anammox activity was inhibited by the presence of DO or the organic

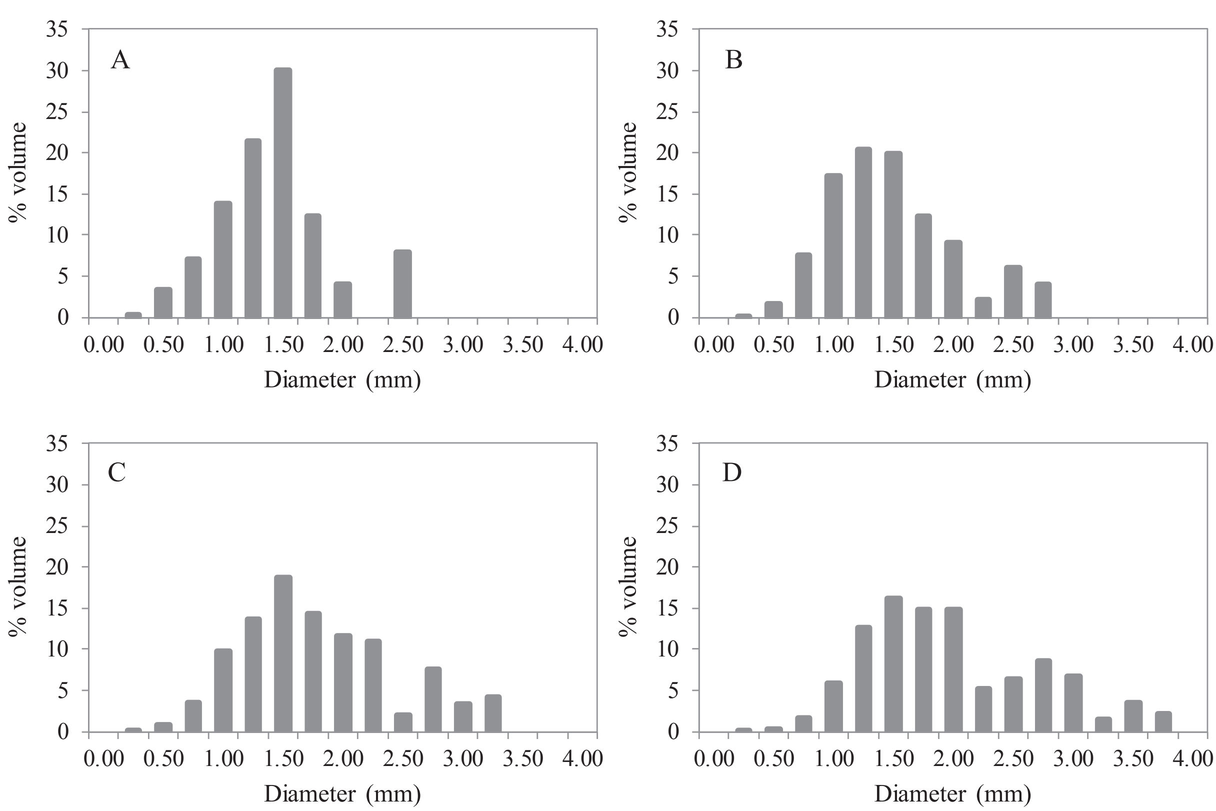

Fig. 4. Evolution of the volume distribution of the granules with respect to the average diameter on days 11 (A), 38 (B), 73 (C) and 93 (D).

matter contained in the wastewater [27].

3.3. Impact of the wastewater composition

The wastewater characteristics in the mainstream of the urban WWTP showed high variability in terms of flow, concentration and temperature (Table 3). It should be noted that no organic removal unit was used in this experimental set up, but the total COD concentration (except for Stage I that was 158.6 ± 28.3 mg COD/L) was comparable to those obtained by other authors where PN/AMX systems are placed after organic matter removal treatment (Table 1). For example, Laureni et al. [11] operated a PN/AMX system, located after an aerobic COD removal unit, at a sludge retention time of 1 day and treating 69 ± 18 mg COD/L comparable to the 51 ± 22 mg COD/L of the present study.

