

Partial Nitrification of Swine Wastewater in View of Its Coupling with the Anammox Process

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Partial nitrification (PN) of swine wastewater was investigated in a sequencing batch reactor (SBR) using a high-performance nitrifying sludge. Characteristics of the wastewater used were low content of biodegradable organic matter and a high alkalinity-to-ammonium ratio. The target oxidation of ammonium nitrogen (NH_4^+-N) to nitrite nitrogen (NO_2^--N) was 57% ($1.32 \text{ g NO}_2^--\text{N g}^{-1} \text{ NH}_4^+-\text{N}$), which corresponds with the reaction ratio of the anaerobic ammonium oxidation (anammox). This target was successfully achieved at 32°C by controlling the inflow rate and the corresponding nitrogen loading rate (NLR). An average NLR of $1.47 \text{ g NH}_4^+-\text{N L}^{-1} \text{ d}^{-1}$ was applied to the partial nitrification sequencing batch reactor during a period of 70 d. The nitrite production rate obtained was $0.91 \text{ g NO}_2^--\text{N L}^{-1} \text{ d}^{-1}$. No nitrate was produced. The PN effluent contained $1.38 \text{ g NO}_2^--\text{N g}^{-1} \text{ NH}_4^+-\text{N}$, which was within 5% of the target ratio. Under steady composition of the wastewater, the pH was shown to be a good indicator of the PN process performance. Furthermore, in a second sequencing batch reactor, the anaerobic ammonium oxidation process was effectively applied to the PN effluent, attaining a nitrogen conversion rate of $0.36 \text{ g N L}^{-1} \text{ d}^{-1}$ (14.3 mg N g^{-1} volatile suspended solids h^{-1}).

APPROPRIATE LAND APPLICATION of manure nitrogen (N) at agronomic rates must be assured to preserve the environmental quality of agricultural ecosystems, atmosphere, and water resources. When land is limiting, the combined bioprocess of nitrification–denitrification (NDN) has been successfully used for the removal of N through the conversion of ammonium (NH_4^+) into dinitrogen gas (N_2) via nitrate (NO_3^-) as intermediate (Béline et al., 2004; Magrí and Flotats, 2008; Vanotti and Szögi, 2008). The operational cost of NDN is an important consideration in livestock effluents. In a full-scale demonstration of a second-generation environmentally superior technology (EST) for the treatment of swine manure in the United States, Vanotti et al. (2009) found that NDN consumed 87% of the total electrical power used by the EST system, which included solids separation, NDN, and phosphorus removal/disinfection unit processes; furthermore, 80% of that requirement was to power the air blower for the nitrification process. They concluded that any significant savings in power requirements by this type of EST system in the future will come from changes in the N treatment, such as the incorporation of anaerobic ammonium oxidation (anammox), which requires about half the aeration required by NDN (Vanotti et al., 2009).

The discovery of the anammox process as an alternative pathway in the N cycle for the biotransformation of the NH_4^+ to N_2 (Mulder et al., 1995) has awakened great expectations for the treatment of animal wastewaters because it entails a more efficient option than conventional NDN. The anammox is a chemolithoautrophic, biologically mediated reaction in which NH_4^+ is oxidized to N gas using nitrite (NO_2^-) as the electron acceptor under anaerobic conditions (Strous et al., 1998). Compared with NDN, this alternative N pathway, which combines partial nitrification (PN) and anammox to eliminate NH_4^+ from wastewater, reduces 58% of the oxygen requirement, 100% of the organic matter requirement, and 83% of the biosolids production due

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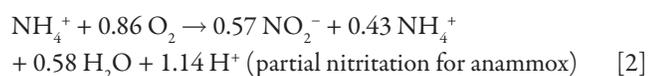
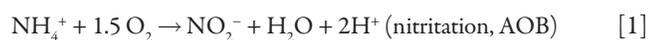
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Abbreviations: A-SBR, anaerobic ammonium oxidation sequencing batch reactor; ALK, alkalinity; anammox, anaerobic ammonium oxidation; AOB, ammonia-oxidizing bacteria; BOD_5 , 5-d biological oxygen demand; COD, chemical oxygen demand; DO, dissolved oxygen; EST, environmentally superior technology; FA, free ammonia; FNA, free nitrous acid; HPNS, high-performance nitrifying sludge; NCR, nitrogen conversion rate; NDN, nitrification–denitrification; NLR, nitrogen loading rate; NOB, nitrite-oxidizing bacteria; NPE, nitrite production efficiency; NPR, nitrite production rate; NRR, nitrogen removal rate; OUR, oxygen uptake rate; PN, partial nitrification; SBR, sequencing batch reactor; SVI, sludge volume index; SW, swine wastewater; TAN, total ammonium nitrogen; TNN, total nitrite nitrogen; TS, total solids; TSS, total suspended solids; VS, volatile solids; VSS, volatile suspended solids.

to the selective growth of autotrophic biomass (Daigger et al., 2011). It also offers the possibility of working with more compact reactors at higher loading rates (Van Hulle et al., 2010). In the case of livestock effluents, the PN–anammox combination offers a more effective integration of biological N removal with waste-to-energy conversion by anaerobic digestion (Ahn et al., 2004; Hwang et al., 2005; 2006; Karakashev et al., 2008; Furukawa et al., 2009; Qiao et al., 2010; Zhang et al., 2011a). In addition to extracting energy, the preceding anaerobic digestion step helps in the reduction of the organic load and the coexistence of heterotrophic activity within PN/anammox reactors, which may negatively affect these autotrophic processes (Mosquera-Corral et al., 2005; Molinuevo et al., 2009; De Prá et al., 2012).

Partial nitrification of NH_4^+ to NO_2^- is needed to produce a suitable influent for the anammox process. This PN conversion is done by ammonia-oxidizing bacteria (AOB) as represented in Eq. [1]. According to the anammox reaction ratio, 1.32 g NO_2^- -N are consumed per g NH_4^+ -N removed (Strous et al., 1998). Thus, optimal performance of PN for its coupling with the anammox process requires 57% oxidation of NH_4^+ -N to NO_2^- -N (Eq. [2]).



The nitrite/ammonium (NO_2^- -N/ NH_4^+ -N) ratio in partially nitrated effluents is usually related to the alkalinity (ALK) of the influent and is linked mainly to its content on inorganic carbon ($\text{CO}_2/\text{HCO}_3^-/\text{CO}_3^{2-}$). According to the stoichiometry of nitrification (Eq. [1]), 2 mol protons are produced per mol of ammonium oxidized, resulting in a theoretical alkalinity requirement of 7.14 g CaCO_3 per g NH_4^+ -N oxidized. Hence, when targeting only 57% oxidation of NH_4^+ -N to NO_2^- -N in PN (Eq. [2]), 4.07 g CaCO_3 would be needed per g NH_4^+ -N supplied. An excess or lack of ALK in the influent may result in effluent NO_2^- -N/ NH_4^+ -N ratios far from the anammox reaction requirements (Magrí et al., 2007b; Zhang et al., 2011b). When ALK is insufficient, the effluent NO_2^- -N/ NH_4^+ -N ratio still can be controlled by adding chemicals and adjusting the influent alkalinity/ammonium (ALK/NH_4^+ -N) ratio to stoichiometric requirements. When ALK is excessive, more nitrite than needed will be produced by PN. In this situation, two main options could be considered based on organic load availability: (i) bypass part of the influent and mix it with the effluent from the PN system or (ii) use heterotrophic denitrification. Both methods result in a decreased NO_2^- -N/ NH_4^+ -N effluent ratio (Ganigué et al., 2008).

In wastewater treatment, the NO_2^- is further converted to NO_3^- by existing nitrite-oxidizing bacteria (NOB). Different strategies have been proposed to avoid NO_3^- formation by NOB in nitrification systems: (i) the combination of moderately high water temperature ($\sim 35^\circ\text{C}$) and low solids retention time in the SHARON (Single Reactor System for High Ammonia Removal Over Nitrite) process that promotes the growth rate of AOB and the wash-out of NOB (Magrí et al., 2007a), (ii) process operation under high concentrations of free ammonia (FA, NH_3) and/or free nitrous acid (FNA, HNO_2) to take advantage of

NOB inhibition to these conditions (Anthonisen et al., 1976), and (iii) process operation under low concentration of dissolved oxygen (DO) to take advantage of the lower affinity for DO of NOB (Yang et al., 2010).

The functioning principle of sequencing batch reactors (SBRs) is based on a cyclic sequence of filling, bioreaction, settling, and withdraw repeated over time. One of the main advantages of SBR technology is its operational flexibility; this technology has been found to be effective to conduct PN (Pambrun et al., 2006; Galí et al., 2007; Ganigué et al., 2007, 2008; Guo et al., 2010) and anammox (Strous et al., 1998; Fux et al., 2002; López et al., 2008) processes in wastewaters from different origins. However, limited information is available on the best approach for PN in view of its coupling with anammox using swine wastewater (SW).

