

Enhanced ammonia removal at room temperature by pH controlled partial nitrification and subsequent anaerobic ammonium oxidation

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Abstract

The Anammox based processes are suitable for the treatment of wastewaters characterized by a low carbon to nitrogen (C/N) ratio. The application of the Anammox process requires the availability of an effluent with a $\text{NO}_2^- \text{-N}/\text{NH}_4^+ \text{-N}$ ratio composition around $1 \text{ g} \cdot \text{g}^{-1}$, which involves the necessity of a previous step where the partial nitrification is performed. In this step the inhibition of the nitrite oxidizing bacteria (NOB) is crucial. In the present work a combined partial nitrification-Anammox two units system operated at room temperature ($20 \text{ }^\circ\text{C}$) has been tested for the nitrogen removal of pre-treated pig slurry. To achieve the successful partial nitrification and inhibit the NOB activity different ammonium/inorganic carbon (NH_4^+/IC) ratios were assayed from 1.19 to $0.82 \text{ g NH}_4^+ \text{-N} \cdot \text{g}^{-1} \text{ HCO}_3^- \text{-C}$. This procedure provoked a decrease of the pH value to 6.0 to regulate the inhibitory effect over ammonia oxidizing bacteria (AOB) caused by free ammonia (FA). Simultaneously the NOB experienced the inhibitory effect of free nitrous acid (FNA) which avoided the presence of nitrate in the effluent. The NH_4^+/IC ratio which allowed the obtaining of the desired effluent composition (50% of both ammonium and nitrite) was of $0.82 \pm 0.02 \text{ g NH}_4^+ \text{-N g}^{-1} \text{ HCO}_3^- \text{-C}$. The Anammox reactor was fed with the effluent of the partial nitrification unit containing a $\text{NO}_2^- \text{-N}/\text{NH}_4^+ \text{-N}$ ratio of $1 \text{ g} \cdot \text{g}^{-1}$ where a nitrogen loading rate (NLR) of $0.1 \text{ g N} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ was efficiently removed.

Keywords

Ammonium/inorganic carbon (NH_4^+/IC) ratio, Anammox, free ammonia (FA), free nitrous acid (FNA), partial nitrification.

Introduction

The nitrogen removal from wastewater is carried out normally by biological nitrification and denitrification processes. However, some kinds of wastewater are characterized by a low carbon to nitrogen (C/N) ratio and their organic matter content is not enough to carry out the denitrification process. Therefore an external source of organic matter must be supplied, increasing the operational costs [1]. For this kind of effluents there are two possible

alternatives to conventional nitrification-denitrification processes based in the so-called “nitrite route”: (1) to combine the partial nitrification and the subsequent heterotrophic denitrification and (2) to combine the partial nitrification of half of the ammonia contained in the wastewater followed by the Anammox (ANaerobic AMmonia OXidation) process. Both alternatives present lower operational costs than the nitrification-denitrification processes. However, the combination of partial nitrification and Anammox processes is the option which allows the highest cost savings in terms of aeration (40%) and external electron donor source addition (100%) [2].

Due to the fact that nitrification is a two stages aerobic process, where ammonium is oxidized to nitrite and this subsequently to nitrate, to obtain the partial nitrification the second reaction must be avoided. For this to happen the ammonium oxidizing bacteria (AOB) should be selectively retained in the system while the nitrite oxidizing bacteria (NOB) should be washed-out or inhibited. Different strategies have been proposed to obtain a stable partial nitrification process: a) to inhibit NOB by free ammonia (FA) and/or free nitrous acid (FNA) [3], b) to limit the amount of supplied dissolved oxygen (DO) to values around 1-2 mg O₂·L⁻¹ working with suspended biomass [1] or by DO regulation in a nitrifying granular reactor [4], or c) to operate at temperatures above 25 °C in a chemostat like in the so called SHARON (single-reactor high-activity ammonia removal over nitrite) system [5].

Most of the used strategies to produce nitrite are based on the aforementioned strategies but all of them have drawbacks. Nitrite accumulation by means of NOB inhibition does not normally last long due to the acclimation of the bacterial populations to the inhibitory conditions. In order to overcome this obstacle a selection strategy to remove NOB from the system can be used. This is the basis of the SHARON process, where the temperature of operation must be controlled at values over 30 °C which favour the AOB growth while NOB are washed out from the system. However in the WWTPs room temperature is frequently under 30 °C and only in tropical zones or when the effluent from a mesophilic and/or thermophilic anaerobic digesters is treated temperatures around this value could be maintained without a significant operational cost. Therefore the partial nitrification at room temperature needs to be studied in order to extend its application range. In this sense some studies showed the possibility to obtain the partial nitrification at low temperatures by the control of the aeration frequency and the pH value [6], the DO concentration [4] or by the addition of hydroxylamine to inhibit the NOB activity [7].