Contrary to other research works where the COD/N ratio in the influent remains almost stable, in the present study this ratio also varied (Table 3). At the beginning of the operation was of 4.4 ± 0.84 g COD_S/g TN close to the recommended value for the heterotrophic denitrification of 5 g COD_S/g N [3]. Throughout the operation, the COD_S/N ratio progressively decreased, favoring the establishment of the autotrophic nitrogen removal processes such as PN/AMX process. Besides the total nitrogen, most of the incoming COD was also removed (> 75%) presumably by both aerobic and anoxic heterotrophic bacteria. Considering the DO concentration maintained inside the reactor (2.0 ± 1.4 mg O_2/L) only limited denitrification (anoxic) would be expected during the aerobic phase. However, the yield of nitrate produced over nitrogen consumed was close to zero and the concentration in the effluent was similar to that in the influent indicating that at least the expected nitrate produced by the anammox bacteria (0.11 NO_3^--N/NH_4^+-N, for combined PN/AMX processes) was denitrified. The absence of nitrite in the effluent can be due to its consumption by anammox bacteria (desirable) but also by the oxidation to nitrate by NOB or the reduction to nitrogen gas by the heterotrophic denitrification.

The average COD_S/TN removal ratio also shows high variability and was of 4.3 ± 2.1 g COD_S/g N close to the minimum theoretical one needed for the denitrification (5 g COD_S/g N). During Stage I, the organic matter content in the wastewater was higher (Table 3) corresponding to COD_S/N removed ratio of 4.4 ± 0.84 g COD_S/g N. The major part of the organic matter was removed anoxically in the feeding and mixing phases (up to 60%) and the nitrogen removal by the heterotrophic denitrification amounted to a maximum of 35% of the total nitrogen removed in the whole cycle. The COD_S/N removed ratio in Stage I during the anoxic feeding was 1.6 g COD_S/g N indicating the coexistence of both autotrophic and heterotrophic processes. Later on, during Stages II and III, from the mass balance to cycle measurements in the different phases (data not shown) it can be stated that COD was mainly removed in the aerobic phase by the aerobic heterotrophs, which indicated the low contribution of denitrification process in the total nitrogen removal (< 10%). Therefore, despite the presence of organic matter, the anammox process was the predominant process responsible for the nitrogen removal.

The autotrophic nitrogen removal is expected to be improved if the organic matter concentration in the wastewater is low. To achieve this, the application of the PN/AMX processes at mainstream conditions will be further optimized if a two-stage approach is used. In this way, in the partial nitritation unit the remaining organic matter will be aerobically oxidized, and the ammonium from the wastewater converted to nitrite. Then, in the anoxic reactor, as no organic matter is available, the anammox process while avoiding the out competition of heterotrophic denitrifiers and the anammox bacteria inhibition by DO, will remove the nitrogen. At the same time, this study also pointed out the promising results of the application of the combined PN/AMX processes obtained without the existence of a previous organic matter removal stage (just primary sedimentation) in those regions with low to moderate loaded wastewaters.

4. Conclusions

To cope with the high variability of the wastewater composition is one of the main challenges to implement the PN-AMX processes in the main line of a WWTP and to achieve the stringent discharge limits.

Despite the high variability of nitrogen load and temperature during the operational period, a granular partial nitritation-anammox pilot plant operated reaching low nitrogen concentrations in the effluent, lower than 10 mg N/L (discharge limit) and nitrate concentrations lower than 3 mg NO_3^--N/L.

The pH based control strategy allowed obtaining a total nitrogen removal efficiency of approximately 50% treating a total nitrogen load of 67 mg N/(L·d). Both biomass concentration and granule size increased during the operational period. An optimization of the pH set point to end up the aeration phase may allow exploiting the AOB activity, increasing the nitrite availability for the anammox process and consequently the nitrogen removal efficiency.

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