The aim of this study was to obtain nitrite through PN of SW and to produce an effluent that is optimized for use in the anammox process as in Eq. [2]. It was conducted at bench-scale using a partial nitrification SBR (PN-SBR) and a high-performance nitrifying sludge (HPNS). The study evaluated the effect of variable inflow rate, aeration rate, FA and FNA concentrations, and wastewater alkalinity on the optimized PN-SBR process. The balanced PN effluent was tested with the anammox process in a second SBR (A-SBR).

Materials and Methods

Swine Wastewater

The SW for the PN experiments was obtained from a second-generation treatment system in a pig farm near Clinton, Sampson County, North Carolina, after solid–liquid separation. The separation module used polyacrylamide flocculation to enhance the separation of fine suspended particles and a rotary press separator for dewatering as described by Vanotti et al. (2009). The separated liquid fraction was collected and stored anaerobically in closed barrels (208 L) at ambient temperature for about 2 mo at the ARS laboratory in Florence, South Carolina before use in the bench PN experiments. The wastewater characteristics shown in Table 1 were determined at the time the wastewater was being fed into the PN reactor. There were two SW samples (SW1 and SW2) used in the PN-SBR corresponding to two different sampling dates. Phase 1 experiments were done with SW1, and Phase 2 experiments were done with SW2.

Partial Nitrification

High-Performance Nitrifying Sludge

For partial nitrification, we used HPNS developed for the treatment of high-ammonium-concentration and low-temperature wastewater (Vanotti et al., 2011a). The HPNS was deposited under the provisions of the Budapest Treaty of the United Nations in the Agricultural Research Service Culture Collection in Peoria, Illinois, on 26 June 2009 (deposit accession number: NRRL B-50298). The HPNS is a composition of bacteria comprised of 35 strains or populations of isolated bacteria (HPNS.1 to HPNS.35; GenBank accession GQ223345 through GQ223379). Based on 16S rRNA analysis, 26 of the bacteria are affiliated with Proteobacteria, seven are affiliated with Bacteroidetes, and two are affiliated with Actinobacteria.

Table 1. Characteristics of the swine wastewater influent used for partial nitrification.

Parameter†‡	SW1§	SW2§
pH	8.26 (0.04)	7.87 (0.03)
EC	15.5 (1.8)	15.4 (0.8)
TS	4921 (252)	6823 (231)
VS	1073 (162)	1093 (50)
TSS	233 (86)	30 (1)
VSS	152 (58)	25 (6)
ALK	7586 (106)	4916 (48)
COD	1453 (97)	3440 (21)
BOD ₅	16 (5)	61 (18)
TKN	1434 (93)	1040 (33)
NH ₄ ⁺ -N	1385 (15)	1026 (27)
NO ₂ ⁻ -N	0	0
NO ₃ ⁻ -N	0	0
P	86 (8)	29 (8)
PO ₄ -P	82 (3)	35 (1)
S	32 (6)	22 (1)
Na	423 (43)	615 (20)
K	1460 (133)	1965 (40)
Mg	3 (1)	53 (6)
Ca	8 (1)	89 (1)
Cu	0.12 (0.06)	0.11 (0.05)
Zn	0.46 (0.02)	0.52 (0.04)

† ALK, alkalinity; BOD₅, 5-d biological oxygen demand; COD, chemical oxygen demand; EC, electrical conductivity; SW, swine wastewater; TKN, total Kjeldahl N; TS, total solids; TSS, total suspended solids; VS, volatile solids; VSS, volatile suspended solids.

‡ Except pH and electrical conductivity (dS m⁻¹), units are in mg L⁻¹.

§ Values are averages (SE in parentheses).

The HPNS was derived from an acclimated lagoon nitrifying sludge originally obtained and described by Vanotti and Hunt (2000) after prolonged cultivation in a suspended biomass reactor at low temperature (10°C). The HPNS was maintained in the USDA-ARS laboratory (Florence, South Carolina) in an aeration tank with fine bubble aeration at 10°C water temperature using the fill-and-draw cultivation method and an inorganic salts medium (Vanotti et al., 2011a). The inorganic salts medium had NH₄⁺-N content of 300 mg L⁻¹, a buffered pH of about 8.5, and sufficient alkalinity for complete nitrification; it was comprised of the chemicals listed in Table 2. Once a week, the aeration was stopped, the suspension was allowed to settle (30 min), the supernatant was withdrawn and replaced with fresh medium, and aeration resumed. The HPNS had the following characteristics (Vanotti et al., 2011a): (i) a specific nitrification activity of 51.0 mg N per g total suspended solids (TSS) per hour (62.1 mg N per g volatile suspended solids [VSS] per hour) obtained at 30°C using inorganic salts medium with 300 mg NH₄⁺-N L⁻¹, biomass concentration 2.01 g VSS L⁻¹, and process DO 5.0 ± 0.6 mg L⁻¹ and (ii) a sludge volume index (SVI) of 62 mL g⁻¹ TSS. A SVI rating of <80 is indicative of excellent sludge settling and compaction properties as a result of a balanced mix of flock-forming and filamentous bacteria (Grady et al., 1999). For the start-up of the PN-SBR, the HPNS was concentrated by settling, and 145 mL of the settled sludge containing 2.1 g VSS (2.5 g TSS) were transferred into the PN-SBR reactor to start the experiments.

Table 2. Composition of inorganic salts medium and trace elements solution used for cultivation of HPNS.

Chemicals	Final concentration
	mg L ⁻¹
Inorganic salts medium	
(NH ₄) ₂ SO ₄	1416
K ₂ HPO ₄	100
NaHCO ₃	2912
Na ₂ CO ₃	391
MgSO ₄ ·7H ₂ O	60
FeSO ₄ ·7H ₂ O	8
CaCl ₂ ·2H ₂ O	8
Trace elements solution	
ZnSO ₄ ·7H ₂ O	1247
MnSO ₄ ·H ₂ O	1119
CuSO ₄ ·5H ₂ O	44
Al ₂ (SO ₄) ₃ ·14H ₂ O	201.5
Na ₂ MoO ₄ ·2H ₂ O	129
CoCl ₂ ·6H ₂ O	30
KCl	100
EDTA‡ disodium salt dihydrate	975

† 0.1 mL L⁻¹ of trace element solution is added to inorganic salts medium.

‡ Ethylenediaminetetraacetic acid.

Partial Nitrification Reactor

Partial nitrification was performed in a 3.5-L jacketed glass reactor (Chemglass Life Sciences) operated under batch mode (Fig. 1). Air was supplied through aquarium air pumps, a flow meter (model 12; Gilmont Instruments), and porous stones, which provided fine bubbles. Biomass suspension and mixing were achieved through uninterrupted aeration during the reaction phases. Influent wastewater was stored in a 15-L tank placed into a cooled water bath at 8°C. Liquid was pumped in and out of the reactor through console drives (Masterflex) equipped with pump heads (Easy-Load II, Cole-Parmer). Process temperature was controlled at 32°C using a water heating circulator (model 9512, PolyScience). Antifoam solution kept in a 0.25-L flask was injected discontinuously to the PN-SBR (1 min/30 min) using a peristaltic pump (model 7553-80, Cole-Parmer). The antifoam solution was stirred only during injection using a magnetic stirrer (Cimarec, Thermo Scientific). The pH of the mixed liquor was monitored using a field measurement system (model 53, GLI International Inc.) connected to a pH/T probe (model 27003-04, Cole-Parmer). Dissolved oxygen was measured using a portable oxygen meter (model 550A, YSI Inc.).

Partial Nitrification Process Operation

The operation schedule for the PN-SBR is shown in Fig. 2. It was implemented using a programmable timer (XT Table Top, ChronTrol). A complete cycle lasted 8 h and consisted of 15 reaction phases of 30 min each and one settling and withdrawing phase. An intermittent-feed strategy (Mace and Mata-Alvarez, 2002) was applied to split the liquid volume to be treated per cycle in several fractions to be added sequentially and consisted of turning on the influent pump during the first 1 min of each reaction phase. At 7.5 h into the cycle, biomass was allowed to settle by interrupting aeration for 25 min. The cycle was completed with the effluent withdrawn for 5 min. To prevent foaming, a 1% (v/v) stirred solution of antifoam (FO-120, ChemTreat Inc.) was

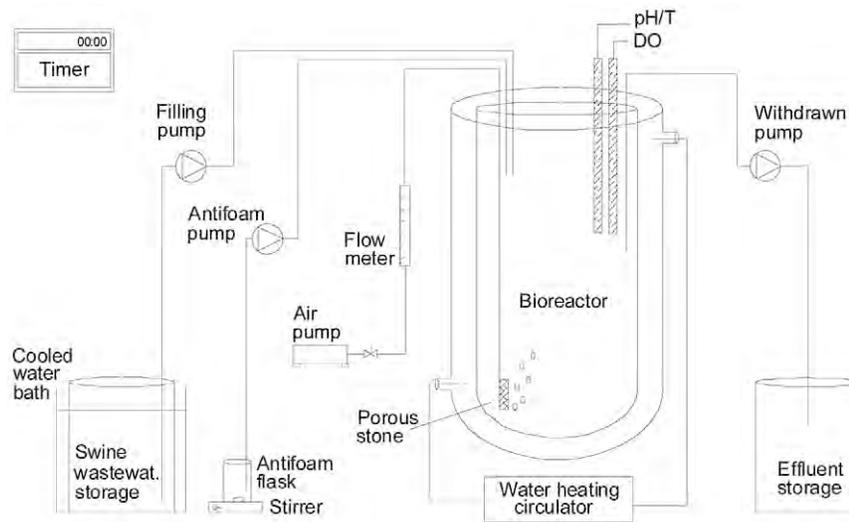


Fig. 1. Schematic diagram of the partial nitritation sequencing batch reactor. DO, dissolved oxygen; T, temperature.