For similar reasons to operate the subsequent Anammox system at low temperatures is also of interest. Despite the fact that the optimum performance of Anammox bacteria is obtained at 30-40 °C [8], some studies demonstrated that it is possible the operation of Anammox systems at lower temperatures [9-11]. In fact this occurs naturally in the environment, since Anammox bacteria have been detected in various marine and freshwater mediums at low temperatures [12].

Therefore, in this study, a new strategy, to obtain the partial nitrification to produce an effluent containing a NO₂⁻-N/NH₄⁺-N ratio of 1 g g⁻¹, was evaluated by regulating the ammonium/inorganic carbon (NH₄⁺/IC) ratio to control the pH and the inhibitory effects of FA

and FNA. The efficiency of nitrogen removal at room temperature from diluted pig slurry of a partial nitrification-Anammox system was studied.

Materials and methods

Partial nitrification-CSTR

The partial nitrification process was carried out in a continuously stirred tank reactor (CSTR) of 2.0 L, operated at a hydraulic retention time (HRT) of 3 days and stirred at 150 rpm, followed by a settling tank of 1.3 L. An amount of 0.45 g VSS·L⁻¹ of nitrifying activated sludge was used as inoculum of the reactor (occupying the 25% of the liquid volume). Air was supplied through air spargers in order to guarantee a dissolved oxygen (DO) concentration higher than 2.0 mg O₂·L⁻¹. The temperature was not controlled and ranged from 22 to 25 °C.

The feeding media consisted of pig slurry pre-treated in an aerobic granular pilot plant as described by Morales et al. [13] and characterized by its low organic matter (103±43 mg COD·L⁻¹) and relatively high ammonia content (399±25 mg NH₄⁺-N·L⁻¹). The applied ammonia nitrogen loading rate (NLR) was of 0.13±0.01 g NH₄⁺-N·L⁻¹·d⁻¹.

The reactor was operated in four different stages (Table 1), following an strategy of decrease of the NH₄⁺/IC ratio in the feeding in order to obtain an effluent with a NO₂⁻-N/ NH₄⁺-N ratio around 1 g·g⁻¹. The NH₄⁺/IC ratio was regulated by means of the addition of bicarbonate without pH control in the influent and maintaining bicarbonate content in the effluent always in excess. In stage IV the biomass retention was not achieved in the settling tank and as a consequence this unit was removed from the system. The solids retention time (SRT) was then similar to the HRT value.

Anammox-SBR

The Anammox process was performed in a sequencing batch reactor (SBR) of 4.0 L and stirred at 120 rpm. Granular biomass (4.1 g VSS·L⁻¹) coming from an Anammox pilot plant was used as inoculum. The exchange volume was of 12.5% resulting in an HRT of 2 days. The reactor was operated during 280 days in anaerobic conditions and 4 cycles/day were performed with the following distribution: fill and reaction (335 min), settle (15 min) and draw (10 min). The temperature was maintained at room values (22-25 °C).

The Anammox SBR was fed with the effluent of the partial nitrification reactor obtained during stage IV, with a composition of NO₂⁻-N/NH₄⁺-N ratio of approximately 1 g·g⁻¹. This effluent was stored at 4 °C previously to its use. The operation of the Anammox SBR was also divided in four stages according to the different applied NLRs (Table 1).

Ammonium, nitrite and Anammox specific activities

Sludge samples were collected from the partial nitrification-CSTR in each operational stage and the specific ammonium oxidizing and nitrite oxidizing activities (SAOA and SNOA, respectively) were determined, through respirometric batch assays, according to the procedure

described by Mosquera-Corral et al. [5]. The initial SAOA and SNOA at 25 °C of the partial nitrification sludge were of $0.49 \pm 0.09 \text{ g NH}_4^+ \cdot \text{N} \cdot \text{g}^{-1} \text{ VSS} \cdot \text{d}^{-1}$ and of $0.35 \pm 0.06 \text{ g NO}_2^- \cdot \text{N} \cdot \text{g}^{-1} \text{ VSS} \cdot \text{d}^{-1}$, respectively.

