Upflow packed bed anammox reactor used in two-stage deammonification of sludge digester effluent

İ. Çelen-Erdem, E. S. Kurt, B. Bozçelik and B. Çallı

ABSTRACT

The sludge digester effluent taken from a full-scale municipal WWTP in Istanbul, Turkey, was successfully deammonified using a lab-scale two-stage Partial Nitritation (PN)/Anammox (A) process and a maximum nitrogen removal rate of 1.02 kg N/m^3/d was achieved. In the PN reactor, 56.8 ± 4% of the influent NH_4-N was oxidized to NO_2-N and the effluent nitrate concentration was kept below 1 mg/L with 0.5–0.7 mg/L of dissolved oxygen and pH of 7.12 ± 12 at 24 ± 4 °C. The effluent of the PN reactor was fed to an upflow packed bed anammox reactor where high removal efficiency was achieved with NO_2-N:NH_4-N and NO_3-N:NH_4-N ratios of 1.32 ± 0.19:1 and 0.22 ± 0.10:1, respectively. The results show that NH_4-N removal efficiency up to 98.7 ± 2.4% and total nitrogen removal of 87.7 ± 6.5% were achieved.

Key words | anammox, digester effluent, nitrogen removal rate, partial nitritation, side-stream

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INTRODUCTION

In many wastewater treatment plants with anaerobic sludge digestion, nitrogen removal from sludge digester effluents in side stream processes is very important with the introduction of more stringent standards for nutrient removal. Since the effluent flows from sludge treatment are about 15–20% of the influent nitrogen load and high ammonium content (500–1,500 mg/L), separate treatment of the digester supernatant would significantly reduce the N load of the main stream and improve nitrogen elimination (Fux *et al.* 2002; Kampschreur *et al.* 2008).

Thus, side-stream biological treatment processes for sludge digester effluent in order to reduce the N loading to the wastewater treatment plant have been suggested by several researchers (Ahn & Choi 2006; Ma *et al.* 2011). The anaerobic ammonium oxidation (Annamox) process is one of the most promising options to improve the energy and cost efficiency compared to the conventional nitrification and denitrification processes. It is a biological process capable of anaerobic transformation of NH_4^+ to dinitrogen (N_2) gas with NO_2 as an electron accepter (Kartal *et al.* 2013). However, in typical industrial wastewater, nitrogen exists in the form of NH_4^+. Therefore, partial nitritation (PN) of ammonium is needed to achieve an effluent of NO_2: NH_4 molar ratio of 1.31 suitable for a subsequent Anammox process (Soliman & Eldyasti 2016). According to Wang *et al.* (2017), anaerobic sludge digester effluent, with high alkalinity (average 1,982 mg/L), temperature (28–30 °C) and low biodegrable compounds, provides favorable conditions for nitritation. Moreover, the recommended control strategies for obtaining AOB enrichment for PN of influent ammonium are mainly focused on high pH and temperature, dissolved oxygen (DO) concentration, real-time aeration control, high free ammonia (FA) and high free nitrous acid (FNA) concentrations, sludge retention time, substrate concentration and inhibitors (Jianlong & Ning 2004; Ge *et al.* 2015; Bao *et al.* 2017).

The major challenge encountered with the application of sludge digester effluent by the PN/Anammox process is the very low growth rates of Anammox bacteria (the average doubling time is 11 days) (Strous *et al.* 1998). Numerous studies have reported that the start-up of the Anammox process in the bioreactor is usually time-consuming and may take from months to years (Ibrahim *et al.* 2015).

The objective of the study was to investigate the treatment of ammonium-rich anaerobic sludge digester effluent with a two-stage partial nitritation/anammox (PN/A) process. A completely stirred PN reactor and an upflow packed bed anammox (UPBAn) reactor were operated in series to determine the optimum operating conditions and to evaluate the total nitrogen removal performance of the system. The novelty of the study lies in the fact that i) the digester effluent of a

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400,000 m^3/d central wastewater treatment plant (WWTP) receiving both municipal and industrial wastewater was used as feed and ii) both the PN and UPBAn reactors were inoculated with the activated sludge taken from a biological nutrient removing WWTP instead of specific seeds.

