



A pilot-scale study on the partial nitrification-anammox process for treatment of anaerobic sludge digester effluent

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ABSTRACT

The treatment of the sludge digester effluent taken from a full-scale municipal wastewater treatment plant (WWTP), in Istanbul, Turkey using a pilot-scale partial nitrification (PN)/anammox (A) reactor was examined and maximum nitrogen removal rate (NRR) of 0.65 kg N/m³/d was achieved in this study. The PN, which was carried out in a continuously stirred tank reactor, successfully converted 56.4±4.8% of NH₄⁺-N to NO₂⁻-N. The effluent NO₃⁻-N concentration kept below 1 mg/L with 0.4–0.8 mg/L of dissolved oxygen (DO) and pH of 7.2±0.1 at 25±3.3°C. Partially nitrified sludge digester effluent was further treated in a continuously stirred tank (CSTAn) anammox reactor, where high ammonium removal efficiency (ARE) achieved with a NO₂⁻-N:NH₄⁺-N and NO₃⁻-N:NH₄⁺-N ratios of 1.28±0.06:1 and 0.29±0.07:1, respectively. The results show that ARE up to 94±0.05% and total nitrogen removal of 75.6±12.6% were achieved. This study also illustrated that in CSTAn reactor, better NRR achieved when pH and DO were 7.72±0.04; 0.02±0.02 mg/L, respectively. However, the NRR was tending to rise below 10 mg/L of NO₂⁻-N. The biomass concentration in the CSTAn reactor was 1075±330 g VSS/L with the SAA 0.38±0.06 kgN/kgVSS/d in the whole study. The anammox activity increased exponentially and correlated with the observed growth in volatile suspended solids.

Keywords: Partial nitrification; Anammox; Sludge digester effluent; Nitrogen removal rate; Anammox activity

1. Introduction

In most of the wastewater treatment operational practices with anaerobic sludge digestion, the ammonia rich centrate (500–1500 mg/L) is recycled back to the main treatment line which increases the NH₄⁺-N loading to the treatment by as much as 10–30% [1,2]. Therefore, separate treatment of the ammonium rich digester supernatant have been suggested by several researchers in order to reduce N loading to the wastewater treatment plant [2–4]. Consequently, the wastewater treatment plant can be operated economically. Anaerobic ammonium oxidation (ANAMMOX) process is one of the most promising options due to the several advantages comparing to the conventional nitrification and denitrification processes: (a) aeration energy

will be saved as only 57% of NH₄⁺-N need to be oxidized to NO₂⁻-N [5]; (b) organic carbon source is not needed during the nitrogen removal process; (c) high potential removal efficiency, high process stability and reliability, low area requirement and moderate cost [6] (d) Anammox bacteria is not a producer of N₂O [7] and it can utilize CO₂ as a carbon source [8], so that less greenhouse gas was released from anammox reactor. However, the application of the anammox process is restricted by some inhibition compounds existing in nitrogen-rich wastewater. A variety of inhibitory substances, such as substrates (ammonia, nitrite), organic matter, salts, phosphate, sulfide and heavy metals [9,10] are commonly present in practical applications.

In typical industrial wastewater, nitrogen exists in the form of NH₄⁺-N. However, anammox process is dependent of availability of nitrite, which is not common in real waste-

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waters. Therefore, nitrite can be generated by the partial nitritation (PN) of ammonium [11]. According to [12], the anaerobic sludge digester effluent with high alkalinity, temperature (28–30°C) and low biodegradable compounds provide favorable conditions for nitritation.

Moreover, various parameters including pH and temperature, dissolved oxygen concentration, real-time aeration control, high free ammonia (FA) and high free nitrous acid (FNA) concentrations, sludge retention time (SRT), substrate concentration and inhibitors are controlled to induce nitrite accumulation [13,14].

The PN/Anammox process can be performed in either two stage or single stage reactors. A one-reactor system is simple in configuration but it requires very tight control in pH range and oxygen concentration [15]. However, in a two-reactor system, optimum conditions can be provided individually which reduces the inhibition problems [4,16]. Also the two-reactor system is the most robust, which may shorten the recovery time after possible system upset such as toxic substances in the feed [15].

In this project, ammonium treatment of a side stream effluent, which was returned to the main water line in a full-scale wastewater treatment plant, was investigated in laboratory and pilot scale experiments by two-stage partial nitritation-anammox process for treatment of anaerobic sludge digester effluent. The aim of the study was scaling up the laboratory PN/A reactors directly to pilot-scale. The novelty of the study is the first pilot-scale PN/A reactor has been taken into operation with real wastewater at the sludge treatment plant Ambarlı, İstanbul, TR. This research is expected to provide the basis for full-scale sludge digester effluent treatment.

The main objectives of this work were to:

- Determine the level of DO concentration, hydraulic retention time (HRT) and pH in the PN reactor
- Study the nitrogen removal performance of anaerobic sludge digester effluent treatment in a CSTAn reactor.
- Evaluate the anammox activities in the CSTAn reactor based on total NRE, stoichiometric ratios, and the biomass concentration.
- Determine the impact of nitrite concentration on NRR in the CSTAn reactor.