The specific Anammox activity (SAA) assays were performed according to the procedure described by Dapena-Mora et al. [14] with the biomass collected from the SBR-Anammox process. The initial SAA of the Anammox sludge at 25 °C was of $0.21 \text{ g N}_2 \cdot \text{N} \cdot \text{g}^{-1} \text{ VSS} \cdot \text{d}^{-1}$.

Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS) and chemical organic demand (COD) were measured according to the Standard Methods [15]. The inorganic carbon (IC) concentration was determined by a Shimadzu Analyzer (TOC-5000). The nitrous oxide in the gas phase was determined by thermal conductivity gas chromatography (TCD) Hewlett Packard 5890 Series II. Ammonium, nitrate and nitrite were analyzed by ion chromatography in an 861 Advanced Compact IC (Metrom AG, 1999). The pH value was measured using a selective electrode Ingold model U-455 connected to a pH/mV measurer Crison 506. The dissolved oxygen (DO) concentration and the temperature were measured using an Aqualytic Oxygen Meter (model OXI-921).

Calculations

Concentrations of FA and FNA were calculated according to Anthonisen et al. [3]. The SRT was estimated as the ratio of the total amount of biomass inside the system and biomass wash-out rate from the reactors.

Results and discussion

Partial nitrification process

The partial nitrification-CSTR was operated in four stages characterized by different NH_4^+/IC ratios in order to obtain the oxidation of 50% of inlet NH_4^+ into NO_2^- (Table 2). During Stage I, approximately 24% of the fed ammonium was oxidized to nitrite and 7% to nitrate. The pH value was slightly alkaline and the $\text{NO}_2^-/\text{NH}_4^+$ ratio was of $0.35 \pm 0.05 \text{ g N} \cdot \text{g}^{-1} \text{ N}$. In the stages II and III, the behaviour was similar, even when the NH_4^+/IC ratio decreased and the ammonium consumption increased, with a $\text{NO}_2^-/\text{NH}_4^+$ of $0.49 \pm 0.07 \text{ g N} \cdot \text{g}^{-1} \text{ N}$. In both stages nitrate was present in the effluent but in small amounts. So far, bicarbonate consumption efficiency remained without changes. In the last stage, the oxidation of 50% of inlet NH_4^+ into NO_2^- was achieved, without significant production of nitrate and the system operated in stable conditions during 140 days maintaining the same effluent composition. The decrease of the NH_4^+/IC ratio was necessary to avoid the nitrite oxidation to nitrate working during stage IV with the optimal value of $0.82 \text{ g N} \cdot \text{g}^{-1} \text{ C}$ when the pH value was of 6.0.

Nitrite accumulation occurred in the reactor due to the inhibitory effects of the FA and FNA. Furthermore the NH_4^+/IC ratio control allowed the production of the desired effluent

composition. In order to define the appropriated conditions for the process to occur the inhibitory zones corresponding to these compounds concentrations were determined by means of the expressions of Anthonisen et al. [3] (Figure 1). The limiting values of each zone were $10 \text{ mg FA} \cdot \text{L}^{-1}$ which inhibit both AOB and NOB and between $0.1 < \text{FA} < 10 \text{ mg FA} \cdot \text{L}^{-1}$ which inhibited the NOB (Figure 1 (a)). In the case of the effect of FNA concentrations over $0.2 \text{ mg FNA} \cdot \text{L}^{-1}$ inhibited the NOB (Figure 1 (b)).

In the present case the free ammonia concentration initially decreased from Stage I to Stage IV due to the diminution of the pH value (Figure 1 (a)). With these operational conditions both AOB and NOB were inhibited by this compound in Stage I, while once the pH value was decreased, in the following stages of operation, only NOB were inhibited. Until the Stage III a small amount of nitrite was oxidized to nitrate. It was only during Stage IV when the concentration of FNA was high enough to avoid the production of nitrate (Figure 1 (b)) and as a consequence the effluent contained almost 50% of ammonium and nitrite, respectively. With respect to the possible inhibitory effect of the FNA on the AOB activity, even though its concentrations were in the levels of those found in the literature to provoke inhibition ($0.20 \text{ mg HNO}_2\text{-N/L}$) [16], this was not detected in the present work.

In this way the composition of the effluent can be regulated by means of the combined effects of FA, in all tested pH values, and FNA at low pH values as it was stated by other authors [1, 5]. Therefore, under the operational conditions established in the reactor to control the partial nitrification process, in order to just oxidize half of the ammonium to nitrite, was possible during 140 days. The analysis performed to gas samples collected from the top of the reactor during the operational period indicated that nitrous oxide was not produced even if nitrite was accumulated in the system.