MATERIAL METHODS

Ambarli Municipal WWTP is one of the largest plants in Istanbul, Turkey, with a capacity of 400,000 m^3/day, and serves 1.6 million population equivalent. It was designed as a biological nutrient removal system according to ATV-DVWK Standards (ATV 131) and has been under operation since 2012. It receives wastewater from both domestic and industrial sources. The design COD, TN and TP loads are 190, 20 and 2.8 tons/day, respectively. It is operated with a total nitrogen discharge limit of 10 mg/l. Primary and secondary sludges generated are dewatered mechanically by centrifugation and then digested in anaerobic sludge digesters. The digested sludge is dewatered by centrifugation up to 25% dry matter and the liquid fraction (digester effluent) is returned back to the inlet of the WWTP. Because of the extra TN load from the side stream, sometimes difficulties are encountered in meeting the TN discharge limit.

Experimental set-up

The liquid fraction of the digestate (which is hereafter called sludge digester effluent) used in this study is taken from Ambarli WWTP in Istanbul, Turkey. The schematic of the laboratory scale experimental set-up consisting of nitritation and anammox reactors is shown in Figure 1. The set-up was operated by feeding the sludge digester effluent to the nitritation reactor and then the partially nitritated digester effluent to the anammox reactor.

Nitritation experiment

The nitritation experiment was performed in a continuously fed and stirred 25-L laboratory scale reactor (20-L active volume) operated with sludge recycle. To stir the reactor, a mechanical mixer (Siemens, S1FL6, Germany) was used. Air was supplied with a fine bubble diffuser placed in the bottom of the reactor to provide the oxygen needed for nitritation. The DO concentration was kept between 0.5 and 0.8 mg/L by monitoring the DO on line (Hamilton 211, USA) and controlling the air flowrate. The reactor was inoculated with 10 L of activated sludge taken from the sludge recycling line of Ambarli WWTP. The MLSS and MLVSS concentrations of the inoculum were 6,800 mg/l and 4,500 mg/L, respectively. The temperature of the reactor was controlled by resistance.

The nitritation experiment was performed in two phases; enrichment, suppression of nitrate formation, and supplying ammonium and nitrite sources for the Anammox reactor. In all phases, the sludge digester effluent of Ambarli WWTP was used as the feed.

In the enrichment phase, the nitritation reactor was initially operated at 35 ± 1 °C. DO and pH were 0.8 mg/L and 7.8 ± 0.05 respectively. The SRT was set at 10 days by withdrawing the excess sludge when necessary. To increase the FA concentration and to suppress the nitrite oxidizing

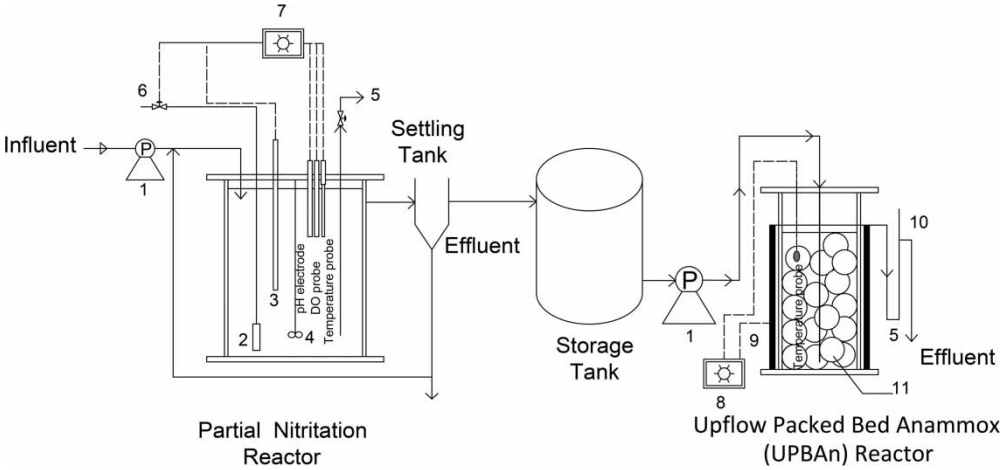

Figure 1 | The schematic drawing of two-stage deammonification process. (1) Pump, (2) Diffuser, (3) Resistance, (4) Mixer, (5) Sampling point, (6) Air, (7) Control panel, (8) Temperature control, (9) Heating mat (10) Siphon breaker, (11) Packing materials.

bacteria (NOB), pH was kept at 7.8 by adding $NaHCO_3$ externally. Between days 19 and 55, the temperature, DO and pH in the reactor decreased to 32 ± 1 °C, 0.5 mg/L and 7.5, respectively.

