High-throughput nitritation of reject water with a novel ammonium control loop: stable effluent generation for anammox or heterotrophic denitritation

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Highlights

- High nitrogen loading rate in a controlled activated sludge for partial nitritation

- Nitrogen loading rate of 5.0 or 9.3 g N L^{-1} d^{-1} for reject or synthetic water

- The new control system allows a suitable and stable effluent for anammox treatment

- Effluent with total nitritation also achieved only modifying the ammonium setpoint

Graphical Abstract
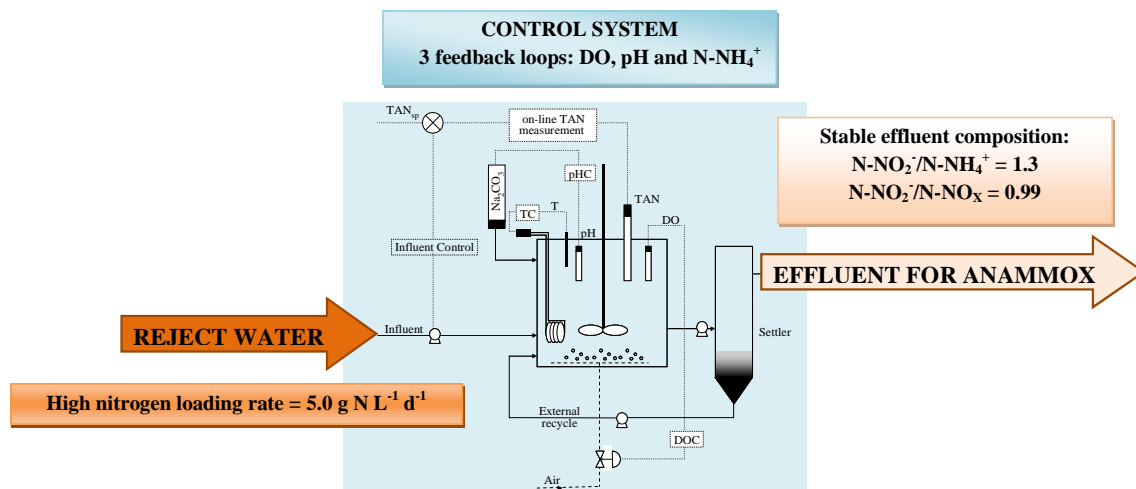
Abstract

This work presents a new control system for the nitritation of high-strength ammonium wastewater as reject water from sludge dewatering. It is based on three independent feedback control loops: i) DO control by manipulating the aeration flow-rate, ii) pH control with the addition of solid Na_2CO_3 and iii) control of NH_4^+-N concentration in the reactor using the influent flow-rate as the manipulated variable. Its application in an activated sludge configuration with one reactor and a settler, demonstrated: i) capability to achieve stable effluent composition with proper NO_2^--N/NH_4^+-N ratio for anammox treatment and ii) possibility to obtain an effluent with full nitritation suitable for heterotrophic denitrification only modifying the ammonium setpoint. A nitrogen loading rate (NLR) up to 5.0 ± 1.0 gN $L^{-1}d^{-1}$ was stably treated using real reject water (T = 30ºC, pH = 7.5) with a NO_2^--N/(NO_2^--N+NO_3^--N) ratio of 99%. NLR reached up to 9.3 ± 0.5 gN $L^{-1}d^{-1}$ with synthetic wastewater.

Keywords: anammox, AOB, control, partial nitrification, nitritation, TAN

Nomenclature

ACR   ammonia consumption rate

AOB   ammonia oxidizing bacteria

CLSM   confocal laser scanning microscopy

DO   dissolved oxygen

FA   free ammonia

FISH   fluorescence in situ hybridization

FNA   free nitrous acid

HRT   hydraulic residence time

NLR   nitrogen loading rate

NOB   nitrite oxidizing bacteria

OUR   oxygen uptake rate

PI   proportional-integral controller

PID   proportional-integral-derivative controller

PN   partial nitritation

SACR   specific ammonia consumption rate

SRT   sludge retention time

TAN   total ammonia nitrogen (TAN = NH_4^+-N+ NH_3-N)

TAN_{SP} TAN setpoint

TIC   total inorganic carbon

TNN   total nitrite nitrogen (TNN = NO_2^--N + HNO_2-N)

TSS   total suspended solids

VSS   volatile suspended solids

WWTP   wastewater treatment plant

μ_{AOB}	specific growth rate of AOB
μ_{NOB}	specific growth rate of NOB;
$\mu_{max,AOB}$	maximum specific growth rate of AOB
$\mu_{max,NOB}$	maximum specific growth rate of NOB
b_{AOB}	decay rate of AOB
b_{NOB}	decay rates of NOB
$b_{max,AOB}$	maximum decay rate of AOB
$b_{max,NOB}$	maximum decay rate of NOB
$K_{I,FA,AOB}$	FA inhibition constant of AOB
$K_{I,FA,NOB}$	FA inhibition constant of NOB
$K_{I,FNA,AOB}$	FNA inhibition constant of AOB
$K_{I,FNA,NOB}$	FNA inhibition constant of NOB
$K_{S,DO,AOB}$	DO affinity constant of AOB
$K_{S,DO,NOB}$	DO affinity constant of NOB
$K_{S,FA,AOB}$	FA affinity constant of AOB
$K_{S,FNA,NOB}$	FNA affinity constant of NOB