Along the operation of the partial nitrification reactor different biomass samples were collected to perform batch activity assays. The SAOA remained without significant changes respect to the initial value of $0.49 \text{ g NH}_4^+\text{-N} \cdot \text{g}^{-1} \text{ VSS} \cdot \text{d}^{-1}$ (Table 2). However, the SNOA decreased progressively from the initial value of $0.35 \pm 0.06 \text{ g NO}_2^-\text{-N} \cdot \text{g}^{-1} \text{ VSS} \cdot \text{d}^{-1}$ to a negligible value in the last stage (data not showed). These results suggest that the operational conditions stimulated the ammonium oxidation while NOB were washed out from the continuous reactor.

The partial nitrification process was successfully operated at room temperature as it has been previously observed by Yamamoto et al. [17]. They reported that the nitrite production rate did not significantly decrease when the temperature remained above $20 \text{ }^\circ\text{C}$, indicating that the partial nitrification at room temperature is possible using another kind of control as the pH. The results of the research corroborate the inhibitory effect of the FNA on NOB, which may be the result of a combination of the pH and the temperature [18], as a strategy that selectively washes out NOB from the reactor, using high bicarbonate concentrations.

Anammox process

The Anammox SBR was operated during 280 days, fed with the effluent of the partial nitrification reactor obtained along stage IV, in order to obtain the complete nitrogen removal

from the pre-treated pig slurry. The operation was divided in four stages according to the different applied NLRs.

In Stage I with an applied NLR of $0.10 \text{ g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ the nitrogen removal efficiency ranged between 80-90% (Figure 2). Then in Stages II and III the applied NLR to the system was progressively increased up to a value of $0.20 \text{ g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, which provoked a worsening in the nitrogen removal efficiency that decreased down to a percentage of only 35%. Therefore in Stage IV the applied NLR was restored to the initial value of $0.10 \text{ g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ and this value was maintained along the rest of the operational period (200 days). The Anammox reactor had a stable operation in these conditions with an average percentage of nitrogen removal of 85%. These results suggest that at room temperature a NLR of $0.10 \text{ g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ (50% of ammonium and nitrite, respectively) could be efficiently removed to dinitrogen in a SBR-anammox reactor with a biomass concentration of 2.4 g VSS L^{-1} .

The measured values of ammonia and nitrite consumption and nitrate production, for each stage of operation, were used to estimate the stoichiometric ratios of the Anammox process: the nitrite production to ammonia consumption ratio ($\text{NO}_2^-/\text{NH}_4^+$ as $\text{g NO}_2^- \cdot \text{N} \cdot \text{g}^{-1} \text{NH}_4^+ \cdot \text{N}$) and the nitrate production to ammonia consumption ratio ($\text{NO}_3^-/\text{NH}_4^+$ as $\text{g NO}_3^- \cdot \text{N} \cdot \text{g}^{-1} \text{NH}_4^+ \cdot \text{N}$). The results obtained are presented in table 3 and compared to the values for Anammox stoichiometry found by Strous et al. [19] ($\text{NO}_2^-/\text{NH}_4^+=1.32$ and $\text{NO}_3^-/\text{NH}_4^+=0.26$). The $\text{NO}_2^-/\text{NH}_4^+$ ratios obtained during stages II and III were as high as 2.69 and 4.03, respectively, related to the increase of the nitrite concentration fed during these stages. Strous et al. [19] stated that the distorted stoichiometry at high nitrite concentrations is due to the fact that the microorganisms do not only use ammonium as the electron donor, but also must have the possibility to generate an internal electron donor to reduce the nitrite. For stages I and IV the $\text{NO}_2^-/\text{NH}_4^+$ ratio observed were close to the theoretical ones, although slightly lower. This difference could be attributed to the operational conditions, like temperature, that was moderate (between 22 and 25 °C) along the operational period. The change of the stoichiometry of the Anammox process with the conditions of operation is still not clear at the moment. Cui [20] by means of Oxitop measurements observed that the stoichiometry of Anammox reaction remained the same at 15 °C and 20 °C, while Dosta et al. [8] observed that with the change of the operational temperature from 30 to 18 °C the $\text{NO}_2^-/\text{NH}_4^+$ ratio decreased from 1.38 to 1.05, similar to the observations made in the present work.