After the enrichment of ammonium oxidizing bacteria (AOB), the nitritation reactor was operated to inhibit NOB as much as possible between days 55 and 93. In this stage, the temperature and DO decreased further to 30 ± 2 °C and 0.4 mg/L, respectively. pH remained stable at 7.50 ±0.05. Throughout the experiment, the HRT was kept constant at 1 day to wash further out the slow growing NOB from the reactor. SRT was raised from 10 days to 30 ± 10 days to increase the MLSS and MLVSS mg/L concentrations.

Between day 94 and day 310, the nitritation reactor was operated to supply ammonium and nitrite source for the Anammox reactor. In the second stage, pH and temperature of the reactor were monitored on-line but not controlled. pH was 7.25 ± 0.05 and the DO was kept in the range of 0.5 and 0.7 mg/L. The alkalinity required was provided by the digester effluent. The reactor was operated at room temperature at 24 ± 2 °C.

To evaluate the performace of the PN process, the Ammonia Removal Efficiency (ARE) and Nitrite Accumulation Ratio (NAR) were calculated according to (Equations (1) and (2) (Soliman & Eldyasti 2016)

$$\text{ARE, \%} = \frac{(NH_4 - N)inf - (NH_4 - N)eff}{(NH_4 - N)inf} \times 100 \quad (1)$$

$$\text{NAR, \%} = \frac{(NO_2 - N)eff}{(NO_2 - N)eff + (NO_3 - N)eff} \times 100 \quad (2)$$

Anaerobic ammonium oxidation experiment

The anaerobic ammonium oxidation experiment was performed in a 4.8-L glass upflow reactor filled with packing materials. The UPBAn reactor was inoculated with 1 L of activated sludge taken from the sludge recycling line of Paşaköy WWTP in Istanbul, Turkey. The MLSS and MLVSS concentrations of the inoculum were 12,040 mg/l and 10,160 mg/L, respectively. The UPBAn reactor was fed with the effluent of the nitritation reactor (partially nitritated digester effluent) which has a nitrite nitrogen to ammonium nitrogen ratio of 1.32 ± 0.19. The effluent of the nitritation reactor was collected in a storage tank and deoxygenated there by flushing with N_2 gas. It is continuously fed to an UPBAn reactor under anoxic conditions with a peristaltic pump. Initially the HRT was 6 days and then gradually decreased to 0.6 days depending on the high Nitrogen Removal Efficiency (NRE). The pH of the influent was usually about 7, therefore no pH adjustment was required in the reactor. The UPBAn reactor was operated at 35 ± 1 °C by using a temperature controlled heating mat wrapped around the reactor.

The NO_2^--N consumed to NH_4^+-N oxidized and NO_3^--N generated to NH_4^+-N oxidized ratios were calculated compared with the theoretical values estimated according to Equation (3). Stoichiometrically for each mole of NH_4^+-N oxidized 1.32 mole of NO_2^--N is consumed and 0.26 mole of NO_3^--N is generated (Strous *et al.* 1998).

$$NH_4^+ + 1.32\,NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O + 0.66CH_2O_{0.5}N_{0.15} \quad (3)$$

To evaluate the performace of anaerobic ammonium oxidation Nitrogen Loading Rate (NLR), Nitrogen Removal Rate (NRR) and NRE were calculated according to Equations (4)–(6).

$$\text{NLR, kg/m}^3 \cdot \text{d} = \frac{(NH_4 - N + NO_2 - N)\,\text{inf} * \text{Flow Rate}}{Reactor\ \ Volume} \quad (4)$$

$$\text{NRR, kg/m}^3 \cdot \text{d} = \frac{(NH_4 - N + NO_2 - N)\,\text{inf} - (NH_4 - N + NO_2 - N - NO_3 - N)eff}{HRT} \quad (5)$$

$$\text{NRE, \%} = \frac{NRR}{NLR} * 100 \quad (6)$$

In addition to calculated NRE and stoichiometric ratios, the effluent pH of the UPBAn reactor was used as a parameter to monitor the performance of anaerobic ammonium oxidation (Jin *et al.* 2013). Because anaerobic ammonium oxidation is a proton consuming process, the effluent pH has to be higher than the influent (van de Graaf *et al.* 1996).