1. Introduction

Reject water is a high-strength ammonium wastewater produced in the sludge dewatering process in wastewater treatment plants (WWTP). This effluent is usually mixed with the influent of the WWTP to be treated in the conventional water line. However, different studies have demonstrated that the specific and separated treatment of reject water is more convenient than its recycle [1]. Among the proposed treatments, biological processes are the most convenient from both economic and ecological points of view. Biological nitrogen removal of reject water can be performed by i) the classical nitrification – denitrification (full ammonium oxidation to nitrate followed by heterotrophic denitrification), ii) nitritation – denitritation (oxidation of ammonium to nitrite followed by nitrite denitrification), which has some advantages compared to the conventional process [2, 3] and iii) partial nitritation (PN) – anammox which is the most novel process and ensures nitrogen removal through an autotrophic process [4, 5]. As a pretreatment of the anammox reactor, the PN reactor has to achieve an effluent ratio of total nitrite nitrogen (TNN = NO_2^--N + N-HNO_2) / total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N) around 1.3, which is the stoichiometric ratio required by anammox:

$$NH_4^+ + 1.3\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$$
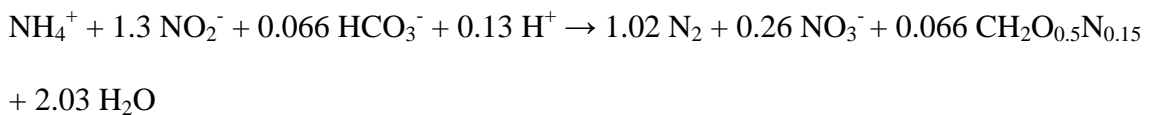
One of the most common PN reactors for achieving the suitable influent for anammox is the SHARON process [4]. However, recent studies have shown that the actual bottleneck in the overall capacity of the autotrophic N-removal process is due to the limiting capacity of the first part of the treatment, that is, PN with the SHARON reactor

[6]. This limitation is due to the low biomass concentration that can be achieved because it works without biomass retention to achieve and maintain PN [7]. Consequently, the development of robust technologies for PN at higher nitrogen loading rates (NLR) is required to improve the capacity of the autotrophic N-removal [8].

PN reactors for anammox systems are usually operated without advanced control loops, as only DO control is usually implemented. The effluent with the required TNN/TAN ratio for the anammox step is achieved thanks to the bicarbonate/TAN ratio of the reject water, which typically contains the stoichiometric alkalinity required to oxidize around 50% of the inlet ammonium [9]. However, the treatment of wastewaters without the proper bicarbonate/TAN ratio or some fluctuations of influent TAN and alkalinity concentrations could strongly affect the TNN/TAN ratio of the effluent and therefore it could disturb the anammox process [10, 11].

Process control is widely recognized in the literature as essential to ensure successful reactor operation under different influent conditions in PN systems [12]. Main control options recommended consider flow adjustment, influent total inorganic carbon (TIC) control and base/bicarbonate dosing in the reactor. Flow adjustment is a feasible option because a large number of sludge dewatering systems in WWTP work only part of the day and hence reject water storage is already available. For example, centrifuges generally operate only during the working hours, and reject water is already stored with the objective of distributing its load during all the day. Many other industries as chemical, pharmaceutical or food industries also produce high-strength ammonium wastewaters discontinuously that must be stored and treated progressively.

In this scenario, the development of a new PN system with a specific control loop is a requirement to produce a proper effluent for anammox treatment from any high-strength

ammonium wastewater, independently of its bicarbonate/TAN ratio. To this aim, a novel automatic control loop able to maintain a specific TAN concentration in the effluent was developed and applied to a single activated sludge nitrifying reactor under continuous operation. The TAN control loop manipulates the influent flow-rate to obtain a more reliable system able to treat reject water at high rates and obtaining an effluent suitable for a subsequent anammox reactor. Moreover, the versatility of the control system was studied for achieving an appropriate effluent for a subsequent heterotrophic denitritation by only decreasing the TAN setpoint (TAN_{SP}).

2. Materials and Methods

2.1. Partial nitritation system setup

The experiments were performed in a continuous activated sludge system consisting of an aerobic mixed reactor with a working volume of 25 L followed by a 25 L settler (Figure 1). The reactor was equipped with measurement systems for dissolved oxygen (DO) (WTW Oxi 340i CellOx 325), pH (Crison pH 52-03) and temperature (Pt-100). TAN was measured with an on-line ammonium ion selective electrode (NH4Dsc Ammonium sensor with a Cartrical cartridge and a SC100 controller, Hach Lange, Düsseldorf, Germany), which provided a stable measurement with low noise. The DO control was based on a proportional-integral derivative (PID) algorithm operated by manipulating a pneumatic control valve which modified the airflow supplied through an air diffuser placed at the bottom of the reactor. DO was controlled at 2.0 mg O_2 L^{-1} throughout all the study. The pH control was an on-off controller adding solid sodium carbonate through a solid dispenser. When operating with this pH control, the selected

setpoint was 7.5. The temperature control, based on an on-off control, was operated by switching an electrical heating device.

The hydraulic residence time (HRT) was not constant, varying in the range 3-8 h during pH controlled operation and increased up to 21 h when pH was not controlled. The sludge retention time (SRT) was kept at different values depending on the operational period. Typical SRT values were in the 3-6 d range.