The $\text{NO}_3^-/\text{NH}_4^+$ ratios during stages I and IV were lower than the theoretical one, which could be due to the occurrence of the denitrification of the nitrate produced in the Anammox process. Although the measurement of the organic matter content in the influent and effluent of the SBR-Anammox system was not performed, the previous characterization of the pre-treated pig slurry used to feed the CSTR-partial nitrification reactor indicated that some slowly biodegradable organic matter was present (around $100 \text{ mg C}\cdot\text{L}^{-1}$) which could be used for denitrification. Another reason of the lower nitrate production respect to the ammonia consumption could be attributed also to the moderate temperatures, since Hu et al. [10] observed that the $\text{NO}_3^-/\text{NH}_4^+$ ratio for the anammox bacteria in a nitrification-anammox SBR decreased from 0.18 to 0.04 when the operational temperature was decreased from 25 to 12 °C.

The activity of the Anammox biomass (SAA) was followed by means of batch activity tests performed at 25 °C. At the beginning of the operation an average value of the SAA of $0.21 \text{ g N}_2\text{-N}\cdot\text{g}^{-1} \text{ VSS}\cdot\text{d}^{-1}$ was obtained, which was maintained along the operational period. Only at the end of the operation, the SAA decreased down to a value around $0.17 \text{ g N}_2\text{-N}\cdot\text{g}^{-1} \text{ VSS}\cdot\text{d}^{-1}$ (Figure 3). Despite this decrease the specific nitrogen loading rate (SNLR) applied to the system remained below the SAA. Therefore the decrease of the nitrogen removal efficiency in Stage III was not due to the fact that the SNLR applied exceeded the maximum SAA, but it was probably related to the high ammonia and nitrite concentrations applied that inhibited the Anammox biomass [21]. It is known that high concentrations of ammonia ($1 \text{ g}\cdot\text{L}^{-1}$) are inhibitory for the Anammox process [19], although the FA is the true inhibitor. Fernández et al. [22] determined that the stable operation of Anammox process can be maintained only at FA concentrations under $20 \text{ mg N}\cdot\text{L}^{-1}$. In the present study the FA concentration was under this limit value, except for Stage III, where it ranged between 35 and $80 \text{ mg N}\cdot\text{L}^{-1}$.

Another important aspect of the Anammox-SBR operation was the fact that the biomass concentration inside the reactor decreased from $4.1 \text{ g VSS}\cdot\text{L}^{-1}$, at the beginning of the operation, to $2.4 \text{ g VSS}\cdot\text{L}^{-1}$, around day 125, then the solids content was maintained until the end of the operational period (Figure 3). The VSS decrease was related to the disintegration of the granular biomass used as inoculum due to the application of mechanical stirring (Figure 4). This mechanical mixture was necessary in order to guaranty a complete mixing inside the reactor. The biomass loss provoked a decrease of the SRT from 300 (Stage I) to 100 days (Stage IV), although it remained still appropriated for an Anammox system.

Comparison between “two-units” and “single-unit” configurations

The removal of nitrogen by Anammox based processes requires the availability of an effluent with adequate $\text{NO}_2^-/\text{NH}_4^+$ ratio of around $1 \text{ g N}\cdot\text{g N}^{-1}$, which is not frequently present in wastewaters. This means that a previous step is necessary to achieve a partial nitrification of the ammonia contained in the wastewater. Both sequential processes can be performed in a “two-units” configuration, where the partial nitrification and Anammox processes take place in separated units, or in a “single-unit” configuration, where both types of microorganisms (AOB and Anammox) coexist in the same unit. Vazquez-Padín et al. [23] compared both types of configurations operated at moderate temperatures and observed that the “two-units” system efficiency was limited by the Anammox step. In this case the achieved nitrogen removal capacity was around ten times lower than the “single-unit” system. These authors reached a nitrogen removal rate of $0.08 \text{ g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ with the “two-units” configuration, which is close to the value obtained in this work ($0.09 \text{ g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$), limited also by the Anammox process. Figueroa et al. [24] used a “single-unit” configuration based on the CANON process to treat a similar effluent to that used this work (pre-treated pig slurry) operated at moderate temperature ($20 \text{ }^\circ\text{C}$) and obtained a nitrogen removal rate of $0.46 \text{ g N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$, which is five times higher than the obtained in the present study with a “two-units” configuration. Therefore the “single-unit” configuration seems to be more adequate in terms of nitrogen removal rate, although both configurations have been implemented successfully at pilot and full-scale [25]. Veys et al. [26] by simulation concluded that a system with separated partial

nitritation and Anammox offered a wider range of optimal process conditions than a one-reactor system.