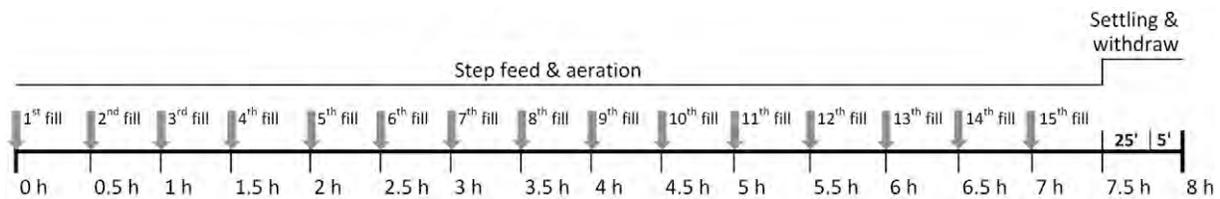


Fig. 2. Operational cycle for the partial nitritation sequencing batch reactor.

added simultaneously with influent wastewater (flow rate, 0.9 mL min^{-1}). The minimum working volume of the reactor was kept at 2 L ($t = 0 \text{ h}$). Maximum working volume was achieved at the end of the cycle ($t = 7 \text{ h}$), with values in the range of 2.33 to 3.81 L depending on the inflow rate applied. Two experimental phases were conducted (Table 3). In Phase 1, the PN-SBR received a constant air flow rate of 2.0 L min^{-1} and a variable influent rate of 1.00 to 5.43 L d^{-1} to adjust process performance. In Phase 2, the PN-SBR received variable aeration rates of 1.5 to 4.5 L min^{-1} and a constant influent rate of 4.11 L d^{-1} . Samples were taken almost daily from the influent tank and the effluent line for chemical analysis including ammonium, nitrite, nitrate, pH, and alkalinity. Liquid samples inside the reactor were taken weekly to measure mixed liquor volatile suspended solids.

Table 3. Operational parameters used in the partial nitritation sequencing batch reactor experiments.

Parameter	Phase 1 experiment	Phase 2 experiment
Time, d	70	70
Target air flow rate, L min^{-1}	2.0	1.5–4.5
Flow rate, L d^{-1}	1.00–5.43	4.11 (0.05)†
Volume exchange ratio, $\# L_{\text{exchanged}} L_{\text{end}}^{-1}$	0.35 (0.01)	0.39 (0.01)
N loading rate, $\text{g NH}_4^+ \text{-N L}^{-1} \text{ d}^{-1}$	1.47 (0.04)	1.25 (0.04)
Hydraulic residence time, d	1.01 (0.04)	0.82 (0.01)
Volatile suspended solids at the end of the cycle, g VSS L^{-1}	1.90 (0.39)	1.88 (0.42)

† Values are averages (SE in parentheses).

‡ Calculated as $1 - (V_0/V_e)$, where V_0 is the liquid volume at the beginning of the first reaction phase of a cycle, and V_e is the liquid volume at the end of the last reaction phase of a cycle.

Batch Tests to Study the Effects of Aeration and Biomass Concentration on Partial Nitritation

Batch tests under constant volume were conducted to assess the combined effect of aeration (2.0 – 5.0 L min^{-1}) and biomass concentration on the nitrite production rate (NPR). The experiment included three levels of VSS: high ($\sim 4.0 \text{ g VSS L}^{-1}$), medium ($\sim 1.5 \text{ g VSS L}^{-1}$), and low ($\sim 0.5 \text{ g VSS L}^{-1}$). Tests with high and medium solids levels were done in the 3.5-L vessel, whereas tests with low solids were done in 1-L vessels as described by Ducey et al. (2010). The aeration rates were also calculated in vvm units ($L_{\text{air}} L_{\text{liquid}}^{-1} \text{ min}^{-1}$), which incorporated the effective water volumes inside the reactors for each treatment. These batch experiments were conducted during the Phase 1 period. Each treatment combination was carried out in duplicate using SW1.

Speciation of Inorganic Nitrogen Forms

The concentrations of un-ionized free ammonia (FA) and un-ionized free nitrous acid (FNA), both as mg N L^{-1} , were calculated using the equations given by Anthonisen et al. (1976) based on temperature (T , $^{\circ}\text{C}$), pH, and total ammonium as N (TAN) or total nitrite as N (TNN) concentration (mg N L^{-1}) values (Eq. [3] and [4]):

$$\text{FA as NH}_3\text{-N (mg L}^{-1}\text{)} = \left(\text{TAN} \cdot 10^{\text{pH}} \right) / \left(\frac{K_b}{K_w} + 10^{\text{pH}} \right) \quad [3]$$

$$\text{FNA as HNO}_2\text{-N (mg L}^{-1}\text{)} = \frac{\text{TNN}}{K_a \cdot 10^{\text{pH}}} \quad [4]$$

where K_b , K_w , and K_a are ionization constants for NH_3 , H_2O , and HNO_2 , respectively. Both the ratio K_b/K_w and K_a may be related to temperature: $K_b/K_w = \exp[6344/(273 + T)]$, and $K_a = \exp[-2300/(273 + T)]$.

Evaluation of the Influence of Free Ammonia and Free Nitrous Acid on the Activity of Ammonia-Oxidizing Bacteria Using Respirometric Tests

The short-term effect of FA and FNA on the activity of the AOB of the HPNS was evaluated using respirometric tests in a watertight respiration chamber completely filled with liquid (in duplicate). This chamber was a 300-mL glass bottle sealed at the top with a rubber stopper. The respiration chamber was placed into a water bath to keep the liquid at a constant temperature of 32°C. A DO probe (Oxyferm VP325, Hamilton) was introduced through a hole in the stopper. The DO probe was plugged into a transmitter (Knick Stratos Eco). Magnetic stirring assured perfectly mixed conditions. Data were acquired every 5 s through a CompactRIO device (National Instruments) custom programmed using Labview 2009 (National Instruments, 2011) and communicated with a laptop as the user interface.

The biomass was extracted from the PN-SBR, kept aerated overnight, and rinsed with tap water before being used in the respirometric tests. The FA tests were conducted in a concentration range of 0 to 500 mg FA-N L⁻¹, using 0 to 1800 mg NH₄⁺-N L⁻¹, 0 mg NO₂⁻-N, and pH 7.0 to 8.7, and the FNA tests were conducted in a concentration range of 0 to 0.5 mg FNA-N L⁻¹ using 0 to 820 mg NO₂⁻-N L⁻¹, 850 mg NH₄⁺-N L⁻¹, and pH 6.5 to 7.0. An inorganic synthetic solution containing NH₄⁺ was prepared according to the general composition previously described for the HPNS cultivation (Table 2) with the following changes in (NH₄)₂SO₄, NaHCO₃, and Na₂CO₃ concentrations: 2000 mg NH₄⁺-N L⁻¹, 10 g NaHCO₃ g⁻¹ NH₄⁺-N, and 0.02 g Na₂CO₃ g⁻¹ NH₄⁺-N using DO-saturated water. This stock solution was then used under different dilution rates in FA and FNA tests. A second stock solution containing NO₂⁻ (2000 mg NO₂⁻-N L⁻¹) was used in the FNA tests. When needed, the pH was adjusted with HCl. The rinsed biomass (final solids concentration, 0.22 g VSS L⁻¹) was added to the respiration chamber after the synthetic substrate, and data recording started immediately. Oxygen uptake rate (OUR) was calculated from the DO decrease with time for DO concentrations between 5 and 2 mg O₂ L⁻¹. Oxygen consumption was entirely attributed to the oxidation of NH₄⁺ to NO₂⁻ because NO₃⁻ production was not detected in a parallel water quality determination.