2.2. TAN control loop

The TAN control loop consisted of a feedback proportional-integral (PI) controller. The controller was initially tuned with the integral of the square error (ISE) criterion [13] based on the modelled response, but the parameters were corrected during the first experimental period to minimize the effect of fast disturbances. PI gain and integral time parameters were set after the tuning period to $Kc = 0.75$ L^2 mg^{-1} N h^{-1} and $\tau_I = 1.67$ h.

The controlled variable was the TAN concentration in the reactor measured with the NH4Dsc on-line ammonium probe. It was measured every 10 minutes and then its 30-minutes moving average value was compared to the TAN$_{SP}$. The difference among these two TAN values was the error fed to the PI controller algorithm, which calculated a new inflow value and, as a result, the new NLR. Finally, the control action (new flow) was transmitted to a process computer that changed the pulse frequency of the inflow pump. Figure 1 schematically shows this control loop, together with the others previously defined.

The TAN control loop was implemented in a supervisory expert control system using Gensym G2$^{©}$ [14] and was run in a Sun workstation, although its implementation in

other control systems is also possible. This control loop is an evolvement of a previously designed control loop based on oxygen uptake rate (OUR) measurements [15-17]. The utilization of OUR as controlled variable allows the maintenance of stable full nitritation (100% oxidation of TAN to TNN), but does not allow working at a high ammonium concentration, avoiding the achievement of an effluent suitable for an anammox reactor (TNN/TAN ratio around 0.5). The reason is that a TAN concentration around 5 mg $N \cdot L^{-1}$ already gives the maximum OUR, and hence it is not possible to distinguish TAN concentrations higher than this value with only OUR measurements. Selecting TAN as controlled variable requires the utilization of an on-line ammonium analyzer, but does not have this limitation and any setpoint inside the measurement range of the equipment can be selected. It provides versatility and theoretically would allow producing an effluent with the proper TNN/TAN ratio to feed an anammox reactor or an effluent with almost 100% of TNN to feed a heterotrophic denitritation reactor if a low TAN_{SP} is selected.

2.3. Wastewater characteristics

The experiments were initially carried out with synthetic wastewater in order to test the viability of the TAN control loop. The synthetic influent mimicked the reject water from the dewatering process of anaerobically digested sludge, except for a lower TIC concentration. It contained a high TAN concentration (1250 ± 150 mg L^{-1}), a low amount of biodegradable organic matter (acetate, 30-35 mg COD L^{-1}) and a TIC concentration of 40.0 ± 1.0 mgC L^{-1}.

After 60 days of operation, the influent was progressively changed to real reject water from a municipal WWTP of Barcelona area (Spain). The reject water was weekly

changed and stored in a 2000 L refrigerated tank at 10ºC. The average TAN

concentration of the reject water was 554±65 mg N L^{-1}.

2.4 Sludge inoculum

The inoculation of the reactor was performed with activated sludge taken from a PN

pilot plant. This pilot plant was composed by three continuous stirred tank reactors and

a settler and was operated at T= 30 ºC, DO = 2.0 mg L^{-1} and pH = 8.3. This system was

controlled to maintain complete nitritation using the original OUR control loop detailed

in section 2.2. Detailed information about this system can be found in Torà et al. [16].

2.5. Microbial and chemical analysis

Fluorescence in situ hybridization (FISH) technique coupled with confocal laser

scanning microscopy (CLSM) was used to investigate the nitrifying population

dynamics. A Leica TCS SP2 AOBS CLSM microscope at a magnification of x63

(objective HCX PL APO ibd.B1 63x1.4 oil) equipped with two HeNe lasers with light

emission at 561 and 633 nm was used for biomass detection. Hybridizations were

carried out using at the same time a Cy3-labeled specific probe and Cy5-labeled

EUBmix probe (general probe). The specific probe used for ammonia oxidizing bacteria

(AOB) detection was Nso190 [18], which identifies Beta-proteobacterial ammonia

oxidizers. Nitrite oxidizing bacteria (NOB) were detected with NIT3 [19],

recommended for Nitrobacter spp. Nso190 and NIT3 were used because they were

found in a previous work [20] as the more representative probes for AOB and NOB in

our operational conditions. EUBmix probe consisted of the mix of probes EUB338,

EUB338 II and EUB338 III [21, 22]. Detailed information about FISH probes can be

found in Supplementary Information (Table S1) and its quantification can be found in Jubany et al. [20].

Off-line TAN analyses were performed with a continuous flow analyzer based on potentiometric determination of ammonia. TNN and nitrate were measured with ionic chromatography using a DIONEX ICS-2000 Integrated Reagent-Free IC System with an auto-sampler AS40. Volatile suspended solids (VSS) and total suspended solids (TSS) concentrations were determined according to standard methods [23].

2.6. Kinetic models for AOB and NOB populations

A kinetic study was performed to analyze the stability of the PN process achieved with the controlled operation. The kinetic models (Equations 1-4) considered DO limitation, Andrews' kinetics for substrate (with limitation and inhibition) and inhibitions of AOB by free nitrous acid (FNA) and NOB by free ammonia (FA).

