Influence of the pretreatment of activated sludge in the aerobic digester as inoculum on rapid anammox enrichment

İ. Çelen-Erdem

Environment and Cleaner Production Institute, The Marmara Research Center TUBITAK, Turkey, email: i_celen@yahoo.com

ABSTRACT

This study aims to determine a shorter start-up period for the anaerobic ammonium oxidation (anammox) reaction which is one of the main obstacles in the application of anammox technology. The results obtained from the two identical laboratory-scale upflow packed bed reactors (R1 and R2) were compared whether the pretreatment of the activated sludge as inoculum plays an important role in the rapid enrichment of anammox bacteria. Pretreatment consists of aerobic digestion and continuous washing of the reactor. Both reactors were fed with synthetic wastewater during 192 d. Anammox activities evaluated by mass balances based on ammonium, nitrite, and nitrate analysis and nitrogen removal rate (NRR). The results demonstrated that the R1 reactor was successfully enriched on day 112 and had an NRR of 30 gN/m³ d. By contrast, it took 58 d to enrich the R2 reactor (48.5 gN/m³ d). It is possible to enrich anammox bacteria within 58 d by applying activated sludge pretreatment to shorten the period for the removal of unwanted organisms and their lysed cell debris.

Keywords: Anaerobic oxidation of ammonium (anammox); Enrichment; Activated sludge; Pretreatment; Upflow packed bed reactors

1. Introduction

Since anaerobic ammonium oxidation (anammox) processes have advantages over conventional biological processes for the treatment of ammonium in the wastewater, anammox-related technologies have been widely reported [1,2]. The anammox process can reduce aeration by 64%, exogenous electron donors by 100% and sludge production by 80%–90% [3]. The biochemical reactions of the anammox process can be described as follows [4].

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O + 0.66CH_2O_{0.5}N_{0.15} \tag{1}$$
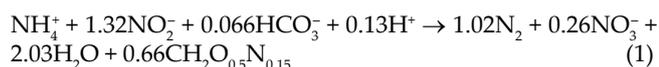
However, the start-up of the anammox process in the bioreactor is usually time-consuming and may take from months to years due to the slow growth rate of anammox bacteria [5–7]. Therefore, many studies have focused on developing techniques for culturing the bacteria with a shorter start-up period to establish a successful anammox culture [8]. The duration of the start-up period of the anammox process is influenced mainly by the seed sludge including nitrifying sludge, methanogenic sludge, single-stage partial nitrification–denitrification-anammox sludge, anaerobic activated sludge and denitrifying sludge from wastewater treatment plant (WWTP) [9]. Moreover, reactor type and optimal operating conditions are key factors in the start-up of the anammox process [10].

In this project, the enrichment of anammox bacteria in two identical laboratory-scale upflow packed bed anammox reactors (R1 and R2) were studied. The performance of the reactors was investigated with chemical analysis and anammox reaction stoichiometry and nitrogen removal rate (NRR).

The results of this work will be very important to those who are planning to begin the anammox process start-up without the anammox seeding sludge. The novelty of this work were to (i) determine the effect of pretreatment of the activated sludge by aeration and washing with tap water to shorten the period for the removal of unwanted organisms and their lysed cell debris which was not reported on anammox start-up by any researcher and (ii) compare the differences between the two reactors (seeded with activated sludge and pretreated activated sludge as inoculum for rapid enrichment).

2. Materials and methods

2.1. Experimental set-up and operating strategies

The laboratory-scale experimental set-up consisting of an aerobic digester, storage tank for tap water, feeding tank for synthetic wastewater and upflow packed bed anammox reactors (R1 and R2) is shown in Fig. 1. The activated sludge was added into R1 and the pretreated activated sludge in an aerobic digester (Fig. 1a) was added into R2. They were operated by feeding with synthetic wastewater (Fig. 1b).

2.2. Pretreatment of activated sludge

Activated sludge taken from the sludge recycling line of Paşaköy WWTP, in Istanbul, Turkey was pretreated before being added to R2 as anammox inoculum. Pretreatment consists of aerobic digestion and continuous washing of the reactor (Fig. 1a) which lasted 4 d. Total suspended solids (TSS) and volatile suspended solids (VSS) of the activated sludge used were 10,620 and 7,080 mg/L, respectively. Aerobic digestion was performed at 35°C ± 3°C in a continuously stirred tank reactor (CSTR) of 25 L capacity. The internal diameter and height of the reactor were 27 and 35 cm, respectively. The activated sludge (16 L) was washed continuously with tap water in the CSTR. The hydraulic retention time (HRT) of the tap water in aerobic digester was 1 d. Floated sludge was washed out of the reactor. Mixing was achieved by paddle type mixers at a speed of about 100 rpm (Siemens, S1FL6, Germany) to provide a homogeneous mixing. Air was supplied at a flow rate of 2 L/min through membrane-type diffuser placed at the bottom of the reactor to provide the oxygen needed for aerobic digestion.