For FA and FNA, a mathematical function $F_i = \text{OUR} / \text{OUR}_{\max}$ ($0 \leq F_i \leq 1$) dependent on the variable tested (FA or FNA) was fitted to the measured data (Wiesmann, 1994). The OUR_{\max} corresponds to the maximum value of OUR measured. In the case of FA, F_{FA} was considered as Haldane kinetics (Eq. [5]), and the affinity and inhibition constants (K_{FA} , $K_{\text{I,FA}}$) (mg FA-N L⁻¹) were estimated. In the case of FNA, F_{FNA} was considered as noncompetitive and reversible inhibition kinetics (Eq. [6]), and the corresponding inhibition constant ($K_{\text{I,FNA}}$) (mg FNA-N L⁻¹) was estimated after the effect of FA was known. Because the aforementioned kinetic parameters were estimated in terms of un-ionized species, they can be considered to be independent of pH and temperature.

$$F_{\text{FA}} = \frac{\text{OUR}}{\text{OUR}_{\max}} = \frac{\text{FA}}{K_{\text{FA}} + \text{FA} + \frac{\text{FA}^2}{K_{\text{I,FA}}}} \quad [5]$$

$$F_{\text{FNA}} = \frac{\text{OUR}}{\text{OUR}_{\max}} = F_{\text{FA}} \cdot \frac{K_{\text{I,FNA}}}{K_{\text{I,FNA}} + \text{FNA}} \quad [6]$$

Anaerobic Ammonium Oxidation

Anammox Sludge

The anaerobic ammonium oxidation (anammox) bacteria used was *Candidatus Brocadia caroliniensis* deposited under the provisions of the Budapest Treaty in the Agricultural Research Service Culture Collection (NRRL) at Peoria, Illinois (accession number: NRRL B-50286) (Vanotti et al., 2011b). It was maintained at the USDA-ARS laboratory in a 10-L jacketed up-flow continuous reactor (120 cm) packed with a biomass carrier to enhance retention of microorganisms (parent reactor). At the time of sludge harvesting, the parent reactor was being fed with synthetic wastewater containing 153 mg NH₄⁺-N L⁻¹ and 153 mg NO₂⁻-N L⁻¹ and operated with a flow rate of 60 L d⁻¹, a N-loading rate (NLR) of 1735 mg N L⁻¹ d⁻¹, and a water temperature of 30°C. Under these conditions, the N-conversion efficiency obtained was 94%, and the total N-removal efficiency was 85% (Vanotti et al., 2011b). The anammox sludge extracted from the parent reactor had a granular structure and a reddish color. The granule size distribution was determined by passing (rinsing) 115 mL of anammox sludge (12.2 g total solids [TS] L⁻¹) through ASTM Standard Sieves nos. 6, 10, 18, 30, and 60 (Dual Manufacturing Co.) and measuring TS retained. The average granule size was 1.51 mm, with 0.6, 18.5, 83.1, 91.2, and 98.5% of the granules (by weight) larger than 3.36, 2.00, 1.00, 0.59, and 0.25 mm, respectively. The initial specific conversion rate (measured after harvesting using synthetic wastewater fed into the parent reactor) in the A-SBR was 16.7 ± 4.4 mg N g⁻¹ VSS h⁻¹ (13.3 ± 3.9 mg N g⁻¹ TSS h⁻¹) (Magrí et al., 2012). Start-up of the A-SBR was done using 700 mL of the anammox sludge containing 7.9 g VSS (9.8 g TSS).

Anammox Reactor

The anammox reactor (A-SBR) set-up to perform the anammox process was similar to the PN-SBR reactor, with a few modifications to guarantee anaerobic conditions. A low-flow peristaltic pump (Manostat Carter 4/8, Thermo Scientific) was used to feed the reactor from a 100-L influent tank. Mixing inside the reactor was achieved through a mechanical stirrer (TempStir, Chemglass) equipped with an impeller (12.5 cm diameter) that was operated at 50 rpm. A 10-L Tedlar bag filled with synthetic N₂ was connected with tubing to the top of the reactor and worked as a gas regulation tank to prevent air from entering the reactor during the withdrawal step. During the reaction phases, the gas produced by anammox flowed from the reactor to the bag, whereas during withdrawal phase gas flowed in the opposite way. When needed, the Tedlar bag was refilled with N₂. A gas relief valve was placed on the reactor upper lid to avoid overpressure inside the reactor. Bubbling with N₂ inside the A-SBR through a porous stone was applied in rare cases

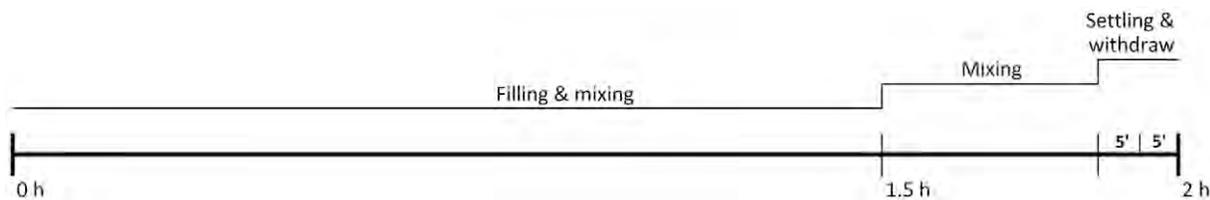


Fig. 3. Operational cycle for the anaerobic ammonium oxidation sequencing batch reactor.

after the reactor was open. The treated effluent was stored in a separate tank before disposal. Process temperature was controlled by the same water heating circulator as the PN-SBR. The A-SBR was kept in the dark using a black polyethylene plastic sheet cover.

Partially Nitritated Swine Wastewater

Partially nitritated effluent was stored at 4°C before use with anammox. It was then diluted approximately five times with distilled water to attain a concentration of 150 mg NO₂⁻-N L⁻¹. The pH was adjusted when needed to 7.5 using HCl (1.19 mol L⁻¹). Bubbling with N₂ was done each time new influent was prepared to reduce DO levels below 1 mg O₂ L⁻¹.

Anammox Process Operation

The A-SBR was operated under 2-h cycles (Fig. 3). Continuous feeding and mixing were supplied during the first 90 min of each cycle to avoid too high concentrations of NO₂⁻ inside the reactor. Feeding was then interrupted while maintaining mixing for another 20 min, providing extra time for the consumption of remaining substrates within the A-SBR. At 110 min into the cycle, mixing was stopped, the anammox sludge was allowed to settle for 5 min, and the effluent was withdrawn during the last 5 min of the cycle. The minimum working volume was kept at 2 L, and the process temperature was 32°C. The A-SBR was fed with synthetic wastewater during the first 20 d. Subsequently, it was fed using partially nitritated SW during the next 80 d. During the first 20 d of trials with the synthetic wastewater, substantial losses of sludge occurred in the effluent due to natural bubbling and sludge resuspension during the effluent withdrawing phase. This problem was solved by the time we started testing with the partially nitritated SW by clarifying the effluent in a separate tank (later in a 1-L settling cone) and reintroducing the settled solids into the A-SBR once a week. The SW testing was done with a stabilized anammox biomass content with an average 2.66 g VSS in the reactor (or 0.94 ± 0.15 g VSS L⁻¹ at the end of the cycle). Targeted NLRs were of 1.00 and 0.50 g N L⁻¹ d⁻¹. Liquid samples were regularly taken from the influent tank and the effluent line for chemical analyses to evaluate process performance. Bulk liquid samples were also periodically taken from the reactor to measure suspended solids.

Analytical Methods

Wastewater analyses were conducted according to the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA, and WEF, 1998). Alkalinity (ALK) was determined by acid titration to an endpoint of pH 4.5 and expressed as CaCO₃ (2320 B). Total solids (TS) were determined after sample drying to constant weight at 105°C (2540 B), and volatile solids (VS) were determined after further ignition in a muffle furnace at 500°C for 15 min (2540 E). Total suspended solids (TSS)

and VSS were measured analogously but after filtration through a 1.5-μm glass microfiber filter (2540 D-E). Chemical organic demand (COD) was determined through the closed reflux colorimetric method (5220 D), and 5-d biodegradable organic demand (BOD₅) was determined through biological incubation tests (5210 B). Inorganic N concentrations were measured after sample filtration to 1.5 μm using an auto-analyzer (Technicon Instruments Corp.). Ammonium-N was determined by the automated phenate method (4500-NH₃ G), NO₂⁻-N + NO₃⁻-N was determined by the automated cadmium reduction method (4500-NO₃⁻ F), and NO₂⁻-N alone was determined by applying the same colorimetric method without the cadmium reduction step. Nitrate-N was then calculated by subtraction. Total Kjeldahl N was determined using the macro-Kjeldahl method (4500-N_{org} B) and using an adaptation of the aforementioned phenate method for acid digested extracts. The orthophosphate phosphorus (PO₄-P) was determined by the automated ascorbic acid method (4500-P F). Total P, S, Na, K, Mg, Ca, Cu, and Zn were determined after acid digestion using an inductively coupled plasma technique (3125 A). Settling characteristics of the nitrifying sludge were determined using the SVI (2710 D). The pH was measured using a pH meter (Pinnacle 540, Corning, Inc.), electrical conductivity referred to 25°C was determined by a portable conductivity meter (model 126, Orion Research, Inc.), and DO was measured by a portable DO meter (model 550A, YSI, Inc.).