2. Materials and methods

Ambarlı municipal wastewater treatment plant (WWTP) is one of the largest plants in İstanbul, Turkey with a capacity of 400.000 m³/d and serves 1.6 million population equivalent. It was designed as a biological nutrient removal system according to ATV-DVWK Standards (ATV 131) and is under operation since 2012. The wastewater treatment plant is located near an industrial zone that includes food, textile, automotive, paper and plastic industries. Therefore, it receives wastewater from both domestic and industrial sources. The digested sludge is dewatered by centrifugation up to 25% dry matter and the digester effluent is returned back to the inlet of Ambarlı WWTP with 4200 m³/d. 11.5% of the nitrogen load and 1.05% hydraulic load were recirculated to the main stream with the return digester effluent from dewatering.

2.1. Flow scheme and reactor configurations

The liquid fraction of digestate (which is hereafter called sludge digester effluent) used in this study is taken from Ambarlı WWTP in İstanbul, Turkey. The flow scheme and reactor configuration of this work is shown in Fig. 1.

After physical precipitation in the sludge digester effluent storage tank, the sludge digester effluent was fed into the biological treatment process. The effluent from the PN process overflowed into the *PN effluent storage tank* and was then pumped to the anammox reactor where nitrogen was removed autotrophically. In order to retain the sludge escaping from reactors, 50 L settling tanks were placed to the outlet of PN and CSTAn reactors. The sludge accumulation in the settling tanks was regularly recycled to the reactors. Thus throughout the operation, no sludge was deliberately removed from the reactors.

All containment vessels were made of propylene material, including the sludge digester effluent storage tank, PN reactor, PN effluent settling tank, PN effluent storage tank, anammox reactor and anammox settling tank. Storage tanks, which were sludge digester effluent tank and partial nitritation effluent tank, had an effective volume 4 m³, with a water height and internal diameter 2 m and 1.6 m, respectively.

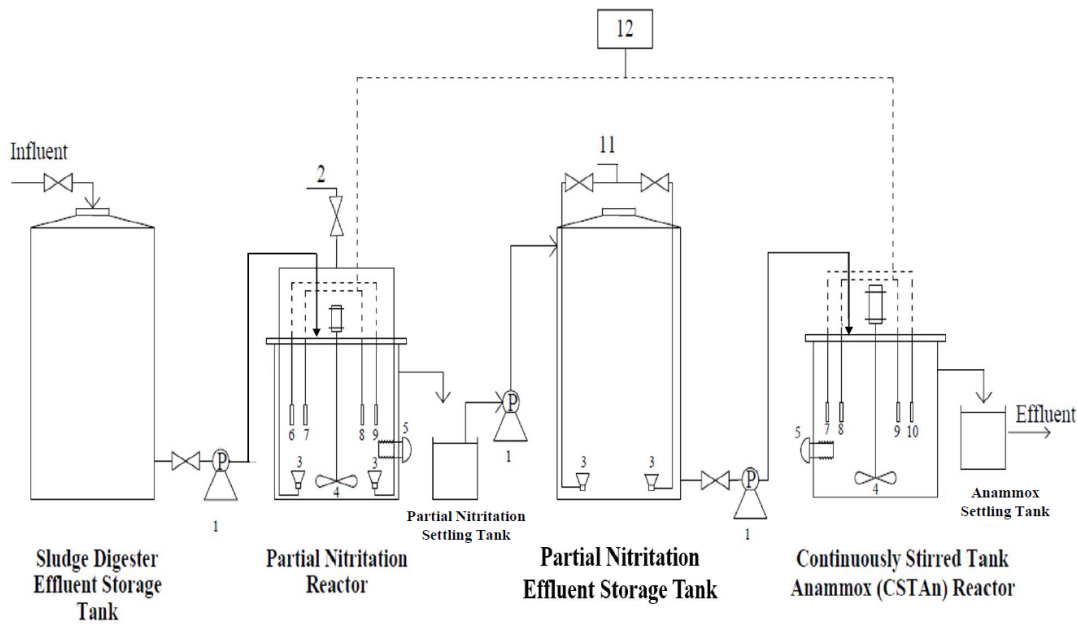
The wastewater inlet to PN, PN effluent storage tank and CSTAn reactor, was provided by diaphragm pumps (Seko Tekna EVO, AKL 803, UK).

There were NH₄⁺-N/NO₃⁻-N, pH, dissolved oxygen and temperature probe in the PN reactor; pH, dissolved oxygen, conductivity, oxidation reduction potential and temperature probe (Hach Lange, Germany) in the CSTAn reactor for system controls. Sensor values of reactors were recorded in the automation system via the Hach Lange SC-1000 controller with 1 in 10 min. The probes were controlled daily and calibrated when they are required.

Based on 5 L laboratory scale experiments with PN/A process (under preparation) on sludge digester effluent from Ambarlı WWTP, the reactors were directly scaled-up to pilot scale.