The pH and the dissolved oxygen (DO) concentration of the reactor were monitored but not controlled.

2.3. Anammox reactors

The experiments were conducted in two identical 4.8 L glass upflow reactors To keep the sludge and create anammox bacteria in each reactor, 100 balls shaped filling materials with 25 mm diameter and "LEVAPOR" biocarrier cubes were used. The two reactors were operated at 35°C ± 1°C by using a temperature-controlled heating mat wrapped around the reactor. Equipped with several ports for gas and liquid at the top. The pH of the influent was usually about 7, therefore no pH adjustment was required in both reactors.

R1 and R2 were inoculated with activated sludge (1 L) and pretreated activated sludge (1 L), respectively. The TSS and VSS concentrations of the sludge in each reactor are shown in Table 1. They were fed synthetic wastewater to

Table 1
Initial TSS and VSS of anammox reactors

Reactor	TSS, mg/L	VSS, mg/L
R1	10,620	7,080
R2	6,663	3,702

enrich anammox bacteria. It was fed into the R1 and R2 at flow rates of 0.9–3.4 L/d.

2.4. Synthetic wastewater

To prevent the oxygen intrusion, synthetic wastewater stored in the feeding tank which had an effective volume of 20 L was deoxygenated by flushing with N_2 gas to control the experiment more precisely (Fig. 1b). The pH was adjusted to 7.5 with carbon dioxide gas. The synthetic wastewater was composed of substrates, trace elements and inorganic solution [11]. Initially, NH_4–N (50 mg/L) and NO_2–N (60 mg/L) were provided in the form of $(NH_4)_2SO_4$ and $NaNO_2$, respectively. The concentrations were increased based on NH_4–N and NO_2–N removal during the operation. Trace elements containing ethylenediaminetetraacetic acid (15 g/L), $ZnSO_4 \cdot 7H_2O$ (0.43 g/L), $CoCl_2 \cdot 6H_2O$ (0.24 g/L), $MnCl_2 \cdot 4H_2O$ (0.99 g/L), $CuSO_4 \cdot 5H_2O$ (0.25 g/L), $NaMoO_4 \cdot 2H_2O$ (0.22 g/L), $NiCl_2 \cdot 2H_2O$ (0.19 g/L), $NaSeO_4 \cdot 10H_2O$ (0.21 g/L), H_3BO_4 (0.014 g/L), and $NaWO_4 \cdot 2H_2O$ (0.050 g/L) were supplied at a dosage of 1.25 mL per liter of wastewater. The inorganic solution consisted of $CaCl_2$ (5.65 mg/L), NaH_2PO_4 (10 mg/L), $MgSO_4 \cdot 7H_2O$ (58.6 mg/L), and $KHCO_3$ (1 g/L).

2.5. Analytical methods

Periodically, samples were taken from the feeding tank, effluent of R1, R2, and effluent of the aerobic digester. TSS, VSS and chemical oxygen demand (COD) analyses were carried out according to Standard Methods [12]. For the analysis of NH_4–N (LCK 303), NO_2–N (LCK 342), NO_3–N (LCK 339) cuvette test kits (Hach Lange GmbH, Germany) and a spectrophotometer (Dr 2800, Hach Lange) were used. All analytical measurements were in duplicate and presented values are corresponding to averages. The pH, DO and temperature was measured with a multimeter (Hach Lange LDO meter, Düsseldorf, Germany).