Characteristics of sludge digester effluent

The physical and chemical characteristics of the sludge digester effluent of Ambarli WWTP are shown in Table 1. The supernatant samples were collected weekly and stored refrigerated before analyses. The characterized samples were used in feeding the laboratory scale PN reactor whose effluent was fed to the laboratory scale UPBAn reactor.

Table 1 | The characteristics of sludge digester effluent used in the sudy

Parameters	
sCOD (mg/L)	236 ± 59.2
BOD_5 (mg/L)	83 ± 13.2
$NH4^+$-N (mg/L)	551 ± 108
PO4 (mg/L)	260 ± 134.6
Total suspended solids (mg/L)	193± 86
Volatile suspended solids (mg/L)	122 ± 47.3
pH	7.50 ± 0.14
Conductivity (μs/cm)	5,710 ± 1,708
Alkalinity (mg/L)	1,982 ± 347

Sample number = 130.

Analytical methods

Periodically, samples were taken from the influent and effluent of both the nitritation reactor and UPBAn reactor. All samples were filtered through 0.45 μm filters before the analyses. COD, BOD_5, MLSS, MLVSS, total alkalinity and phosphate analyses were carried out according to Standard Methods (APHA 2005). For the analysis of NH_4-N (LCK 303), NO_2-N (LCK 342), and NO_3-N (LCK 339), cuvette test kits (Hach Lange GmbH, Germany) and a spectrophotometer (Dr 2800, Hach Lang) were used. The COD concentrations were corrected according to nitrite values (1.1 g COD/g NO_2). The pH, DO and temperature was measured with a multimeter (Hach-Lange LDO meter, Dusseldorf, Germany). The FA and FNA concentrations in the reactor were calculated according to Equations (7) and (8) proposed by Anthonisen *et al.* (1976).

$$\text{FA, mg } NH_3 - N/L = \frac{[NH_4^+ - N]10^{pH}}{e^{(6344/T+273)} + 10^{pH}} \quad (7)$$

$$\text{FNA, mg } HNO_2 - N/L = \frac{[NO_2^- - N]10^{-pH}}{e^{(-2300/T+273)} + 10^{-pH}} \quad (8)$$

RESULTS AND DISCUSSION

PN reactor

Phase I: Enrichment and suppression of nitrate formation

Between days 1 and 19, influent ammonia concentration was 651.70 ± 72.69 mg/L, corresponding to an NLR of 0.68 ± 0.16 kg N/m^3/day. The average NRR was 0.25 ± 0.19 kg N/m^3/day due to the high rate of nitrification of inoculation sludge taken from the aeration line. After day 19, the activity of NOB was suppressed by the synergistic effect of FA (3.84–12.54 mg/L) and FNA (0.01–0.03 mg/L) (Figure 2(b)). Since the growth rate of AOB is less affected than that of NOB under an oxygen limitation condition (Wyffels *et al.* 2004), the DO was decreased from 0.8 mg/L to 0.5 mg/L on days 19 – 55. This provided an advantage for the growth of AOB rather than NOB.

Between days 55 and 93, the temperature was decreased to 30 ± 2 °C. DO and pH remained stable at 0.4 mg/L and 7.50 ± 0.05, respectively. Influent ammonia concentration was 617.17 ± 65.12 mg NH_4-N/L, corresponding to an NLR of 0.65 ± 0.13 kg N/m^3/day, and accordingly the effluent NO_3-N concentration decreased from 122 mg/L to below 34.7 mg/L. FA and FNA concentrations in the PN reactor were below inhibitory levels for AOB but high enough to suppress NOB activity. The low nitrate concentrations (<1 mg/L) in the last part of this phase indicate that the NOB activity in the PN reactor ceased almost completely and NOB were competed out by AOB (Figure 2(a)).

Phase II: Feed for anammox reactor

After day 94, the PN reactor was operated to obtain an NH_4:NO_2 molar ratio of 1.32 (Strous *et al.* 1998) in the feed of the Anammox phase. pH remained stable at 7.25 ± 0.05. The temperature was room temperature (24 °C), and DO was between 0.5–0.7 mg/L. The NAR, which was 99.6%, also remained constant indicating the capability of the system to maintain a successful PN treating variable nitrogen loads in digester effluent.