2.1.1. Nitritation experiment

The nitritation experiment was realized in a CST reactor of 0.5 m³ capacity with a water height and internal diameter of 0.83 m and 0.88 m, respectively. The pH, NH₄⁺-N/NO₃⁻-N (Hach Lange, Germany) and temperature of the reactor were monitored on-line but not controlled. The dissolved oxygen (DO) concentration kept between 0.4 and 0.8 mg/L by monitoring the DO on-line and controlling the air flowrate at 6 m³/h. Two membrane type diffusers (Aquaflux Add230-9") at the bottom of the PN tank were installed for air supply. Paddle type mixers (Gamak, AGM2E 90 L 6, Turkey) were used in PN and CSTAn reactor to provide homogeneous sludge mixture and to avoid bacterial damage. The sludges in the tanks were mixed at a speed of about 200 rpm. pH fluctuated between 7.0 and 7.3. The alkalinity required was provided by the digester effluent. The reactor was operated at room temperature at 25±3.3°C. The PN reactor was inoculated with the 300 L of activated sludge taken from İstanbul-Ambarlı WWTP for the acclimation and enrichment of ammonia oxidizing bacteria (AOB).



(1) Pump, (2) Air, (3) Diffuser, (4) Mixer, (5) Resistance, (6) $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ probe, (7) pH probe, (8) DO probe, (9) Temperature probe, (10) Conductivity probe, (11) $\text{N}_2/\text{CO}_2(\text{g})$, (12) Control panel

Fig. 1. Pilot plant flow diagram.

In typical municipal wastewater, nitrogen exists in the form of NH_4^+ . Therefore, partial nitritation of ammonium is needed to achieve an effluent of $\text{NO}_2^-\text{:NH}_4$ molar ratio of 1.32 suitable for a subsequent Anammox process. During partial nitritation, physiological differences between ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) are very important. Nitrite accumulation would occur under low dissolved oxygen because AOB have a higher affinity for oxygen than NOB [11,16].

To evaluate the performance of PN process the ammonia removal efficiency (ARE) and nitrite accumulation ratio (NAR) were calculated according to Eqs. (1) and (2) [11].

$$\text{ARE, \%} = \frac{(\text{NH}_4 - \text{N})_{\text{inf}} - (\text{NH}_4 - \text{N})_{\text{eff}}}{(\text{NH}_4 - \text{N})_{\text{inf}}} \times 100 \quad (1)$$

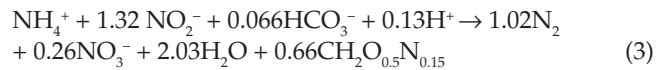
$$\text{NAR, \%} = \frac{(\text{NO}_2 - \text{N})_{\text{eff}}}{(\text{NO}_2 - \text{N})_{\text{eff}} + (\text{NO}_3 - \text{N})_{\text{eff}}} \times 100 \quad (2)$$

2.1.2. Anaerobic ammonium oxidation experiment

The CSTAn reactor (0.5 m^3) was inoculated with 100 L of deammonification sludge from a lab-scale two stage PN/Anammox reactor of Environment and Cleaner Production Institute of the TUBITAK Marmara Research Center Kocaeli, TURKEY. Initial TSS and VSS concentrations of reactor were 724 mg/L and 426 mg/L, respectively. The CSTAn reactor was fed with the effluent of partial nitritation reactor (partially nitritated digester effluent) which has $\text{NH}_4^+\text{-N}$ to $\text{NO}_2^-\text{-N}$ ratio of about 1:1.30±0.08. The effluent of nitritation reactor was collected in a storage tank. It is continuously fed to the CSTAn reactor under anoxic conditions

with a pump. Initially the HRT was 5 d and then gradually decreased to 1 d. In order to keep the reactor pH between 7.60 and 7.90, the pH of influent (taken from PN effluent) set between 7.25 and 7.50 flushing with N_2 and CO_2 gas. The CSTAn reactor was operated at $35^\circ\text{C} \pm 1^\circ\text{C}$ by using a heater. Paddle type mixers (Gamak, AGM2E 90 L 6, Turkey) was used in the CSTAn reactor to provide homogeneous sludge mixture and to avoid bacterial damage. The sludges in the tanks were mixed at a speed of about 200 rpm.

The $\text{NO}_2^-\text{-N}$ consumed to $\text{NH}_4^+\text{-N}$ oxidized and $\text{NO}_3^-\text{-N}$ generated to $\text{NH}_4^+\text{-N}$ oxidized ratios of were calculated compared with the theoretical values estimated according to Eq. (3). Stoichiometrically for each mole of $\text{NH}_4^+\text{-N}$ oxidized 1.32 mole of $\text{NO}_2^-\text{-N}$ is consumed and 0.26 mole of $\text{NO}_3^-\text{-N}$ is generated [17].



To evaluate the performance of anaerobic ammonium oxidation nitrogen loading rate (NLR), nitrogen removal rate (NRR) and nitrogen removal efficiency (NRE) were calculated according to Eqs. (4), (5) and (6).

$$\text{NLR, kg N / m}^3 \text{ / d} = \frac{(\text{NH}_4 - \text{N} + \text{NO}_2 - \text{N})_{\text{inf}} \text{ inf}^* \text{ Flow Rate}}{\text{Reactor Volume}} \quad (4)$$

$$\text{NRR, kg N / m}^3 \text{ / d} = \frac{(\text{NH}_4 - \text{N} + \text{NO}_2 - \text{N})_{\text{inf}} - (\text{NH}_4 - \text{N} + \text{NO}_2 - \text{N} - \text{NO}_3 - \text{N})_{\text{eff}}}{\text{HRT}} \quad (5)$$

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

2.2. Characteristics of sludge digester effluent

The digester liquor originated from the dewatering of anaerobically digested sludge from a sludge treatment plant. During the dewatering process, approximately 0.3 g/L of anionic polyelectrolyte was added to the sludge digestion effluent. After dewatering, the sludge digester effluent was turbid and dark brown in color.