$$\mu_{AOB} = \mu_{max,AOB} \frac{[DO]}{K_{S,DO,AOB} + [DO]} \frac{[FA]}{K_{S,FA,AOB} + [FA] + \frac{[FA]^2}{K_{I,FA,AOB}}} \frac{K_{I,FNA,AOB}}{K_{I,FNA,AOB} + [FNA]} \quad (1)$$

$$\mu_{NOB} = \mu_{max,NOB} \frac{[DO]}{K_{S,DO,NOB} + [DO]} \frac{[FNA]}{K_{S,FNA,NOB} + [FNA] + \frac{[FNA]^2}{K_{I,FNA,NOB}}} \frac{K_{I,FA,NOB}}{K_{I,FA,NOB} + [FA]} \quad (2)$$

$$b_{AOB} = b_{max,AOB} \frac{[DO]}{K_{S,DO,AOB} + [DO]} \quad (3)$$

$$b_{NOB} = b_{max,NOB} \frac{[DO]}{K_{S,DO,NOB} + [DO]} \quad (4)$$

μ_{AOB} and μ_{NOB} are the specific growth rates of AOB and NOB respectively; b_{AOB} and b_{NOB} the decay rates; $\mu_{max,AOB}$ and $\mu_{max,NOB}$ the maximum specific growth rates, $b_{max,AOB}$ and $b_{max,NOB}$ the maximum decay rates; $K_{S,DO,AOB}$ and $K_{S,DO,NOB}$ the DO affinity constants; $K_{S,FA,AOB}$ the FA affinity constant of AOB; $K_{S,FNA,NOB}$ the FNA affinity constant of NOB; $K_{I,FA,AOB}$ and $K_{I,FA,NOB}$ the FA inhibition constants and $K_{I,FNA,AOB}$ and $K_{I,FNA,NOB}$ the FNA inhibition constants. An extended description and justification of both kinetic models and parameters can be found elsewhere [15]. The kinetic parameters for AOB and NOB populations were adequately modified to the temperature and pH used in this study according to the equations described in [24]. FA and FNA concentrations were calculated from the measured TAN and TNN concentrations using the acid-base equilibria (equations 5 and 6) [25].

$$FA = \frac{TAN \cdot 10^{pH}}{\exp\left(\dfrac{6344}{273+T}\right)+10^{pH}} \cdot \frac{17}{14} \tag{5}$$

$$FNA = \frac{TNN}{\exp\left(\dfrac{-2300}{273+T}\right)\cdot 10^{pH}+1} \cdot \frac{47}{14} \tag{6}$$

3. Results and Discussion

3.1. Controlled operation to obtain a suitable anammox influent using synthetic wastewater

The nitrifying reactor was inoculated with 25 L of the activated sludge inoculum detailed in section 2.4, which was composed by 77±13% of AOB, <1% of NOB and the rest being considered heterotrophic biomass. The reactor worked with the control loops previously defined. The TAN concentration in the influent was regularly measured and

the TAN_{SP} of the control loop was modified (between 600-800 mg N L^{-1}) when the influent concentration changed to achieve the TNN/TAN ratio of 1.3 stoichiometrically required for the anammox process.

The reactor was operated for 2 months with the synthetic influent at 30ºC, pH setpoint of 7.5 and SRT around 3 d (Period I). During this period, the NLR increased from the initial 3.7 g N L^{-1} d^{-1} up to an average value of 9.3±0.5 g N L^{-1} d^{-1} for the last 20 days (Figure 2). This significant increase of the NLR was related to the biomass concentration, which increased from an initial value of 450 mg VSS L^{-1} up to 2000 mg VSS L^{-1} (data not shown), maintaining a VSS/TSS ratio of 0.95±0.03 during all period I. The proper TNN/TAN ratio of 1.3 was achieved at day 15 and maintained by the TAN control loop at 1.3±0.1 during the rest of period I (Figure 3). The HRT during this period changed automatically from 8 h to 3 h. This period was enough to demonstrate the stability and robustness of the TAN control loop to achieve a proper influent for anammox reactors.

3.2. Controlled operation to obtain a suitable anammox influent using real reject water

When the steady state with synthetic wastewater was achieved, the influent was progressively changed to reject water to demonstrate the viability of the TAN control loop for treating real wastewater. During 10 days the influent was a mixture of 50% synthetic wastewater and 50% real reject water whereas the temperature, pH and SRT were maintained at 30ºC, 7.5 and 3 d, respectively (Period II). During this period, the NLR experimented a slight decrease compared to the achieved treating the synthetic wastewater, while the TNN/TAN ratio was maintained at 1.4±0.1 with <1% of nitrate in

the effluent (Table 1). After this short period till the end of this study, the reactor was fed only with the real reject water.

The viability of the nitrifying reactor with the TAN control loop was tested during one month (Period III), maintaining the temperature, pH and SRT at 30ºC, 7.5 and 3 d, respectively. Comparing the results obtained at the same temperature and pH using the synthetic wastewater (Period I) and the reject water (Period III), it was observed that the capacity of the system was reduced treating real wastewater. Comparing both volumetric NLRs (Table 1), the capacity of the system decreased around 40% for reject water (from 9.0 to 5.0 g N L^{-1} d^{-1}). However, this decrease was not only caused by the wastewater change, but also was related to a decrease of the biomass concentration between both periods. The specific ammonia consumption rate (SACR) decreased only 20% from period I (2.6 g N g^{-1} VSS d^{-1}) to period III (2.0 g N g^{-1} VSS d^{-1}). This decrease could be caused by differences in the conductivity and organic matter and salts content between the synthetic and real wastewaters. Nevertheless, the NLR achieved treating the reject water was very high compared with most of the bibliographic references of PN systems [5, 9, 26, 27]. Moreover, the TNN/TAN ratio, which is the most important parameter for a subsequent anammox reactor, was stably maintained at 1.3±0.3, while the nitrate concentration in the effluent was always lower than 5 mg N L^{-1}. The VSS/TSS ratio decreased from the initial 94% to a value of 51% during the 30 days of this period. The reason for this significant mineralization of the activated sludge was an important input of inorganic content in the reject water. However, the increased sludge mineralization was not a problem for the proper system operation and hence it is not expected a significant effect of this change in sludge characteristics in full-scale

applications. This low VSS/TSS ratio was maintained constant during the rest of operation with real reject water. The HRT during this period was 3.9±0.6 h.