As a result, the influent NH_4-N was partially oxidized to NO_2-N (56.8 ± 4%) with low nitrate concentrations (<1 mg/L) (Figure 2(a)–(c)) in the PN reactor. This suggests that a stable Anammox-suited effluent was obtained by the PN process in this phase. Moreover, Wang *et al.* (2017) reported that inhibition of NOB was observed at FA concentrations from 1.7 to 8.4 mg/L and FNA concentrations from 0.02 to 1 mg/L. Similarly, in this work at this phase the inhibition of NOB can be attributed to the combined effect of FA (4.48–8.40) mg/L and FNA (0.02) mg/L (Figure 2(b)).

Anammox reactor

The anaerobic AOB existing in the activated sludge inoculum was successfully enriched within 60 days before starting this study (under preparation). After enrichment of

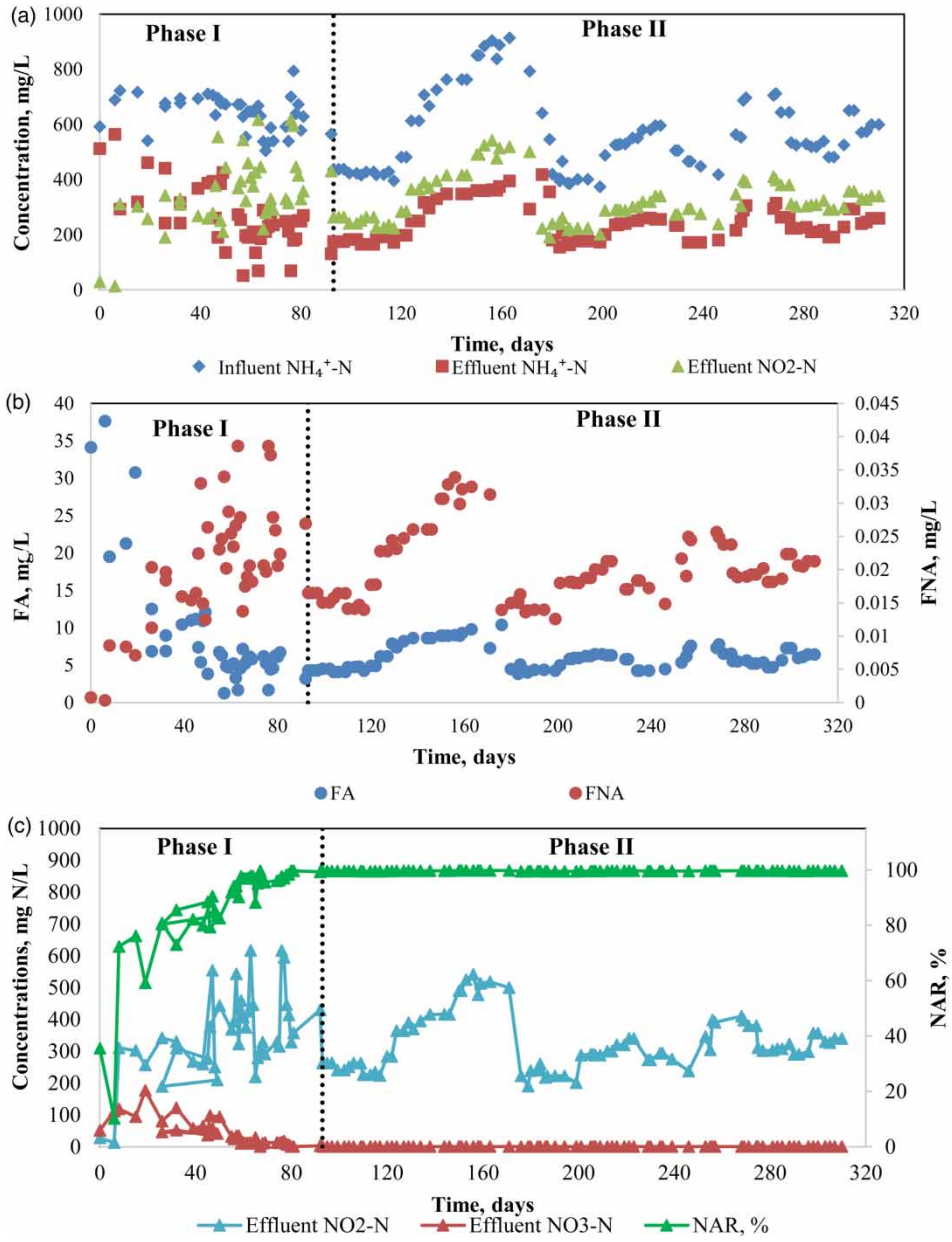

Figure 2 | (a) Ammonium conversion in the nitritation reactor with continuous operation; (b) time course of FA and free nitrite acid in the nitritation reactor; (c) NO_2-N and NO_3-N concentrations in the effluent and NAR (%).