3. Results

3.1. Pretreatment of the activated sludge

The anammox bacteria are enriched from activated sludge usually under completely anaerobic conditions [5,8,9,13]. However, in this study, activated sludge was firstly treated in an aerobic tank operated as an aerobic digester as shown in Fig. 1a. The aim of using this process was reducing the duration of the cell lysis phase (with effluent NH_4^+ concentration > influent concentration) in the anammox tank. Ammonium, nitrite, and nitrate concentrations were not available before aerobic digestion in the activated sludge. Therefore, 50 mg/L NH_4^+–N was added externally to follow the AOB and NOB activities. During the operation, pH was between 7.55 and 7.89. The NH_4^+–N, NO_2–N, NO_3–N, COD
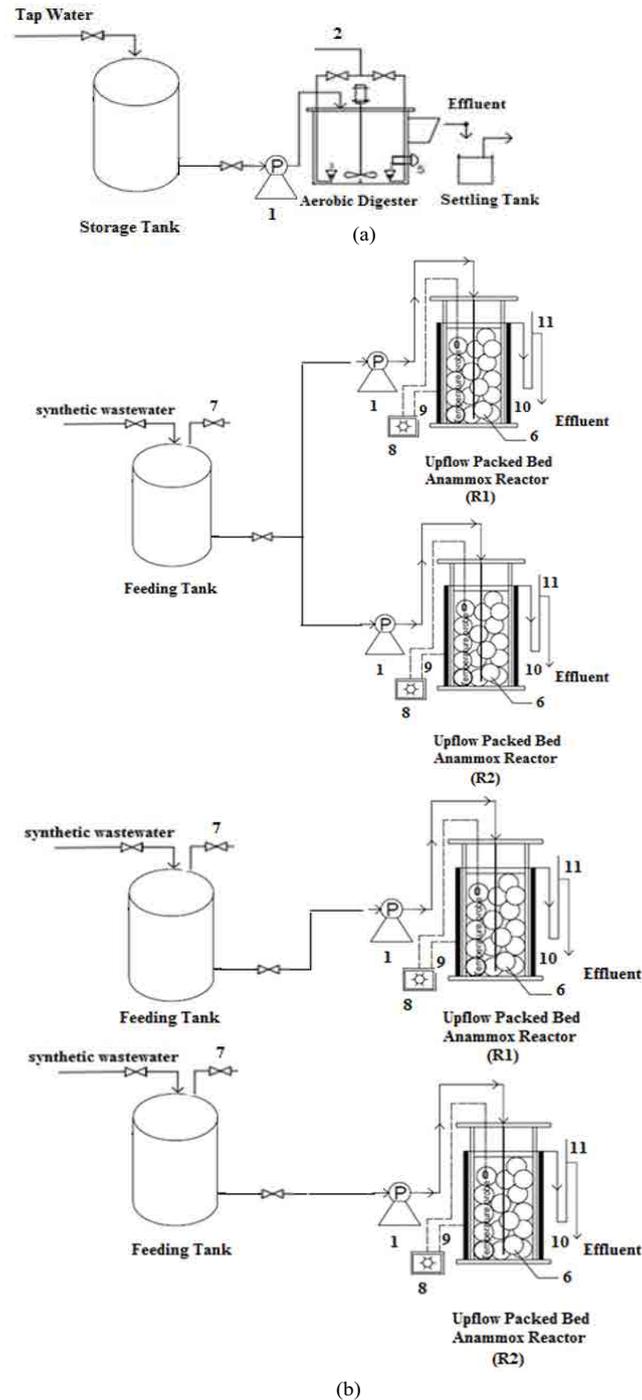
Fig. 1. Schematic drawing of (a) pretreatment of activated sludge and (b) R1 and R2. (1) Pump, (2) air supply, (3) diffuser, (4) mixer, (5) resistance (heater), (6) packed material, (7) gas supply (N_2, CO_2), (8) temperature control, (9) heating mat, (10) sampling point and (11) siphon breaker.

and DO concentrations in the aerobic digester are shown in Table 2.

During the sludge digestion organics and nitrogen compound released from cell lysis. Therefore at the end of the 4th day NO_3–N concentration was higher than the initial ammonium concentration (Table 2). Since no organic carbon source is supplied and washed from the aerobic digester, denitrification activity soon diminished when organics from cell lysis were completely consumed. COD concentration was reduced from 539 to 24 mg/L in a day. Also, the accumulation of the NO_3–N was supposed to be caused by the low denitrification activity in the reactor. Moreover, since there was not

Table 2
Characteristics of the pretreated seeding sludge

Day	DO, mg/L	NH_4^+–N, mg/L	NO_2–N, mg/L	NO_3–N, mg/L	COD, mg/L
0		50.0	–	–	539
1	0.2	24.5	–	–	24
2	0.6	13.7	–	–	–
3	0.4	1.43	–	–	–
4	1.5	0.30	1.1	75.6	–

any substrate for AOB and NOB's in the media, oxygen was not consumed and started to accumulate in the 4th day of the phase. The DO concentration was between 0.2–0.6 mg/L within the 3 d. It is known that anammox bacteria could survive in these concentrations considering a large number of full-scale implementations of one-stage nitritation/anammox process [14]. However, high DO concentration (2–3 mg/L) is supposed to be toxic [5]. Therefore, the pretreatment phase was stopped when DO concentration increased to 1.5 mg/L. The VSS/TSS ratio was about 55%. As soon as DO started to increase as an indication of the complete removal of biodegradable organics and nitrogen-based electron donors, the sludge was settled and the supernatant was decanted. The remaining sludge was used for inoculating the R2.