When the viability of our system treating the reject water at 30ºC was demonstrated, and considering that other systems as SHARON recommend the range of 30-40ºC for maintaining its performance, the temperature in our system was decreased to check the achievement of a suitable effluent for anammox reactor at lower temperatures. Then, it was decreased to 24ºC and the pH was maintained at 7.5 for Period IV. In spite of the temperature decrease, the TNN/TAN ratio was maintained at 1.3±0.2 while the NLR and ammonia consumption rate (ACR) were slightly lower than Period III (Table 1). However, the biomass concentration increased notably during this period due to the high solid concentration in the reject water and a significant improvement of the settleability of the nitrifying activated sludge, which resulted in an increased SRT of 6 d. This change in the solids concentration caused an important decrease of the SACR (around 70%) between periods III and IV.

It should be emphasized the extremely high NLR treated with this novel ammonium control loop (Table 1). As stated in several studies, the actual bottleneck in the overall capacity of N-removal advanced treatment systems is the limiting capacity of the PN compared to anammox capacity [6, 8, 28]. Consequently, the novel technology presented in this study can be useful to improve the capacity of N-removal via nitrite. The FISH technique was used to detect the microbial populations presented in the nitrifying reactor through the study. These analyses determined that the bacterial populations were almost the same treating synthetic wastewater or reject water. For example, AOB population was quantified in 73±10% at the end of Period I using synthetic wastewater and 75±6% during Period V with reject water, while NOB

population was undetected (<1%) in both periods. This NOB washout was also reflected in the absence of nitrate production during both periods.

3.3. Is automatic pH control convenient for partial nitritation?

A disagreement point in the design and operation of PN reactors is the application of pH control loops. The use of pH control means an increase in the consumption of reagents but also an improvement of the ambient conditions for nitritation. An experiment was designed and carried out to clarify this controversy. The experiment consisted on the deactivation of the pH control loop of the nitrifying reactor (period V). During this period, the alkalinity of the system was only the alkalinity that contained the reject water and the pH decreased to a stable value of 6.8. Furthermore, significant decreases of the NLR (from 4.1 to 0.8 g N L^{-1} d^{-1}) and the ACR (from 2.4 to 0.5 g N L^{-1} d^{-1}) were observed. As a consequence, the inflow rate strongly decreased and the HRT was stabilized to 21±2 h. The TNN/TAN ratio was kept at 1.2±0.3 without nitrate formation in the effluent despite the change of operational conditions.

Hence, the nitrifying system could work without pH control but with a treatment capacity 5-fold lower than the achieved using a pH control loop. Finally, in spite of the lower rates achieved without pH control, these rates are much higher than those typically observed in SHARON reactors [9, 29].

The observed decrease in the capacity of the system during Period V could be caused by the lower alkalinity of the system with the consequence of lower pH under operating conditions [30] or the TIC limitation [31, 32]. The TIC limitation could increase, at the same time, the inhibitory effects of FA and FNA on AOB [33]. If the decrease of activity was only due to the effect of pH over AOB, the ACR should decrease around

22% [34] and not 80% as it was observed. This significant difference could be explained because the concentration of FA decreased from 5.0 mg NH_3 L^{-1} obtained at pH 7.5 to 1.2 mg NH_3 L^{-1} at pH 6.8 and the FNA concentration increased from 0.08 to 0.39 mg HNO_2 L^{-1}. As previously reported, the affinity for FA decreases and the inhibition by FNA is specially amplified under TIC limitation [33]. These two factors explain the important decrease in the ACR when the pH control was deactivated.

The final decision of using pH control in PN reactors would rely on the alkalinity content of a given wastewater. In the case studied, the investment and operational costs of maintaining a controlled pH with the addition of base should be economically evaluated and compared to the costs associated to a reactor with a volume five times higher to compensate for the 80% reduction of the ACR.

3.4. Controlled operation to obtain a suitable influent for heterotrophic denitritation.

The last objective of this study was to check if the TAN control loop was able to produce a proper effluent for a subsequent heterotrophic denitritation by just changing the TAN_{SP} (period VI). In this case, the effluent should contain most of the total nitrogen as TNN and therefore the TAN_{SP} was decreased to 20-30 mg N L^{-1}. This value allowed to achieve an effluent with low TAN concentration and complete nitritation. The pH control loop was again applied and the temperature and pH were maintained at 24ºC and 7.5, respectively throughout this period.

As Figure 3 shows, TAN was completely oxidized to TNN with very low nitrate formation during this period and consequently, the NLR and the ACR were very similar (Figure 2). The NLR decreased compared to period IV, in which the pH and temperature were the same and only the TAN_{SP} was different. This decrease was only a

consequence of the NLR measurement, because it is calculated based on the influent TAN concentration. During period IV only 56% of this TAN was oxidized to TNN, while during period VI more than 95% of this TAN was oxidized to TNN. However, the obtained ACR was the same in both periods, which means that the capacity of the nitrifying system was the same independently of the fixed TAN_{SP}: high TAN_{SP} to achieve a suitable influent for anammox process or low TAN_{SP} when a proper effluent for heterotrophic denitritation is the target. As a consequence, it can be assumed that the high free ammonia (FA) concentration in the reactor (5.1 ± 0.8 mg FA L^{-1}) during period IV and the high free nitrous acid (FNA) concentration during period VI (0.034 ± 0.006 mg FNA L^{-1}) were not inhibitory for AOB.