the Anammox bacteria, the UPBAn reactor was operated for about 280 days to deammonify the sludge digester effluent that was partially nitritated in the PN reactor. The effluent of the PN reactor was fed to the UPBAn reactor in a $1{:}1.32 \pm 0.19$ $NH_4{:}NO_2$ ratio. The deammonification performance of the UPBAn reactor was monitored based on NRE, $NO_2\text{-}N_{consumed}/NH_4\text{-}N_{consumed}$ and $NO_3\text{-}N_{produced}/NH_4\text{-}N_{consumed}$ ratios and effluent pH. The results are presented in Figures 3(a) and 3(b), 4 and 5.

Nitrogen removal performance

In the first 55 days, the UPBAn reactor was operated with an NLR of 0.06 kg N/m^3/day. Between days 23 and 56, the considerably high effluent NO_2-N concentrations (37.5 ± 10.4 mg/l) have resulted from NO_2-N/NH_4-N ratios of over 1.6 in the feed (PN reactor effluent) because of a problem in the PN reactor. After this period, the NLR was progressively increased from 0.06 to 0.36 kg N/m^3/d. On day 65,

the total NRE reached approximately 91%. On day 85, the DO concentration unintentionally increased to 0.15 mg/L in the reactor. Accordingly, the NH_4-N concentration reduced from 19.8 mg/L to 3.3 mg/L and NO_2-N concentrations rose up to 36.5 from 0.44 mg/L. It was speculated that the elevated DO concentration stimulated the AOB existing in the UPBAn reactor and resulted in nitritation of a portion of the incoming NH_4-N to NO_2-N (Figure 3(a)). Accordingly, the NRE decreased from 93% to 88% and pH was increased to 8.0 from 7.85. To prevent the oxygen intrusion, the effluent of the PN reactor stored in the feed tank was flushed with N_2 gas starting from day 140 and the influent DO concentration was consistently kept below 0.05 mg/L. On day 115, the NLR increased back to 0.34 kg N/m^3/d and until day 203 fluctuated between 0.32 and 0.49 kg N/m^3/d depending on the treatment performance of the PN reactor. In this period, the effluent NH_4-N concentration was always quite low, but a few times the effluent NO_2-N concetration exceeded 20 mg/l.

On day 206, the NLR increased to 0.68 kg N/m^3/d and until the end of the study kept at 0.74 ± 0.11 kg N/m^3/d. In this period, the maximum NRR achieved was 1.02 kg N/m^3/d and the average was 0.68 ± 0.11 kg N/m^3/d (Figure 3(b)). This NRR was lower than the NRR of 1.23 kg N/m^3/d reported by Wang *et al.* (2017). They achieved this NRR using a two-phase partial nitrification-Anammox (PN/A) reactor fed with sludge digester effluent. The NRR we obtained was similar to the NRR of 0.60 ± 0.0 kg N/m^3/d reported by Fux *et al.* (2002) and lower than the 0.75 kg N/m^3/d reported by van Dongen *et al.* (2001). They obtained these results using a two-phase partial nitrification-Anammox (PN/A) reactor fed with sludge digester effluent.