Jaroszynski and Oleskiewicz [27] also studied the advantages and/or drawbacks of the two configurations without obtaining a clear conclusion about the most suitable technology. These authors underlined the simplicity of the “single-unit” configuration, although they indicated that it requires a very tight operational control (oxygen and pH) for an adequate interaction between AOB and Anammox bacteria. They explained that the “two-units” configuration is more complex from the implementation point of view, but the operation is more robust. This fact might shorten the recovery time after a possible instability of the system.

Despite the limitation of the “two-units” system due to the low nitrogen removal rates of the Anammox process in the present study an efficient strategy to obtain the partial nitrification by controlling the NH_4^+/IC ratio was developed. Bagchi et al. [25] pointed out that to accumulate nitrite the growth of NOB can be inhibited by means of FA or FNA toxicity by the control of the pH value. In this case the control of pH was achieved indirectly providing an adequate NH_4^+/IC ratio in the feeding of the partial nitrification reactor. Furthermore the “two-units” configuration to remove nitrogen by Anammox based process operated successfully at moderate temperature.

In the present study the combination of partial nitrification and Anammox processes has been successfully used to remove the nitrogen from pre-treated pig slurry. The combination of both processes results in up to 60% savings in aeration costs and 100% savings in external organic carbon dosing compared with conventional nitrification-denitrification processes. At the same time the production of CO_2 and sludge are minimized [25, 28].

Conclusions

The control of the NH_4^+/IC ratio around $0.82 \text{ g}\cdot\text{g}^{-1}$ was selected as the strategy to obtain the oxidation of ammonia, contained in pre-treated pig slurry, to nitrite. The value of the $\text{NH}_4^+/\text{NO}_2^-$ ratio was around $1 \text{ g}\cdot\text{g}^{-1}$ and it depended on the inhibitory effects of FA. The presence of nitrate in the effluent was controlled by the inhibition of NOB by FA and FNA. Stable accumulation of 50% ammonia and nitrite was obtained during more than 100 days in a reactor operated at room temperature indicating a stable operation of the partial nitrification system.

The effluent obtained in the partial nitrification reactor was successfully treated in an Anammox reactor operated at room temperature and fed with a NLR of $0.1 \text{ g}\cdot\text{N}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. The obtained average nitrogen removal efficiency was around 85%.

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Table 1. Characteristics of the feeding along the different stages of operation of the partial nitrification and Anammox reactors.

| <i>Partial nitrification-CSTR</i> | | | | |
|--|---------------|------------|------------|-----------|
| Parameters | Stages | | | |
| | I | II | III | IV |
| pH _{influent} | 7.42±0.3 | 7.76±0.2 | 8.10±0.4 | 7.79±0.2 |
| T _{reactor} (°C) | 23.8±1.5 | 25.7±1.0 | 23.6±0.9 | 24.5±1.2 |
| NH ₄ ⁺ -N (mg·L ⁻¹) | 419.4±23.7 | 387.3±10.2 | 386.1±12.6 | 396.0±7.2 |
| NH ₄ ⁺ /IC ratio (g N·g ⁻¹ C) | 1.19 | 0.95 | 0.89 | 0.82 |

| <i>Anammox-SBR</i> | | | | |
|--|---------------|-----------|------------|-----------|
| Parameters | Stages | | | |
| | I | II | III | IV |
| pH _{influent} | 7.01±0.2 | 7.13±0.1 | 7.09±0.1 | 7.02±0.2 |
| T _{reactor} (°C) | 24.0±1.7 | 24.8±0.8 | 22.8±0.7 | 23.9±0.9 |
| NO ₂ ⁻ -N (mg·L ⁻¹) | 97.4±3.9 | 145.1±6.7 | 196.2±7.7 | 98.3±3.5 |
| NH ₄ ⁺ -N (mg·L ⁻¹) | 97.0±2.6 | 153.1±5.2 | 194.4±5.1 | 98.8±4.1 |
| NO ₂ ⁻ /NH ₄ ⁺ ratio (g N·g ⁻¹ N) | 1.00 | 0.95 | 1.01 | 0.99 |
| NLR (g N·L ⁻¹ ·d ⁻¹) | 0.10±0.01 | 0.15±0.01 | 0.20±0.01 | 0.10±0.01 |

Table 2. Summary of experimental results of the partial nitrification-CSTR during the four operational stages.