The physical and chemical characteristics of sludge digester effluent of Ambarli WWTP are shown in Table 1.

2.3. Analytical methods

Influent and effluent samples of both nitrification reactor and CST An reactor were collected daily and analyzed immediately. All samples were filtered through 0.45 µm filters before the analyses. sCOD, BOD₅, TSS, VSS, alkalinity and PO₄ analyses were carried out according to Standard Methods (APHA, 2005). For the analysis of NH₄⁺-N (LCK 303), NO₂⁻-N (LCK 342), NO₃⁻-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 sCOD/g NO₂⁻-N). The pH, DO and temperature was measured with a multimeter (Hach-Lange, Germany). The free ammonia (FA) and free nitrous acid (FNA) concentrations in the reactor were calculated according to Eqs. (7) and (8) proposed by [18].

$$FA = \frac{[NH_4^+ - N]10^{pH}}{e^{\left(\frac{6344}{T+273}\right)} + 10^{pH}} \quad (7)$$

$$FNA = \frac{[NO_2^- - N]10^{-pH}}{e^{\left(\frac{-2300}{T+273}\right)} + 10^{-pH}} \quad (8)$$

3. Results and discussion

3.1. Partial nitrification (PN) treatment

In the two-stage autotrophic nitrogen removal process, nitrification reactor is used to convert 57% of the ammonium to nitrite, resulting in an appropriate ratio of nitrite to ammonium for the subsequent anammox reactor [16]. In the PN

Table 1

The physical and chemical characteristics of sludge digester effluent of Ambarli WWTP

Parameters	
sCOD (mg/L)	374.5±180
BOD ₅ (mg/L)	218±124
NH ₄ ⁺ -N (mg/L)	574±124.6
Total suspended solids (mg/L)	605.8±170.3
Volatile suspended solids (mg/L)	343.2±124
pH	7.57±0.2
Conductivity (µs/cm)	5634.3±1686.9
Alkalinity (mg/L)	2129.8±567.8

reactor, AOB became the dominant species while NOB was mostly suppressed by manipulating of pH, DO and temperature. Since the growth rate of AOB is less affected than that of NOB under oxygen limitation condition, high pH and temperature [16,19], the DO, pH and temperature remained stable at 0.8 mg/L, 7.8±0.05 and 35±1°C, respectively. After these manipulations the effluent NO₃⁻-N concentration was decreased under 1 mg/L and 99.7% of NAR was observed.

After achieving a stable PN process by adjusting the DO in combination with FA and FNA, the stable running performance was evaluated. During the study, PN effluent of DO was between 0.4–0.8 mg/L, NAR was stable at 99.8±0.09% (Fig. 2c). According to [20], 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, FA and FNA of PN effluent kept 1.93±0.34 mg/L and 0.07±0.01 mg/L, respectively (Fig. 2b). While anammox reactor was fed from PN, pH was not adjusted; it fluctuated between 7 and 7.30. The temperature was at room temperature (25±3.3°C), the HRT changed between 0.5–1 (0.88±0.14) d due to the influent NH₄⁺-N concentration. The VSS concentration increased to 4050 mg/L (180th day) from 1215 mg/L (1st day) due to no sludge was deliberately removed from the reactor.

In this study, the influent NH₄⁺-N was oxidized to NO₂⁻-N partially (56.4±5.8%) with low nitrate concentra-

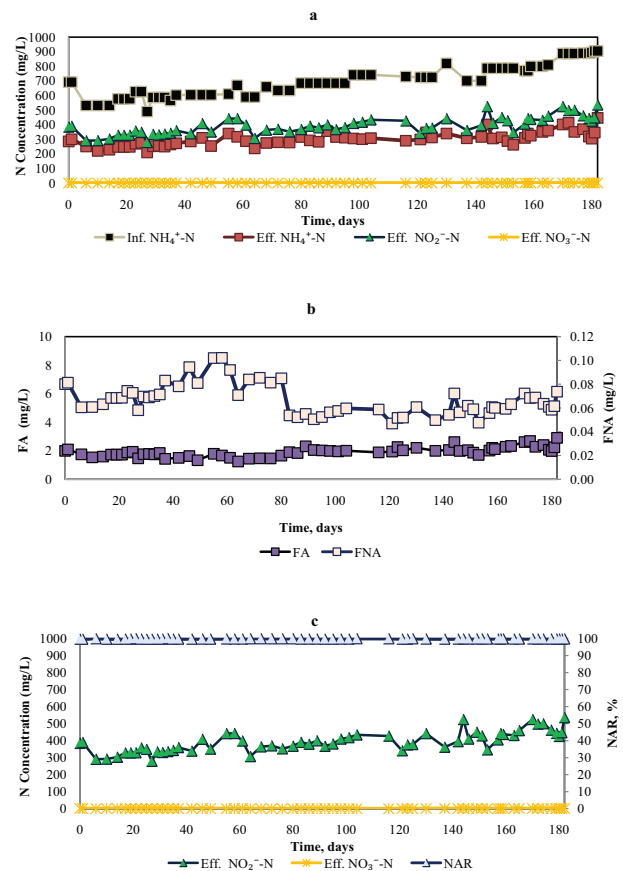


Fig. 2. (a) Ammonium conversion in the Nitrification Reactor with continuous operation (b) Time course of free ammonia and free nitrous acid in the nitrification reactor (c) NO₂-N and NO₃-N concentrations in the effluent and NAR (%).

tions (<1 mg/L) (Fig. 2a) in the PN reactor which are very close to the findings reported by [16]. This suggests that the PN process was successfully started up and that a stable anammox-suited effluent was obtained.