Statistical Analysis

Data were analyzed by means of standard error. Linear regression analyses (Draper and Smith, 1981) were used to quantify the NPR in nitritation batch tests and to estimate the OUR in respirometric tests. Data related to (i) PN-SBR performance under variable aeration rate during Phase 2 and (ii) the study of the combined effect of aeration rate and VSS content within the reactor were subjected to ANOVA using the GLIMMIX procedure in SAS (SAS Institute, 2008). Significant differences among treatment means were evaluated using least squares means (α = 0.05). Exponential fitting was used to correlate pH and ALK from PN effluent during Phase 1. Kinetic constants in respirometric tests were estimated by fitting the F_{FA} and F_{FNA} functions to the measured data by maximizing the nonlinear R^2 value.

Results and Discussion

Swine Wastewater

The SW used for PN (Table 1) was raw manure that received solid-liquid separation treatment with polymer from the second-generation EST system described by Vanotti et al. (2009). The on-farm polymer solid-liquid separation treatment was effective at separating organic particles (90% VSS removal) but was ineffective at removing the NH₄⁺ (only 5% removal). At the same

site, the separated liquid manure received biological N treatment using the NDN process and HPNS with average NH_4^+ removal efficiency of 91% for separated liquid containing $1190 \pm 455 \text{ mg NH}_4^+-\text{N L}^{-1}$ (Vanotti et al., 2009). In this laboratory experiment, we tested the PN process using samples of the separated liquid that could be coupled with anammox to reduce the cost of biological N treatment. The separated liquid (SW1 and SW2) contained high N strength, mostly ($\geq 97\%$) in the form of NH_4^+ ($1026\text{--}1385 \text{ mg NH}_4^+-\text{N L}^{-1}$), and $< 3\%$ organic N. Nitrite and nitrate were not detected. In comparison, indicators of organic strength (VS, COD, and BOD_5) were present in low concentrations and were not expected to affect PN and anammox processes due to high organic load. Alkalinity concentration, mainly attributable to the inorganic carbon buffer system, was $7586 \text{ mg CaCO}_3 \text{ L}^{-1}$ in SW1 and $4916 \text{ mg CaCO}_3 \text{ L}^{-1}$ in SW2. The $\text{ALK}/\text{NH}_4^+-\text{N}$ ratio of the wastewater is very important when planning PN because it determines the amount of NH_4^+ potentially oxidizable to nitrite. Very high or low alkalinity concentration (i.e., inorganic carbon) with respect to the NH_4^+ availability may result in a PN effluent with a quality far from that required for the anammox process. The $\text{ALK}/\text{NH}_4^+-\text{N}$ ratio of SW1 and SW2 were 5.48 and 4.79 $\text{g CaCO}_3 \text{ g}^{-1} \text{NH}_4^+-\text{N}$, respectively; they are 35 and 18% higher than the theoretical optimum for PN purposes ($4.07 \text{ g CaCO}_3 \text{ g}^{-1} \text{NH}_4^+-\text{N}$). Therefore, supplemental alkali was not required in the SW to achieve PN.

Partial Nitritation

Performance under Variable Loading Rate (Phase 1)

The addition of HPNS into the PN-SBR resulted in an initial solids content of $0.80 \text{ g VSS L}^{-1}$ (measured at Day 1 at the end of the first cycle with 2.6-L volume). No sludge was wasted during the first 8 wk. Such management resulted in a progressive increase of solids up to $3.38 \text{ g VSS L}^{-1}$ (end of a cycle at Day 57) (Fig. 4). At that point, sludge wasting was performed to adjust solids to the initial level. Excellent to good settling properties of the biomass were maintained, with SVIs ranging from 45 to $119 \text{ mL g}^{-1} \text{TSS}$.

Partial nitritation was successfully achieved by controlling the inflow rate and the corresponding NLR. Average N concentrations in the effluent were $645 \pm 13 \text{ mg NH}_4^+-\text{N L}^{-1}$ and $866 \pm 12 \text{ mg NO}_2^--\text{N L}^{-1}$ (Fig. 4; Table 4), which was equivalent to a $\text{NO}_2^--\text{N}/\text{NH}_4^+-\text{N}$ ratio of $1.38 \pm 0.04 \text{ g NO}_2^--\text{N g}^{-1} \text{NH}_4^+-\text{N}$. The nitrite production efficiency (NPE; i.e., the ratio between nitrite concentration in the effluent and ammonium concentration in the influent [Table 4]) was $62 \pm 1\%$, and the NPR was $0.91 \pm 0.03 \text{ g NO}_2^--\text{N L}^{-1} \text{ d}^{-1}$. No nitrate formation was detected. Measured values for COD removal were about 10%. Although wasting of solids performed at Day 57 reduced VSS from 3.38 to $0.87 \text{ g VSS L}^{-1}$, this reduction did not affect process performance of the PN-SBR. On the other hand, the PN-SBR was sensitive to N overload episodes ($\text{NLR} > 1.6 \text{ g NH}_4^+-\text{N L}^{-1} \text{ d}^{-1}$ at approximately Days 20 and 40 [Fig. 4]). However, recovery of the system was quickly achieved by decreasing the inflow rate.

Inhibition of NOB by FA was identified as the key factor avoiding NO_3^- formation. Free ammonia (FA) concentration at the end of the cycle was $52.4 \pm 4.4 \text{ mg FA-N L}^{-1}$ (range, $1.6\text{--}168.1 \text{ mg FA-N L}^{-1}$), whereas FNA was $0.05 \pm 0.01 \text{ mg FNA-N L}^{-1}$ (range, $0.005\text{--}0.64 \text{ mg FNA-N L}^{-1}$). In comparison, values given by Anthonisen et al. (1976) as inhibitory to NOB were

Table 4. Summary of the partial nitritation sequencing batch reactor effluent characteristics and performance during Phase 1.

Parameter†	Value‡
NH_4^+-N , mg L^{-1}	645 (13)
NO_2^--N , mg L^{-1}	866 (12)
NO_3^--N , mg L^{-1}	0
FA-N, mg L^{-1}	52.4 (4.4)
FNA-N, mg L^{-1}	0.05 (0.01)
pH	7.83 (0.05)
ALK, $\text{mg CaCO}_3 \text{ L}^{-1}$	1359 (77)
$\text{NO}_2^--\text{N}/\text{NH}_4^+-\text{N}$, $\text{g NO}_2^--\text{N g}^{-1} \text{NH}_4^+-\text{N}$	1.38 (0.04)
NPR, $\text{g NO}_2^--\text{N L}^{-1} \text{ d}^{-1}$	0.91 (0.03)
NPE, %	62 (1)

† ALK, alkalinity; FA, free ammonia; FNA, free nitrous acid; NPE, nitrite production efficiency; NPR, nitrite production rate.

‡ Values are averages (SE in parentheses).

0.1 to 1.0 mg FA L^{-1} ($0.08\text{--}0.8 \text{ mg FA-N L}^{-1}$) and 0.2 to $2.8 \text{ mg FNA L}^{-1}$ ($0.06\text{--}0.83 \text{ mg FNA-N L}^{-1}$).

Alkalinity and pH Considerations

The operational conditions applied did not result in the complete consumption of ALK (effluent ALK ranged from 269 to $2848 \text{ mg CaCO}_3 \text{ L}^{-1}$), allowing the regulation of the effluent $\text{NO}_2^--\text{N}/\text{NH}_4^+-\text{N}$ ratio (Fig. 5). The ALK consumption was $8.10 \pm 0.09 \text{ g CaCO}_3 \text{ g}^{-1} \text{NH}_4^+-\text{N}$ oxidized (13% higher than the theoretical value of $7.14 \text{ g CaCO}_3 \text{ g}^{-1} \text{NH}_4^+-\text{N}$). A high correlation between the effluent pH and its composition in terms of ALK and $\text{NO}_2^--\text{N}/\text{NH}_4^+-\text{N}$ ratio was observed (Fig. 5). This finding indicates that the pH could be a fast indicator of the effluent quality. In the case of treating SW1, the optimum $\text{NO}_2^--\text{N}/\text{NH}_4^+-\text{N}$ ratio of 1.32 could be obtained when effluent pH is near 8.0 (Fig. 5). In the case of the aforementioned overload episodes and deteriorated $\text{NO}_2^--\text{N}/\text{NH}_4^+-\text{N}$ ratio (0.91 ± 0.07), the pH rose to values of 8.25 to 8.40. This indicates that control of a balanced PN process when treating SW could be achieved through the implementation of automated supervision strategy based on pH monitoring.