| Stage | NH ₄ ⁺ /IC (g N·g ⁻¹ C) | Time (days) | pH | Nitrogen and carbon species in effluent (%) | | | | ^c SAOA (g NH ₄ ⁺ -N·g ⁻¹ VSS·d ⁻¹) | SRT (days) |
|-------|---|----------------|---------|--|--|--|---|--|----------------|
| | | | | ^a NH ₄ ⁺ -N | ^b NO ₂ ⁻ -N | ^c NO ₃ ⁻ -N | ^d HCO ₃ ⁻ -C | | |
| I | 1.19±0.05 | 0-21 | 7.4±1.4 | 69.8±11 | 23.9±9 | 6.7±3 | 66.5±10 | 0.49±0.05 | 7.35±1.2 |
| II | 0.95±0.04 | 22-29 | 6.5±0.3 | 63.4±9 | 30.9±8 | 5.1±2 | 63.8±12 | 0.52±0.04 | 4.87±0.7 |
| III | 0.89±0.04 | 30-42 | 6.3±0.4 | 63.2±5 | 30.9±5 | 4.6±4 | 67.9±11 | 0.49±0.07 | 4.10±1.1 |
| IV | 0.82±0.02 | 43-183 | 6.0±0.3 | 50.3±4 | 48.2±6 | 0.8±1 | 44.3±8 | 0.52±0.02 | 3 [*] |

^{a,b,c,d} Calculated as percentage composition of the effluent referred to the influent composition: (Concentration in the effluent/Concentration in the influent)*100

^cSAOA was determined from biomass samples collected from the reactor during steady state operational conditions

^{*}No settler after the CSTR (SRT=HRT)

Table 3. Stoichiometric ratios obtained for each stage of operation of the Anammox-SBR system compared with the theoretical values.

| | $\text{NO}_2^-/\text{NH}_4^+$ (g N·g N ⁻¹) | $\text{NO}_3^-/\text{NH}_4^+$ (g N·g N ⁻¹) |
|--------------|---|---|
| Theoretical* | 1.32 | 0.26 |
| Stage I | 1.14±0.07 | 0.09±0.03 |
| Stage II | 2.69±0.41 | 0.36±0.15 |
| Stage III | 4.03±1.42 | 0.76±0.29 |
| Stage IV | 1.01±0.09 | 0.07±0.03 |

*According to Strous et al. [19]

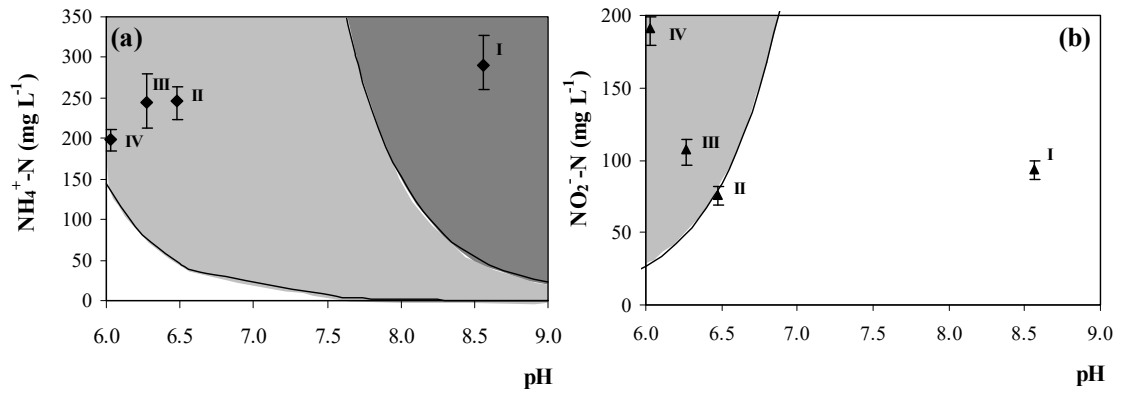


Figure 1. The experimental values of FA (\blacklozenge), FNA (\blacktriangle) and the zones of inhibition corresponding to: (a) between 0.1 and 10 mg FA L^{-1} (\square) and $\geq 10 \text{ mg FA L}^{-1}$ (\blacksquare) and (b) $\geq 0.2 \text{ mg FNA L}^{-1}$ (\square) were calculated using the expressions from Anthonisen et al. ([3]).