3.5. Kinetic study

The growth rates of AOB and NOB populations under non-limiting FA or FNA concentrations are influenced by three factors: the inhibitions by FA and FNA and the limitation by DO. The total and complete washout of the NOB from the system to achieve stable PN is possible using the combination of these factors and a proper SRT. The minimum SRT (SRT_{min}) to maintain the AOB and NOB populations in the system can be calculated with equation 7 [15, 35].

$$SRT_{min,i} = \frac{1}{\mu_i - b_i} \tag{7}$$

μ_i and b_i represent the specific growth and decay rates of AOB or NOB for a given experimental condition. The particular μ_{AOB} and μ_{NOB} values for each period were calculated with equations 1 and 2 using the average experimental DO, FA and FNA concentrations in the reactor during that period. b_{AOB} and b_{NOB} values were calculated with equations 3-4 using the average experimental DO concentration for each period.

The SRT_{min} values obtained for AOB and NOB with equation 7 for each experimental period were compared to the operational SRT in table 2. $SRT_{min,AOB}$ was always lower than the operational SRT, which means that it was sufficient to sustain the AOB population in the pilot plant. On the contrary, during periods I-V the high ammonium concentration in the reactor combined with the controlled pH favored the formation of FA, which is extremely inhibitory for NOB. Under these conditions, μ_{NOB} was always lower than b_{NOB}, which means than even with an infinite SRT the net growth of NOB was not possible, leading to the wash out of this population from the system. In period V, the pH control was deactivated and consequently the pH decreased from 7.5 to 6.8, which reduced the FA concentration and as a consequence the $SRT_{min,NOB}$ decreased to 27.4 d. However, the SRT was still lower and the PN was maintained. During period VI, the TAN_{SP} was decreased to 20-30 mg N L^{-1} in order to produce a suitable effluent for a subsequent heterotrophic denitritation. This operational change reduced the FA concentration in the reactor and as a consequence the $SRT_{min,NOB}$ decreased to 5.5, which was lower than the operational SRT in that period and thus, NOB were able to grow in the system. This result completely agrees with the low increase of nitrate concentration detected at the end of this period (Figure 3). Under these conditions, operation with SRT lower than 5.5 would has been required to eliminate completely the nitrate presence in the effluent.

3.6 Practical implications

The first step to apply this control system to a given wastewater would be measuring its TAN concentration and calculating the required TAN setpoint to obtain the desired TNN/TAN ratio in the effluent. Once this value is fixed, the experimental results of this

study demonstrate that choosing proper operational conditions (DO and pH setpoints of the conventional control loops) joint to the TAN control loop allows the build-up of FA in the reactor. FA has a higher inhibitory effect on NOB than on AOB, reflected on the required SRT to survive in the system for both populations ($SRT_{min,AOB}$ and $SRT_{min,NOB}$). If the operational SRT is selected lower that the required SRT for NOB survive ($SRT_{min,NOB}$), NOB will be undoubtedly washed out of the system, as it is corroborated with the FISH detection and quantification in this study.

An additional interesting characteristic of the TAN control loop is that it allows working at the maximum capacity of the system, avoiding any non-desired accumulation of ammonia. The controlled flow always matches the applied load to the ACR capacity in a given set of environmental conditions. For example, decreasing DO concentration in a non-controlled system would lead to ammonia accumulation and eventually to substrate inhibition. However, a similar DO decrease in a system with the developed TAN control loop would cause no problem, as the influent flow-rate would be automatically reduced. The same protective effect would be provided in the case of a pH change. Therefore, the process stability achieved with the TAN control loop, combined with the proper selection of DO and pH setpoints to induce growing conditions more favorable to AOB than NOB is the key point for the success of the developed control system.

One concern about the application of this system to wastewaters with variable composition is that information about the ammonium concentration in the influent should be provided to calculate the required ammonium in the reactor to achieve a desired effluent TNN/TAN ratio. This information can be provided with off-line analysis if stored wastewater is used, or it can be measured on-line with another ammonium sensor or changing the sampling point of a single analyzer. The utilization

of on-line data to automatically calculate a new setpoint for the slave control loop is the typical ratio control structure found in the literature [13]. In any case, the cost involved for this implementation is not extremely high, as ammonium selective electrodes as the one used in this work are reducing its price and nowadays its cost is only around twice the price of a typical DO sensor for a WWTP.

Regarding the versatility of this control system, it is not limited by the stoichiometric bicarbonate/ammonium ratio for achieving a suitable influent for anammox reactor, as required in other treatments as SHARON. For example, the synthetic wastewater used had much lower alkalinity than the typical reject water, and extremely high NLR of 9.3 ± 0.5 g N L^{-1} d^{-1} was obtained. When using real reject water, a very high NLR of 5.0 ± 1.0 g N L^{-1} d^{-1} was also obtained (T = 30ºC, pH = 7.5), with an ACR of 2.8 ± 0.8 g N L^{-1} d^{-1} and a $NO_2^--N/(NO_2^--N+NO_3^--N)$ ratio of 99%. Moreover, this system is also able to work at lower temperatures (24ºC) than the ones required for other systems (e.g. SHAROH among others), maintaining a high NLR.