Reduction of natural buffer agents (i.e., inorganic carbon and ammonium) in SWs occurring during PN treatment may help in subsequent processing based on selective precipitation of calcium phosphates (Vanotti et al., 2003; Szogi and Vanotti, 2009).

Partial Nitritation Sequencing Batch Reactor Performance under Variable Aeration Rate (Phase 2)

Aeration rate treatments applied to the PN-SBR significantly affected the NPE (Table 5). The lower aeration rate ($1.56 \pm 0.09 \text{ L min}^{-1}$) provided insufficient DO ($< 0.3 \text{ mg O}_2 \text{ L}^{-1}$), and consequently the attained NPE was only $26 \pm 1\%$ (compared with target of 57%). Increased aeration rates increased DO during a PN-SBR cycle and provided more balanced NPE (56–63%) compared with the 57% target (Table 5). The highest aeration rate ensured nonlimiting conditions for AOB activity due to DO availability at the end of reaction phases ($3.5 \text{ mg O}_2 \text{ L}^{-1}$). Even though the aeration rate varied in the range of 1.92 to 4.51 L min^{-1} , the NPE changed little (56–63%). Higher levels of DO did not produce a significant amount of nitrate (effluent contained $< 1 \text{ mg NO}_3^--\text{N L}^{-1}$). With increased aeration rate, the pH of the effluent decreased from 8.47 to 6.19, and ALK decreased

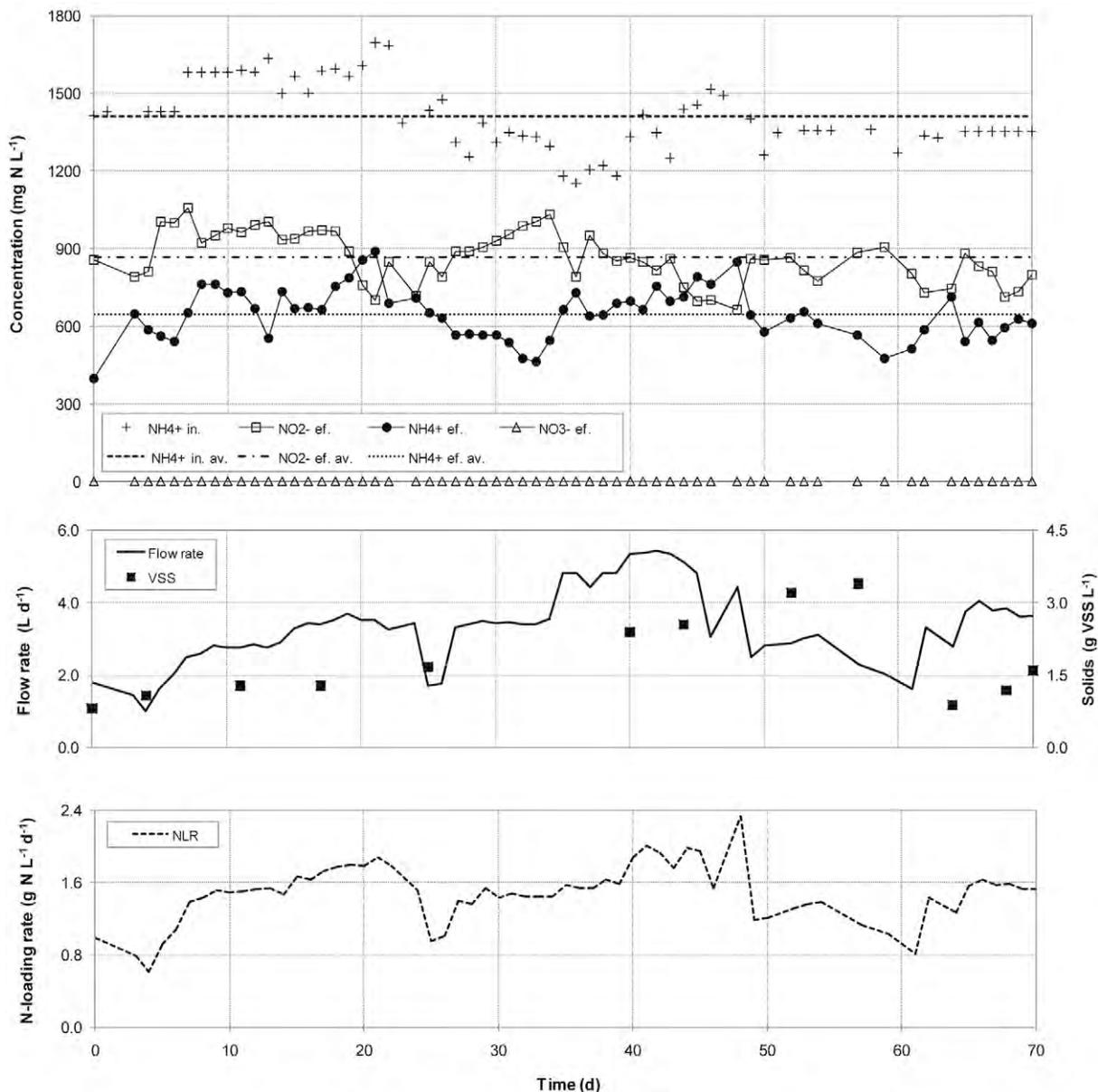


Fig. 4. Top: Concentration profiles for $\text{NH}_4^+\text{-N}$ influent and $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$ effluent. Horizontal lines are 70-d averages. Middle: Flow rate and volatile suspended solids (VSS) content inside the partial nitrification sequencing batch reactor measured at the end of an operational cycle (VSS is a measure of the nitrifying sludge concentration). Bottom: Nitrogen loading rate. NLR, nitrogen loading rate.

from 2337 to 293 $\text{mg CaCO}_3 \text{ L}^{-1}$. Concentrations of $\text{NH}_4^+\text{-N}$ in the effluent under the five aeration rates tested (1.56, 1.92, 2.41, 3.00, and 4.51 L min^{-1}) were 769 ± 41 , 523 ± 69 , 493 ± 11 , 358 ± 24 , and $611 \pm 18 \text{ mg NH}_4^+\text{-N L}^{-1}$, respectively. Corresponding $\text{NO}_2^-\text{-N}$ effluent concentrations were 280 ± 10 , 618 ± 21 , 718 ± 8 , 622 ± 17 , and $664 \pm 16 \text{ mg NO}_2^-\text{-N L}^{-1}$. Hence, FA and FNA concentrations changed significantly during Phase 2, from $170.7 \pm 9.0 \text{ mg FA-N L}^{-1}$ and $0.002 \pm 0.0001 \text{ mg FNA-N L}^{-1}$ at aeration rate of 1.56 L min^{-1} to $1.1 \pm 0.3 \text{ mg FA-N L}^{-1}$ and $0.94 \pm 0.17 \text{ mg FNA-N L}^{-1}$ at 4.51 L min^{-1} (values measured at the end of the batch cycle). These results showed that NPE can be optimized under a wide range of aeration rates.

Effect of Aeration Combined with Solids Concentration

Higher levels of biomass in the reactor (VSS) combined with lower aeration rates resulted in low DO concentrations in the bulk liquid, which limited the NPR (Table 6). For a given

concentration level of VSS, the NPR increased with increased DO. In fact, the NPR was more affected by changes in aeration rate than by changes in VSS content. On the other hand, specific NPR rates showed that the AOB activity was highly conditioned by the levels of DO. Thus, within an appropriate concentration range of VSS in the PN-SBR, the NPR attained a maximum value dependent on the aeration rate and irrespective of the biomass content. This suggests that the biomass specific activity would vary according to the aeration rate, acting as a buffer agent in the nitrifying system and providing process stability. Hence, under these tested conditions, some flexibility seems to be possible in the control of the sludge wasting rate during operation of a PN-SBR plant for SW without affecting the NO_2^- production rate.