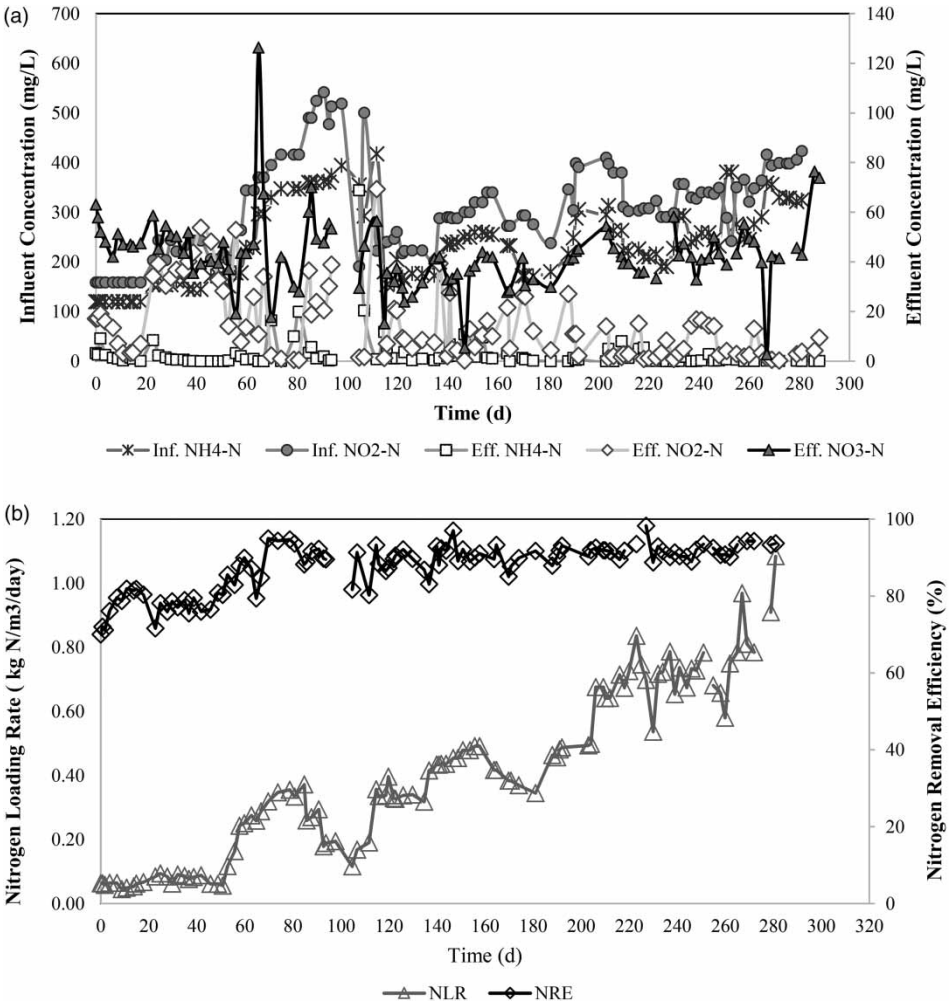

Figure 3 | (a) Profiles of influent ammonium, nitrite nitrogen concentrations, effluent nitrite, nitrate and ammonium nitrogen concentrations in Anammox reactor. (b) Profiles of NLR and NRE in the Anammox reactor.

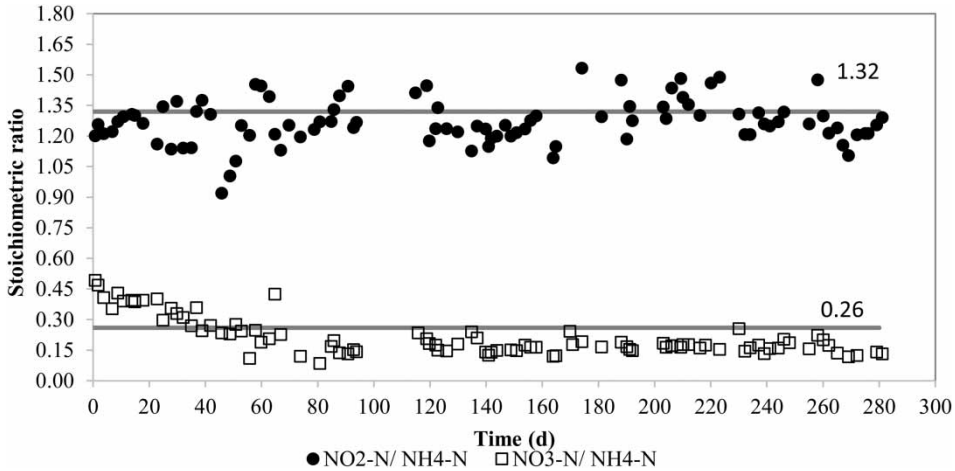

Figure 4 | The evolution of the stoichiometric ratios in the effluent.

In this work, NH_4-N removal efficiency of up to 98.7 ± 2.4% and to a total NRE of 87.7 ± 6.5% was achieved. Dosta *et al.* (2015) reported total NRE of 88.1% from the lab-scale of the combined PN and Anammox treatment. Similarly, over 90% NRE was achieved in a full scale plant (Joss *et al.* 2009) and 84% on average in a pilot plant for sludge digester effluent treatment by PN/Anammox (Gut *et al.* 2006).