The time course of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ for the PN process is shown in Fig. 2a. The initial $\text{NH}_4^+\text{-N}$ concentration of the sludge digester effluent was changed between 692 mg/L and 905 mg/L from day 1 to 180 (Fig. 2a). The sludge digester was out of order between days 100 and 115 due to a malfunction. Therefore, the sludge digester effluent could not be collected and the PN was not fed with wastewater during this period. However, CSTAn reactor was fed from the storage tank continuously, since the PN effluent was stored in the PN Effluent Storage Tank (Fig. 1).

3.2. Anammox reactor treatment

CSTAn reactor was operated for about 180 d to deammonify the sludge digester effluent that was partially nitrified in the PN reactor. The effluent of PN reactor was fed to CSTAn reactor in a $1:1.30 \pm 0.08 \text{NH}_{4\text{removal}} : \text{NO}_{2\text{produced}}$ ratio with low nitrate concentrations (<1 mg/L). Similar to [21] and [22], it is found that the performance of the first stage PN (i.e. effluent $\text{NO}_2^-/\text{NH}_4^+$ ratio and nitrate concentration) determines removal efficiency. Fig. 3 shows the applied NLR and observed NRR, the influent and effluent nitrogen composition of the Anammox reactor for the whole experimental period. The deammonification performance of CSTAn reactor was monitored based on NLR, NRR, the

stoichiometric ratio of $\text{NH}_4^+\text{-N}:\text{NO}_2^-\text{-N}:\text{NO}_3^-\text{-N}$, the effect of effluent nitrite concentration on NRR and Anammox growth versus nitrogen removal efficiency.

3.2.1. Nitrogen removal performance

CSTAn reactor was started up with a NLR of 0.08 ± 0.01 kgN/m³/d due to the low concentration of VSS. Between days 27 and 51, as shown in Fig. 3a, the DO concentration was fluctuated between 0.06 and 0.17 mg/L (0.12 ± 0.03 mg/L) due to the leakage of sampling port leading to the penetration of oxygen which was detrimental for the anammox process. This DO value changed the diversity of bacterial activity. It was speculated that the elevated DO concentration stimulated the NOB existing in CSTAn reactor due to the high $\text{NO}_3^-\text{-N}$ production rate in these days. However, the performance of reactor resumed quickly after the recovery of normal operation, probably demonstrating a reversible inhibitory effect of oxygen on anammox activity. The NRE increased from 50.4% to 84.7% on day 63 while the NLR was 0.15 ± 0.02 kg N/m³/d after NOB suppression with low DO. It shows that rigorous dissolved oxygen control was extremely crucial for operation of an anammox bio-reactor, especially at low bacterial activity.

A failure of the heating system of the CSTAn reactor on day 45, caused a decrease in the working temperature to $22 \pm 2^\circ\text{C}$ for 5 d which negatively affected anammox performance and promoted the rise of $\text{NO}_2^-\text{-N}$ concentration up to 42.3 mg $\text{NO}_2^-\text{-N/L}$.

The NLR increased to 0.63 kgN/m³/d on day 153 and until the end of the study kept at 0.66 ± 0.05 kgN/m³/d. In this period, the maximum NRR achieved was 0.65 kg N/m³/d and the average was 0.57 ± 0.06 kg N/m³/d (Fig. 3b). This NRR was lower than the NRR of 1.23 kg N/m³/d reported by [12]. 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 ± 0.04 kg N/m³/d reported by [23] and close to the 0.75 kg N/m³/d reported by [24]. They obtained these results using two-phase partial nitrification-anammox (PN/A) reactor fed with sludge digester effluent.

Between days 150 and 180, the suppression of NOB was successfully achieved again by keeping very low DO concentration values (0.02 ± 0.02 mg/L). The performance of the CSTAn reactor was observed based on total nitrogen removal efficiency and stoichiometric ratios. The NRE and ARE were determined as $85.3 \pm 4\%$ and $96 \pm 0.02\%$, respectively. The observed Anammox stoichiometry ($\text{NH}_{4\text{removal}}:\text{NO}_{2\text{removal}}:\text{NO}_{3\text{produced}}$) was $1:1.28 \pm 0.06:0.29 \pm 0.07$ which was a ratio very close to the theoretical anammox stoichiometry reported by [4,23,24].

In this work, the combined PN and anammox treatment lead to a maximum ARE up to $94 \pm 0.05\%$ and a total NRE up to $75.6 \pm 12.6\%$. [4] reported total NRE of 88.1% from the lab-scale of the combined PN and anammox treatment. Similarly, over 90% NRE in a full-scale plant [25] and 84% on average in a pilot plant for sludge digester effluent treatment by PN/anammox were achieved [26].