4. Conclusions

An activated sludge nitrifying reactor with a novel TAN control loop was able to stably treat reject water producing a suitable effluent for a subsequent anammox process. The TNN/TAN ratio in the effluent was steadily maintained at 1.3 with an extremely high NLR of 5.0 ± 1.0 g N L^{-1} d^{-1} (T= 30ºC, pH = 7.5). The system was also able to achieve a high NLR with the proper TNN/TAN ratio when the temperature was decreased to 24ºC. Finally, the TAN control loop also permitted to produce an effluent appropriate for a subsequent heterotrophic denitritation process by only changing the TAN setpoint.

5. Acknowledgements

6. References

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Table 1. Summary of the main operational parameters and results obtained for the different operational periods of the nitrification system. TSS: total suspended solids, VSS: volatile suspended solids, NLR: nitrogen loading rate, ACR: ammonia consumption rate, TNN: total nitrite nitrogen, TAN: total ammonia nitrogen, SACR: specific ammonia consumption rate.

Period	Wastewater	T (°C)	pH control	pH	TSS (g L^{-1})	VSS (g L^{-1})	VSS/TSS (%)	NLR (g N L^{-1} d^{-1})	ACR (g N L^{-1} d^{-1})	SACR (g N g^{-1}VSS d^{-1})	TNN/TAN Ratio
I	Synthetic	30	Yes	7.5±0.1	2.1±0.5	2.0±0.5	95	9.3±0.5	5.2±0.6	2.6±0.3	1.3±0.1
II	50% Synthetic 50% Reject water	30	Yes	7.5±0.1	1.8±0.2	1.7±0.2	94	8.1±0.6	4.6±0.5	2.7±0.3	1.4±0.1
III	Reject water	30	Yes	7.5±0.1	2.2±1.0	1.4±0.6	64	5.0±1.0	2.8±0.8	2.0±0.4	1.3±0.3
IV	Reject water	24	Yes	7.5±0.1	8.1±3.2	4.1±1.5	51	4.1±0.6	2.4±0.4	0.6±0.1	1.3±0.2
V	Reject water	24	No	6.8±0.2	11.3±1.7	5.8±0.7	51	0.8±0.2	0.5±0.2	0.2±0.1	1.2±0.3
VI	Reject water	24	Yes	7.5±0.1	11.5±2.3	5.6±0.9	49	2.5±0.8	2.3±0.4	0.4±0.1	100% TNN

Table 2. Minimum SRT required to sustain AOB and NOB populations and the operational SRT in the reactor.

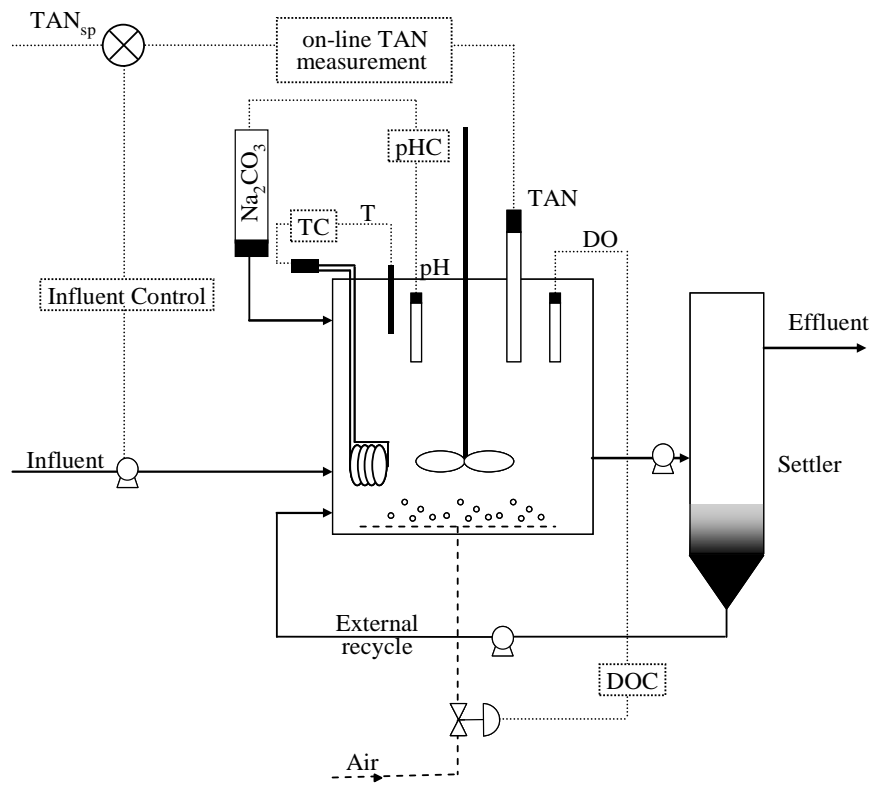| Period	$SRT_{min,AOB}$ (d)	$SRT_{min,NOB}$ (d)	$SRT_{operational}$ (d)
I	1.3	∞	3
II	1.2	∞	3
III	1.2	∞	3
IV	1.2	∞	6
V	1.9	27.4	24
VI	1.9	5.5	12