Mass balances for ammonium, nitrite and nitrate have been used to determine the anammox activity. The NO_2-$N_{consumed}$/NH_4-$N_{consumed}$ and NO_3-$N_{produced}$/$NH_4N_{consumed}$ ratios achieved in the UPBAn reactor are shown as a function of nitrogen conversion in Figure 4. The average value for NO_2^-/NH_4^+ is 1.27 ± 0.11 and the NO_3^-/NH_4^+ is 0.22 ± 0.10, which is a ratio very close to the Anammox stoichiometry reported by Van Dongen *et al.* (2001) and Dosta *et al.* (2015). Strous *et al.* (1998) found that the nitrite and ammonium was 1.32; nitrate and ammonium was 0.26 in a sequencing batch reactor (SBR) treating synthetic wastewater. The variation might be due to the microbiological structure under the real digester effluent conditions. Fux *et al.* (2002) found an NO_2/NH_4^+ ratio of 1.3 in the Anammox reactor effluent but a substantial amount of the produced nitrate was denitrified by heterotrophs in the Anammox reactor.

As shown in Figure 5, the effluent pH of the UPBAn reactor was always higher than the influent pH. Throughout the study, the influent pH was in the range of 6.5 and 7.5 and on average 6.99 ± 0.22. Depending on the influent pH, the effluent pH fluctuated between 7.3 and 8.1 and after day 181, when the NRR reached maximum, the effluent pH was stabilized at about 7.73 ± 0.2. In the meantime, the influent pH was 7.08 ± 0.15.

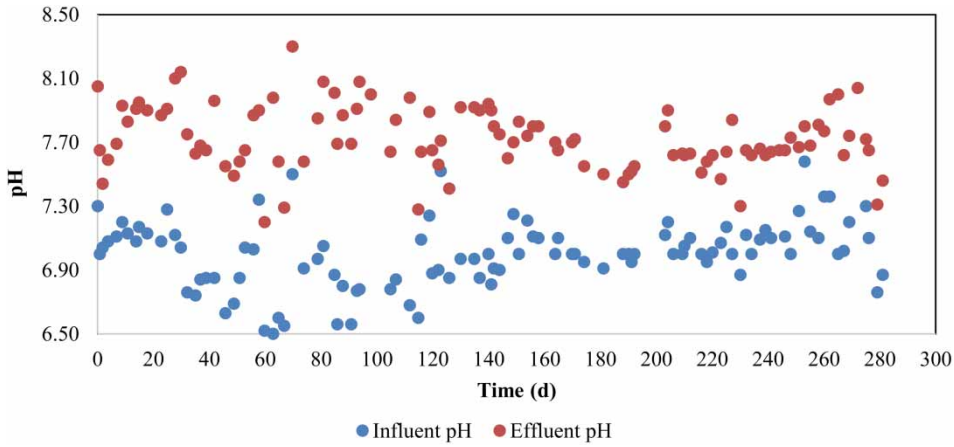

Figure 5 | The influent and effluent pH values.

The optimum pH in the UPBAn was determined to be in between 7.6–7.9. Rosenthal *et al.* (2009) utilized an SBR for the Anammox proceses. The feed to the SBR was anaerobic digester effluent from the Ward's Island Water Pollution Control Plant (New York city, NY, USA) that was partially nitritated in a preceding bench scale reactor. According to their findings, the optimum pH interval was between 7.5–7.8, which is very close to the pH value determined in this work.

CONCLUSION

The anaerobic sludge digester effluent of a central WWTP was successfully deammonified in a laboratory scale two-stage system consisting of a completely stirred PN reactor and an UPBAn reactor. Both reactors were inoculated with the activated sludge taken from a biological nutrient removing WWTP instead of specific PN and Anammox seeds. After the enrichment phase, reactors were operated in series for about 200 days and $98.7 \pm 2.4\%$ of NH_4-N removal, $87.7 \pm 6.5\%$ of TN removal and $1.02\ kg\ N/m^3/d$ of maximum NRR were achieved. The NO_2-N:NH_4-N and NO_3-N:NH_4-N ratios in the effluent were 1.32 ± 0.19:1 and 0.22 ± 0.10:1, respectively.

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