Nitrite is one of the substrates of anammox but above a certain threshold value will obviously have suppressive effects on the activity [10]. Nitrite inhibition threshold concentrations vary between 5 and 280 mg/L under differ-

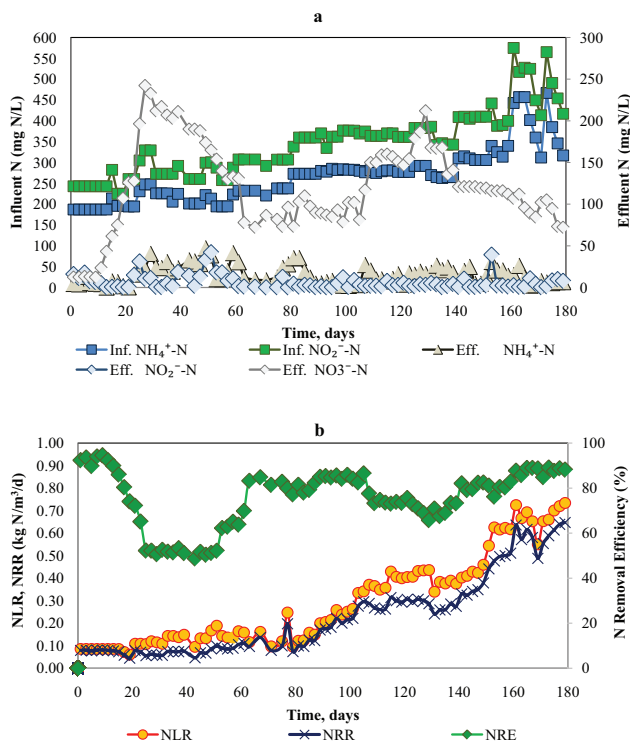


Fig. 3. (a) Profiles of influent ammonium, nitrite nitrogen concentrations, effluent nitrite, nitrate and ammonium nitrogen concentrations in anammox reactor. (b) Profiles of Nitrogen Loading Rate (NLR), Nitrogen Removal Rate (NRR) and Nitrogen Removal Efficiency (NRE) in the anammox reactor.

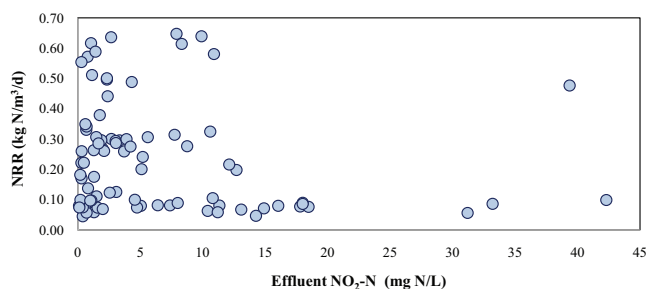


Fig. 4. The relation between the effluent nitrite concentration and the NRR.

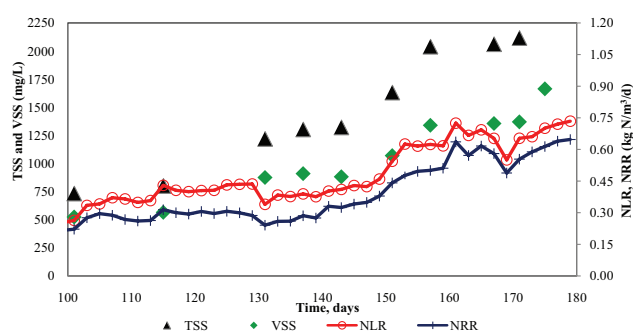


Fig. 5. Profiles of Nitrogen Loading Rate (NLR), Nitrogen Removal Rate (NRR), Total Suspended Solid (TSS) and Volatile Suspended Solid (VSS) concentrations in the anammox reactor.

ent experimental conditions and operating modes [15,27]. Although the threshold values change, low NO_2^- -N concentration has been considered critical to preventing anammox bacteria inhibition [28,29]. The NRR was plotted against nitrite concentration to evaluate any inhibitory effect of elevated NO_2^- -N levels (Fig. 4). In this study NO_2^- -N was not causing directly inhibition within the range tested, since a clear declining trend for NRR was not observed (Fig. 4). However, the increasing of NRR was determined under 10 mg/L of NO_2^- -N concentration. Besides above the 30 mg/L of NO_2^- -N concentration the NRR rise began to decline.

3.2.2. Anammox activity (Growth rate)

Anammox activity is defined as the nitrogen removal rate (sum of NH_4^+ -N and NO_2^- -N) in the absence of oxygen and non-limiting concentrations of NH_4^+ -N and NO_2^- -N. The anammox activity was calculated on a daily basis directly during operation. Following a normal feeding of NH_4^+ -N and NO_2^- -N consumption were calculated, and VSS was analyzed to figure out the specific anammox activity (SAA) in CSTAn reactor. Anammox activities are reported only as volumetric rates, in gN/gVSS/d. Besides kinetic parameters such as biomass growth yield ($Y; \text{gVSS}_{\text{produced}}/\text{g}(\text{NH}_4^+\text{-N}+\text{NO}_2^-\text{-N})_{\text{consumed}}$) of anammox bacteria was calculated in the same way. The VSS/TSS ratios of the anammox sludge in CSTAn reactor was in the range of 0.67 ± 0.04 . SAA was 0.36 ± 0.06 g NH_4^+ -N/g VSS/d and Y was 0.04 ± 0.02 g VSS/g N between 100 and 180 d. The biomass growth yield of CSTAn reactor in this study was close to [30] (0.07 g VSS/g NH_4^+ -N) and [31] (0.066 ± 0.01

g VSS/g NH_4^+ -N). The day 180th NRR was up to 0.65 kg N/ m^3/d from 0.22 kg N/ m^3/d (100th day) and VSS was up to 1664 mg/L from 566 mg/L (100th day). The anammox activity increased exponentially and correlated with the observed growth in volatile suspended solids which is shown in Fig. 5.