Figure 1. Schematic diagram of the partial nitritation system showing the pH, DO and temperature control loops (pHC, DOC and TC respectively) and the inflow control loop with TAN as the measured variable.
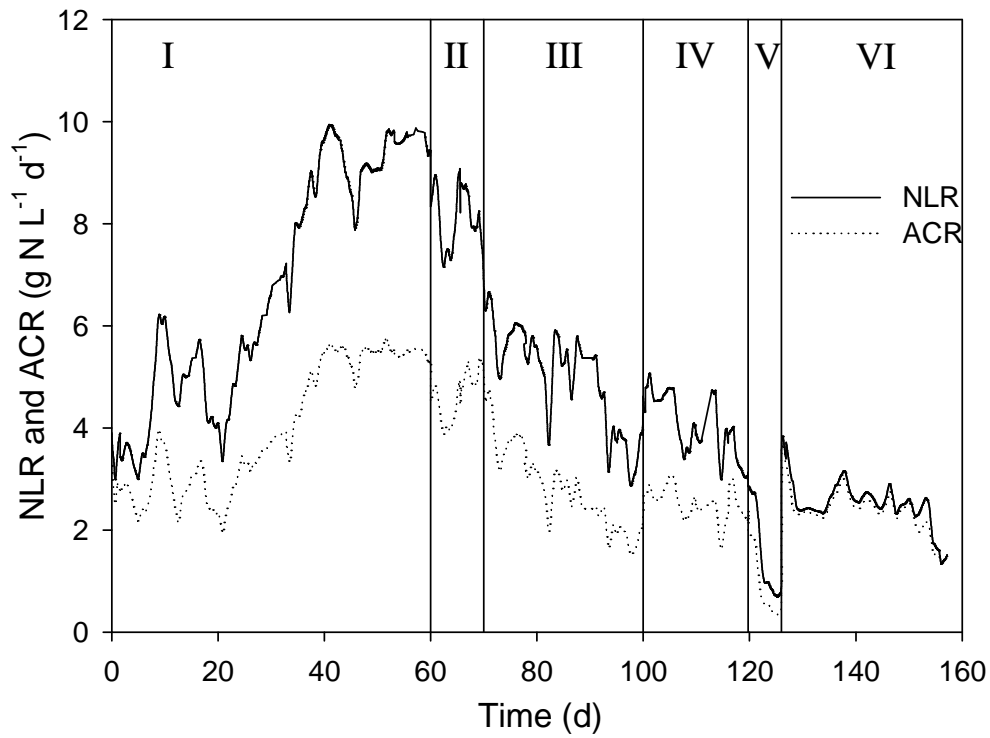
Figure 2. Nitrogen loading rate (NLR) and ammonia consumption rate (ACR) in the partial nitritation system during this study. I. Synthetic wastewater; II. 50% synthetic wastewater and 50% reject water; III. Reject water at 30ºC; IV. Reject water at 24ºC; V. Reject water without pH control; VI. Reject water with an effluent mostly nitrite.
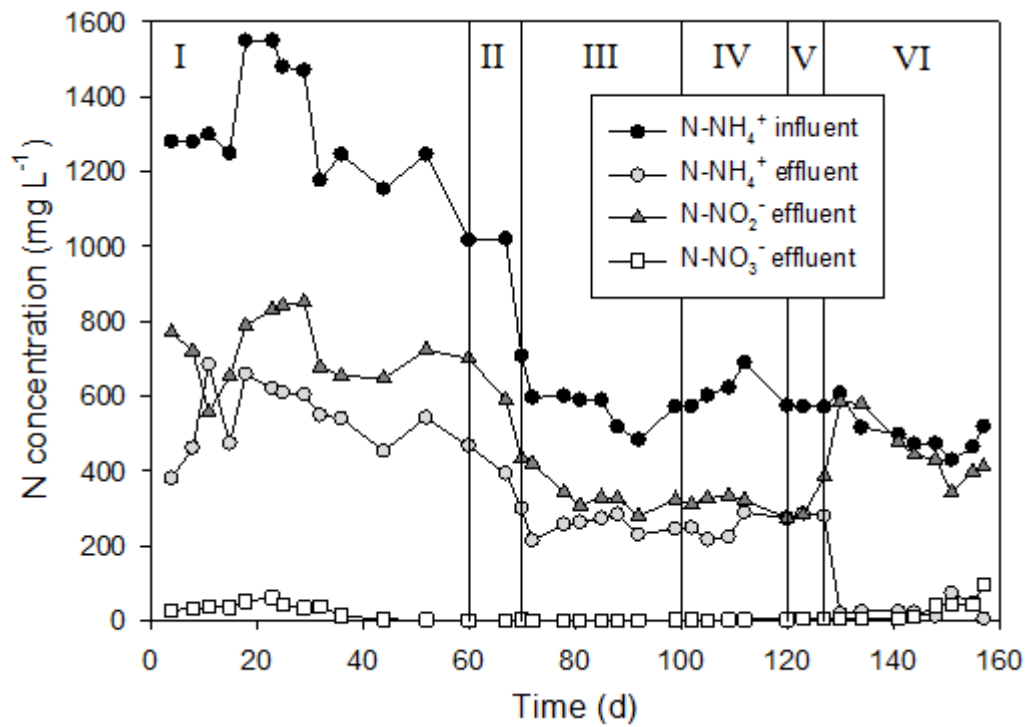
Figure 3. Time course of nitrogen compounds concentrations through the study. I. Synthetic wastewater; II. 50% synthetic wastewater and 50% reject water; III. Reject water at 30ºC; IV. Reject water at 24ºC; V. Reject water without pH control; VI. Reject water with an effluent mostly nitrite.