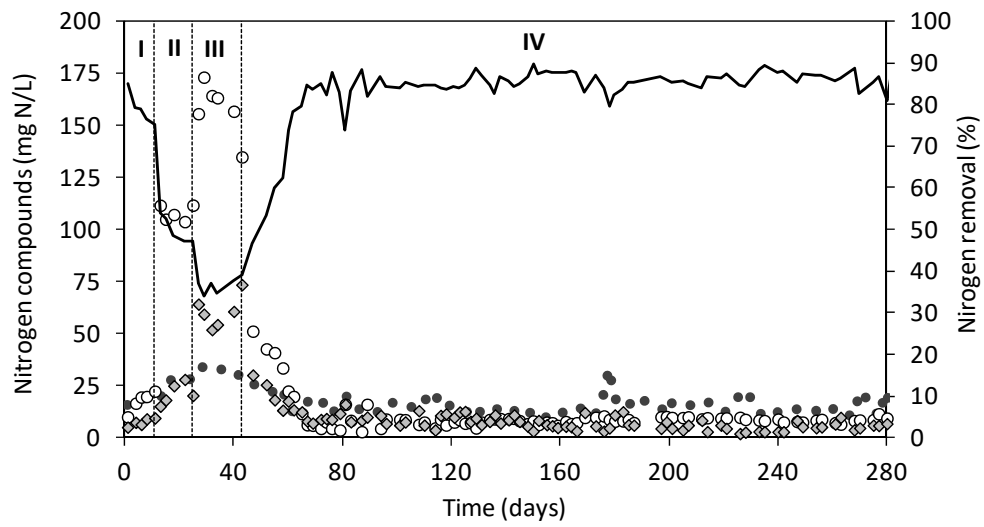


Figure 2. Concentration of nitrogen compounds in the effluent: ammonium (○), nitrite (◆) and nitrate (●) and percentage of nitrogen removal (—).

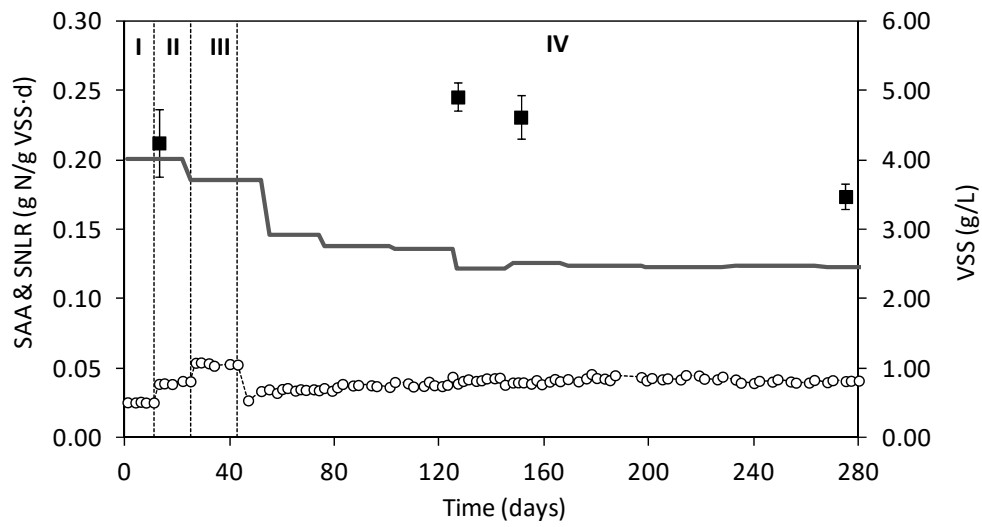


Figure 3. Evolution of SAA (■) in batch tests at 25 °C, applied SNLR (○) and concentration of biomass as VSS (□) inside the Anammox reactor.

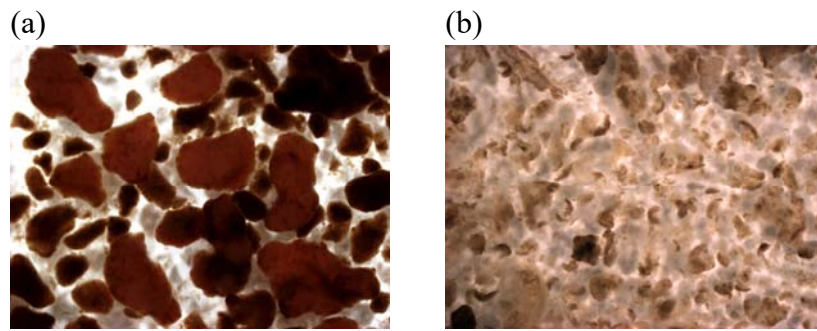


Figure 4. Images of Anammox biomass used as inocula (a) and at the end of the operation of SBR (b).