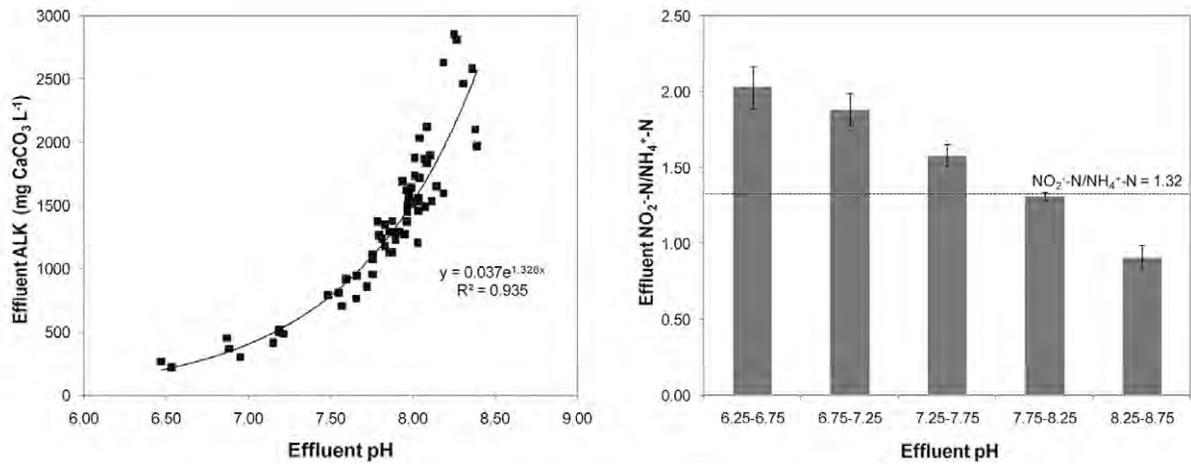


Fig. 5. Left: Relationship between effluent pH and alkalinity (ALK). Right: Relationship between effluent pH and $\text{NO}_2^- \text{-N}/\text{NH}_4^+ \text{-N}$ ratio. Error bars represent SE.

Table 5. Effect of aeration rate supplied to the partial nitrification sequencing batch reactor on the partial nitrification.

Aeration rate	NLR†	ALK‡/NH ₄ ⁺ -N influent	DO§ range	pH effluent	ALK effluent	NPE¶	NO ₂ ⁻ -N/NH ₄ ⁺ -N effluent
L min ⁻¹	g NH ₄ ⁺ -N L ⁻¹ d ⁻¹	g CaCO ₃ g ⁻¹ NH ₄ ⁺ -N	mg O ₂ L ⁻¹		mg CaCO ₃ L ⁻¹	%	g NO ₂ ⁻ -N g ⁻¹ NH ₄ ⁺ -N
1.56 (0.09)§	1.33 (0.04)#a††	4.23 (0.06)a	<0.3	8.47 (0.01)a	2337 (143)a	26 (1)a	0.37 (0.03)a
1.92 (0.06)	1.15 (0.07)ab	4.36 (0.45)a	0.3–0.5	7.71 (0.31)b	634 (208)b	59 (2)bc	1.24 (0.23)bc
2.41 (0.04)	1.40 (0.03)a	4.27 (0.13)a	0.3–0.5	7.88 (0.04)b	764 (9)b	63 (1)c	1.46 (0.04)c
3.00 (0.00)	0.97 (0.08)b	4.84 (0.39)a	0.3–1.0	6.40 (0.29)c	207 (50)c	60 (2)bc	1.76 (0.09)d
4.51 (0.02)	1.32 (0.11)a	4.20 (0.17)a	0.3–3.5	6.19 (0.10)c	293 (41)c	56 (2)b	1.09 (0.02)b

† Nitrogen loading rate.

‡ Alkalinity.

§ Dissolved oxygen (DO range describes the DO at the beginning of the first reaction phase of a cycle and at the end of the last reaction phase of a cycle).

¶ Nitrite production efficiency.

Values are averages (SE in parentheses).

†† Values in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 6. Effect of biomass solids content and aeration rate on nitrite production rate.

VSS†	Aeration rate‡	Aeration rate (vvm)	DO§	NPR¶	Specific NPR
g L ⁻¹	L _{air} min ⁻¹	L _{air} L _{liquid} ⁻¹ min ⁻¹	mg O ₂ L ⁻¹	mg NO ₂ ⁻ -N L ⁻¹ h ⁻¹	mg N g ⁻¹ VSS h ⁻¹
High level of solids (~4.0 g VSS L ⁻¹)					
4.27 (0.00)#	2.0	0.9	0.58 (0.08)a††	43.5 (2.4)ab	10.2 (0.6)a
3.92	4.0	1.7	1.16a	82.2cd	20.9a
Medium level of solids (~1.5 g VSS L ⁻¹)					
1.82 (0.00)	2.0	0.7	0.40 (0.03)a	40.0 (6.9)a	22.0 (3.8)a
1.59 (0.24)	4.0	1.5	1.15 (0.09)a	92.9 (0.1)d	59.9 (9.1)b
1.26 (0.00)	5.0	1.4	2.65 (0.43)b	104.6 (8.9)d	83.1 (7.1)c
Low level of solids (~0.5 g VSS L ⁻¹)					
0.59 (0.01)	2.0	2.0	4.77 (0.26)c	59.3 (2.4)bc	100.9 (2.5)c

† Volatile suspended solids.

‡ Aeration rate applied to a 3.5-L reactor in high and medium level of solids treatments and to a 1-L reactor in the low solids treatment.

§ Dissolved oxygen.

¶ Nitrite production rate.

Values are averages (SE in parentheses).

†† Values followed by the same letter are not significantly different.

Evaluation of the Influence of Free Ammonia and Free Nitrous Acid on the Activity of Ammonia-Oxidizing Bacteria Using Respirometric Tests

Anthonisen et al. (1976) reported that FA starts inhibiting the activity of AOB within the range of 10 to 150 mg FA L⁻¹ (8.2–123.5 mg FA-N L⁻¹). However, AOB activity can vary highly depending on its acclimatization to the presence of such

un-ionized species (Villaverde et al., 2000). In this research, the effects of FA and FNA on the activity of AOB were evaluated using short-term respirometric tests. Oxygen uptake rates were measured under different FA and FNA concentrations; three examples (out of 56 tests conducted) are depicted in Fig. 6. The maximum OUR (OUR_{max}) in these tests was measured

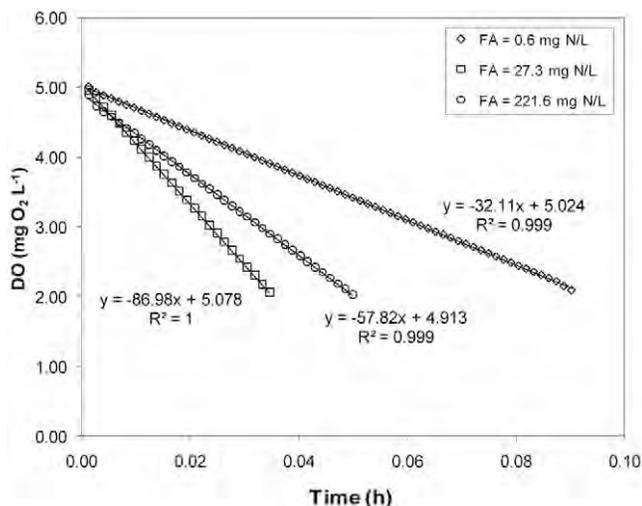


Fig. 6. Examples of oxygen uptake rate (OUR) experiments. Data show decrease of dissolved oxygen (DO) concentrations with time obtained at three concentrations of free ammonia (FA). The OUR value obtained at FA = 27.3 mg N L⁻¹ was considered as the maximum OUR in the subsequent mathematical analysis (OUR_{max} obtained after considering duplicates was 84.6 ± 2.4 mg O₂ L⁻¹ h⁻¹).

at 27.3 mg FA-N L⁻¹, with a value of 84.6 ± 2.4 mg O₂ L⁻¹ h⁻¹ (NPR = 24.7 ± 0.7 mg NO₂⁻-N L⁻¹ h⁻¹ if considering 3.43 g O₂ consumed per g NO₂⁻-N produced).

According to Fig. 7, K_{FA} was 0.76 mg FA-N L⁻¹. This value was within the range of 0.50 to 0.85 mg FA-N L⁻¹ reported in other PN systems working with high concentrations of FA (Wyffels et al., 2004; Pambrun et al., 2006). Thus, low FA concentration might become a limiting substrate only under high aeration conditions, such as those found in Phase 2 with FA-N concentrations in the PN-SBR near 1 mg L⁻¹. On the other hand, $K_{L,FA}$ was 450 mg FA-N L⁻¹ (Fig. 7). A wide range of values was found in the literature for this constant, ranging from 6 to 605 mg FA-N L⁻¹ (Carrera et al., 2004; Ganigué et al., 2007), most probably because of the different operational conditions of the cited studies. In our research, inhibition by high concentrations of FA in the PN-SBR was not an important factor. For example, considering the FA levels (average, 52.4 mg FA-N L⁻¹; maximum, 168.1 mg FA-N L⁻¹) found during Phase 1, the expected reduction on nitrifying activity according to the proposed Haldane kinetics would be only 12% in average, with a maximum of 27%.

High concentrations of FNA may also play an inhibitory role for AOB (Sharma and Ahlert, 1977). The inhibition constant for FNA was fitted to 0.78 mg FNA-N L⁻¹ (Fig. 7). This value was within the range of 0.05 to 2.04 mg FNA-N L⁻¹ found in the literature for PN systems (Pambrun et al., 2006; Van Hulle et al., 2007). Because in Phase 2 the higher levels of FNA were measured under the higher aeration rates, reversible inhibition due to FNA of up to 55% might occur (FNA inhibition quantified as 1 - 0.78/0.78 + 0.94). Although higher aeration rates resulted in higher DO levels inside the reactor, the lower pH and higher NO₂⁻-N concentrations attained may negatively affect the process conversion rate.