4. Conclusion

The anaerobic sludge digester effluent of a central WWTP was successfully deammonified in the first pilot scale two-stage system operated in Istanbul, TR consisting of a continuously stirred partial nitrification (PN) reactor and a continuously stirred tank Anammox (CSTAn) reactor. A stable PN process was achieved in a wide DO spectrum, ranging from 0.4 to 0.8 mg/L. The HRT changed between 0.5 – 1 (0.88 ± 0.14) day due to the influent NH_4^+ -N concentration. The pilot scale PN/A reactor fed with sludge digester effluent performed stable between days 150 and 180 due to the suppression of NOB under the low DO concentration values (0.02 ± 0.02 mg/L) with a NRE of $96\pm 0.02\%$. Globally, the combined PN and anammox treatment lead to a maximum ARE up to $94\pm 0.05\%$ and a total NRE up to $75.6\pm 12.6\%$. The biomass concentration in the CSTAn reactor was 1075 ± 330 g VSS/L with the SAA 0.38 ± 0.06 kgN/kgVSS/d. Nitrite as high as 42.3 mg/L did not cause deactivation of anammox consortium, however the NRR was tending to rise under 10 mg/L nitrite. In order to provide all-sided description of PN/anammox, such as relevant variables, their interactions, and finding the best experimental conditions for the proper functioning of the reactors, a multivariate study need to be done in future works [32–34].

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References

- [1] C. Fux, H. Siegrist, Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitrification/anammox: environmental and economical considerations, *Water Sci. Technol.*, 50(10) (2004) 19–26.
- [2] S. Lackner, E.M. Gilbert, S.E. Vlaeminck, A. Joss, H. Horn, van Loosdrecht MCM, Full-scale partial nitrification/anammox experiences – An application survey, *Water Res.*, 55 (2014) 292–303.
- [3] B. Ma, S. Zhang, L. Zhang, P. Yi, J. Wang, S. Wang, Y. Peng, The feasibility of using a two-stage autotrophic nitrogen removal process to treat sewage, *Bioresour. Technol.*, 102(17) (2011) 8331–8334.
- [4] J. Dosta, J. Vila, I. Sancho, N. Basset, M. Grifoll, J. Mata-Alvarez, Two-step partial nitrification/anammox process in granulation reactors: Start-up operation and microbial characterization, *J. Environ. Manage.*, 164 (2015) 196–205.
- [5] S.F. Kosari, B. Rezaei, K.V. Lo, D.S. Mavinic, Operational strategy for nitrogen removal from centrate in a two-stage partial nitrification–anammox process, *Env. Technol.*, 35(9) (2014) 1110–1120.
- [6] S.W.H. Van Hulle, H.J.P. Vandeweyer, B.D. Meesschaer, P.A. Vanrolleghem, P. Dejana, A. Dumoulin, Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams, *Chem. Eng. J.*, 162 (2010) 1–20.

- [7] M.J. Kampschreur, R. Poldermans, R. Kleerebezem, W.R.L. van der Star, R. Haarhuis, W.R. Abma, M.S.M. Jetten, M.C.M. van Loosdrecht, Emission of nitrous oxide and nitric oxide from a full-scale single-stage nitrification-anammox reactor, *Water Sci. Technol.*, 60(12) (2008) 3211–3217.
- [8] A. van der Graaf, P. Bruijn, L. Robertson, M. Jetten, J. Kuenen, Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor, *Microbiology*, 142 (1996) 2187–2196.
- [9] R.C. Jin, G.F. Yang, J.J. Yu, P. Zheng, The inhibition of the Anammox process: A review, *Chem. Eng. J.*, 197 (2012) 67–79.
- [10] T. Lotti, W.R. van der Star, R. Kleerebezem, C. Lubello, M.C. vanLoosdrecht, The effect of nitrite inhibition on the anammox process, *Water Res.*, 46(8) (2012) 2559–2569.
- [11] M. Soliman, A. Eldyasti, Development of partial nitrification as a first step of nitrite shunt process in a Sequential Batch Reactor (SBR) using Ammonium Oxidizing Bacteria (AOB) controlled by mixing regime, *Bioresour. Technol.*, 221 (2016) 85–95.
- [12] G. Wang, X. Xu, L. Zhou, C. Wang, F. Yang, A pilot-scale study on the start-up of partial nitrification-anammox process for anaerobic sludge digester liquor treatment, *Bioresour. Technol.*, 241 (2017) 181–189.
- [13] Y. Xue, F. Yang, S. Liu, Z. Fu, The influence of controlling factors on the start-up and operation for partial nitrification in membrane bioreactor, *Bioresour. Technol.*, 100(3) (2009) 1055–1060.
- [14] B.S. Xing, Y.X. Ji, G.Y. Yang, H. Chen, W.M. Ni, R.C. Jin, Start-up and stable operation of partial nitrification prior to ANAMMOX in an internal-loop airlift reactor, *Separ. Puri. Technol.*, 120(13) (2013) 458–466.
- [15] L.W. Jaroszynski, N. Cicek, R. Sparling, J.A. Oleszkiewicz, Importance of the operating pH in maintaining the stability of anoxic ammonium oxidation (anammox) activity in moving bed biofilm reactors, *Bioresour. Technol.*, 102(14) (2011) 7051–7056.
- [16] İ. Çelen-Erdem, E.S. Kurt, B. Bozçelik, B. Çallı, Upflow packed bed anammox reactor used in two-stage deammonification of sludge digester effluent, *Water Sci. Technol.*, 78 (9) (2018) 1843–1851.
- [17] M. Strous, J. Heijnen, J. Kuenen, M. Jetten, The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, *Microbiol. Biotechnol.*, 50 (1998) 589–596.
- [18] A. Anthonisen, R. Loehr, T. Prakasam, E. Srinath, Inhibition of nitrification by ammonia and nitrous acid, *J. Water Pollut. Contr. Fed.*, 48(5) (1976) 835–852.
- [19] S. Wyffels, P. Boeckx, K. Pynaert, D. Zhang, O. van Cleemput, G. Chen, W. Verstraete, Nitrogen removal from sludge reject water by a two-stage oxygen-limited autotrophic nitrification denitrification process, *Water Sci. Technol.*, 49(5–6) (2004) 57–64.
- [20] G. Wang, X. Xu, L. Zhou, C. Wang, F. Yang, A pilot-scale study on the start-up of partial nitrification-anammox process for anaerobic sludge digester liquor treatment, *Bioresour. Technol.*, 241 (2017) 181–189.
- [21] W. Liu, D. Yang, Y. Shen, J. Wang, Two sludge partial nitrification-anammox process for high-rate mainstream deammonification, *Appl. Microbiol. Biotechnol.*, 102 (2018) 8079–8091.
- [22] A. Bartroli, J. Perez, J. Carrera, Applying ratio control in a continuous granular reactor to achieve full nitrification under stable operating conditions, *Environ. Sci. Technol.*, 44 (2010) 8930–8935.
- [23] C. Fux, M. Boehler, P. Huber, I. Brunner, H. Siegrist, Biological treatment of ammonium-rich wastewater by partial nitrification and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant, *J. Biotechnol.*, 99(3) (2002) 295–306.
- [24] U. van Dongen, M.S.M. Jetten, M.C.M. van Loosdrecht, The SHARON-Anammox process for treatment of ammonium rich wastewater, *Water Sci. Technol.*, 44(1) (2001) 153–160.
- [25] A. Joss, D. Salzgeber, J. Eugster, R. König, K. Rottermann, S. Burger, P. Fabijan, S. Leumann, J. Mohn, H. Siegrist, Full-scale nitrogen removal from digester liquid with partial nitrification and anammox in one SBR, *Environ. Sci. Technol.*, 43 (2009) 5301–5306.
- [26] T. Gut, E. Plaza, J. Trela, B. Hultman, J. Bosander, Combined partial nitrification/anammox system for treatment of digester supernatant, *Water Sci. Technol.*, 53(12) (2006) 149–159.
- [27] K. Isaka, T. Sumino, S. Tsuneda, High nitrogen removal performance at moderately low temperature utilizing anaerobic ammonium oxidation reactions, *J. Biosci. Bioeng.*, 103(5) (2007) 486–490.
- [28] B. Wett, S. Murthy, I. Takacs, M. Hell, G. Bowden, A. Deur, M. O'Shaughnessy, Key parameters for control of DEMON deammonification process, *Water Practice.*, 1(5) (2007) 1–11.
- [29] B. Szatkowska, E. Plaza, J. Trela, B. Hultman, J. Bosander, Combined partial nitrification and anammox biofilm system as a sustainable solution for supernatant treatment, *Water Pract. Technol.*, 53(12) (2006) 149–159.
- [30] C. Trigo, J.L. Campos, J.M. Garrido, R. Mendez, Start-up of the Anammox process in a membrane bioreactor, *J. Biotechnol.*, 126(4) (2006) 457–487.
- [31] M. Strous, E. Van Gerven, P. Zheng, J.G. Kuenen, M.S.M. Jetten, Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (anammox) process in different reactor configurations, *Water Res.*, 31(8) (1997) 1955–1962.
- [32] D. Wang, G. Wang, F. Yang, C. Liu, Treatment of municipal sewage with low carbon to nitrogen ratio via a novel integrated process, *Chem. Eng. J.*, 341 (2018) 58–64.
- [33] D. Wang, G. Wang, F. Yang, C. Liu, L. Kong, Y. Liu, Treatment of municipal sewage with low carbon to nitrogen ratio via simultaneous partial nitrification, anaerobic ammonia oxidation, and denitrification (SANAD) in a non-woven rotating biological contactor, *Chemosphere*, 208 (2018) 854–861.
- [34] D. Wang, G. Wang, X. Xu, F. Yang, Multiple factors influencing anaerobic acidogenic pretreatment in an up-flow non-woven biofilm reactor, *Chem. Eng. J.*, 221 (2013) 37–43.