Anammox Reaction Using Partial Nitritation Effluent

Treatment of the nitritated wastewater effluent was demonstrated as feasible in the A-SBR during an 80-d operation period. A NLR of 1.0 g N L⁻¹ d⁻¹ was targeted in the A-SBR during the first 64 d and decreased afterward to 0.5 g N L⁻¹ d⁻¹ to match

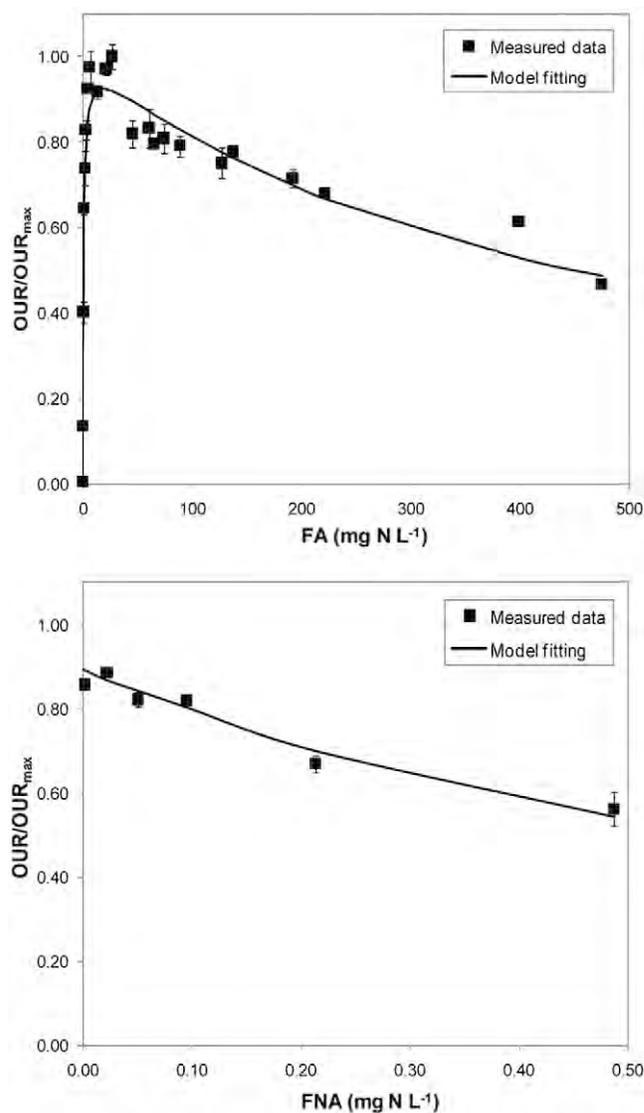


Fig. 7. Top: Influence of free ammonia (FA). Bottom: Influence of free nitrous acid (FNA) on the activity of the nitrifying sludge. Error bars represent SE. OUR, oxygen uptake rate.

the measured N conversion rate (NCR) (Fig. 8). The average NCR attained was 0.36 ± 0.002 g N L⁻¹ d⁻¹. The aforementioned change in NLR resulted in a significant enhancement of the N conversion efficiency, from 40 to 70% (Table 7). The color of the biomass changed from red when treating synthetic wastewater to brownish when using partially nitritated SW effluent. Accounting for a biomass solids content within the reactor of 0.94 ± 0.15 g VSS L⁻¹ (end of the cycle), the specific NCR was estimated to be 14.3 ± 2.2 mg N g⁻¹ VSS h⁻¹. Van Hulle et al. (2010) summarized up to 14 different studies regarding the anammox process, which provided an average value for specific N removal of 17.0 mg N g⁻¹ VSS h⁻¹ (SD, 18.9; range, 2.5–66.7). Thus, the anammox sludge NRRL B-50286 contained in the A-SBR presented a relatively good activity. Moreover, the specific NCR obtained with PN effluent (14.3 ± 2.2 mg N g⁻¹ VSS h⁻¹) was consistent with the specific NCR of the same sludge obtained at the beginning with inorganic synthetic wastewater (16.7 ± 2.2 mg N g⁻¹ VSS h⁻¹). Higher N conversion rates in the A-SBR seem to be feasible by increasing the biomass content while preventing bio-

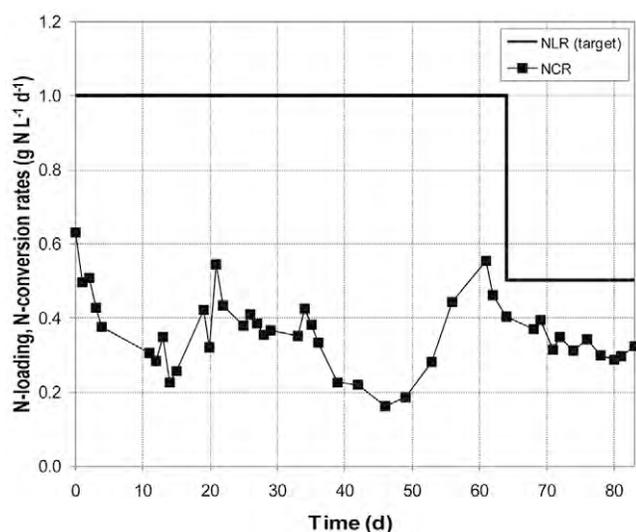


Fig. 8. Time courses of targeted N loading rate (NLR) and measured N conversion rate (NCR) in the anaerobic ammonium oxidation sequencing batch reactor treating partially nitrated swine wastewater.

Table 7. Summary of the operating conditions and performance for the anaerobic ammonium oxidation sequencing batch reactor treating partially nitrated effluent according to nitrogen loading rate.

Parameter	NLR† =	NLR =
	1.00 g N L ⁻¹ d ⁻¹	0.50 g N L ⁻¹ d ⁻¹
NH ₄ ⁺ -N influent, mg L ⁻¹	123 (6)‡	97 (3)
NO ₂ ⁻ -N influent, mg L ⁻¹	148 (2)	150 (4)
NO ₃ ⁻ -N influent, mg L ⁻¹	0 (0)	4 (1)
Inflow rate, L d ⁻¹	10.4 (0.4)	5.4 (0.3)
Hydraulic residence time, h	6.9 (0.2)	12.7 (0.6)
Volume exchange ratio, L _{exchanged} L _{end} ⁻¹	0.30 (0.01)	0.18 (0.01)
N loading rate, g N L ⁻¹ d ⁻¹	0.96 (0.03)	0.49 (0.02)
N conversion rate, g N L ⁻¹ d ⁻¹	0.37 (0.02)	0.33 (0.01)
N removal rate, g N L ⁻¹ d ⁻¹	0.35 (0.02)	0.29 (0.01)
N conversion efficiency, %	40 (2)	70 (3)
N removal efficiency, %	37 (2)	61 (2)
Reaction ratios (NH ₄ ⁺ -N/NO ₂ ⁻ -N/NO ₃ ⁻ -N/N ₂ -N)	1.00/1.32/0.15/1.08	

† Nitrogen loading rate.

‡ Values are averages (SE in parentheses).

mass losses during reactor withdraws. Reaction ratios obtained (NH₄⁺-N consumed, NO₂⁻-N consumed, NO₃⁻-N produced, and N₂-N produced) were 1.00/1.32/0.15/1.08, which are very similar to those of 1.00/1.32/0.26/1.02 proposed by Strous et al. (1998) and those of 1.00/1.30/0.18/1.06 measured by Vanotti et al. (2011b) for *Candidatus B. caroliniensis* used in this study. Because the specific activity and reaction ratios of the anammox was maintained with PN effluent, we conclude that the PN-SBR process and the corresponding effluent obtained are suitable for the treatment of SW using the anammox process.

Conclusions

Partial nitritation of SW was feasible using a HPNS in a SBR operating under a timed schedule and constant aeration. The amount of NH₄⁺ oxidized to NO₂⁻ was optimized through inflow rate control. Under steady composition of the wastewa-

ter, the pH was shown as a good indicator of PN process performance. In this study, an average NLR of 1.47 g NH₄⁺-N L⁻¹ d⁻¹ was applied at 32°C, attaining a NPR of 0.91 g NO₂⁻-N L⁻¹ d⁻¹ and producing an effluent containing 1.38 g NO₂⁻-N g⁻¹ NH₄⁺-N and 0 nitrates. Anammox treatment of the partially nitrated effluent was also demonstrated as feasible by attaining a NCR of 0.36 g N L⁻¹ d⁻¹, equivalent to a specific rate of 14.3 mg N g⁻¹ VSS h⁻¹, and reaction ratios of 1.00/1.32/0.15/1.08, showing good performance of the anammox process.

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