3.2. Anammox reactors

The enrichment experiment with activated sludge was performed in R1 with the same conditions as in R2 which contains pretreated activated sludge as inoculum. Nitrogen removal in R1 was considerably different than that of R2 during 195 d. Synthetic wastewater contains NH_4–N and NO_2–N at a ratio of 1:1.32. Two reactors were operated by increasing the influent NH_4^+–N and NO_2–N concentrations stepwise as shown in Figs. 2 and 3. The flow rate of the feeding solution was increased based on the removal rate of each system. R1 did not show stability until 107 d (Fig. 2a). It reached a steady-state after 112 d with effluent NH_4^+–N and NO_2–N concentrations reduced and had an NRR of 30 g N/m³/d. Also, the ratio of NH_4–N:NO_2–N:NO_3–N was found closer to its stoichiometric value. This good performance indicated the domination of anammox activity in the R1 reactor. However, NH_4^+–N and NO_2–N removed simultaneously from the beginning in the R2 reactor which was inoculated with pretreated activated sludge (Fig. 3a). It was successfully enriched on day 58 and had an NRR of 48.52 g N/m³/d. The nitrogen loading rate (NLR) was doubled to stimulate the growth of anammox after day 105 in the R2 reactor (Fig. 3b). Whereas NLR was increased after day 130 in the R1 reactor (Fig. 2b). After this change, a continuous increase in the NRR was observed. Therefore, the NLR was increased after day 158 with 90.64% nitrogen removal efficiencies. The NRR of the R2 reactor (209.4 gN/m³ d) was higher than the R1 reactor (80.19 gN/m³ d) on day 115 (Figs. 2b and 3b). On day 192, the NRR of R1 and R2 reactors were increased to 244.78 and 700 gN/m³ d, respectively.

According to previous studies, the anammox enrichment process can be divided into four stages: cell lysis
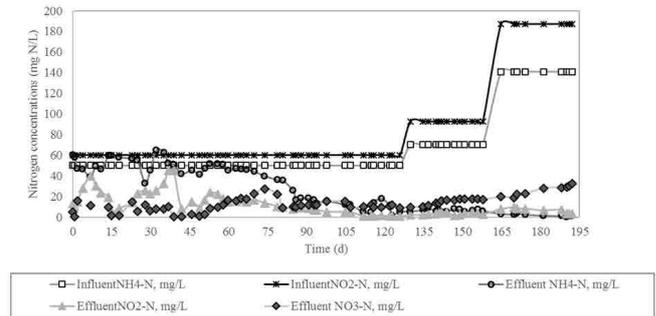
(a)
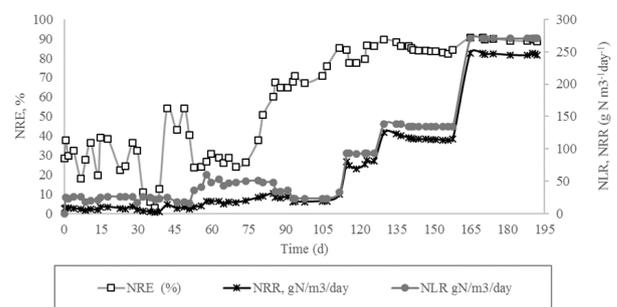
(b)

Fig. 2. Performance of R1. (a) Influent concentrations of ammonium and nitrite, effluent concentrations of ammonium, nitrite and nitrate and (b) nitrogen loading rates (NLRs), nitrogen removal rates (NRRs) and nitrogen removal efficiencies (NREs).

phase, the lag phase, the transition phase, and activity elevation phase [9,15]. During 58 d, the effluent ammonium concentration was higher than the influent one (the cell lysis phase) in R1. However, there was no sign of the cell lysis phase in R2 which was commonly found in many other studies [16,17]. The cell lysis phase probably was completed very fast in the pretreatment of the activated sludge with air and moderately low HRT (1 d) with tap water in aerobic digester which indicates the basic advantage of the pre-aerobic enrichment strategy.

3.3. Stoichiometric ratio

In this study, mass balances for NH_4–N, NO_2^-–N, and NO_3–N have been used as an indicator of the anammox reaction. The NO_2^-–$N_{consumed}$/NH_4–$N_{consumed}$ and NO_3–$N_{produced}$/NH_4–$N_{consumed}$ ratios achieved in R1 (Fig. 4a) and R2 (Fig. 4b) are shown as a function of nitrogen conversion in Fig. 4. The ratios of nitrite/ammonia and nitrate/ammonia were compared to the classical theoretical value [4,18,19]. In R1, on day 112, stoichiometry molar ratios of NO_2–N conversion and NO_3–N production to NH_4–N conversion were calculated to be 1.31 ± 0.2 and 0.22 ± 0.05, respectively. The average value in R2, on day 58 for NO_2^-/NH_4^+ was 1.32 ± 0.07 and the NO_3/NH_4^+ was 0.26 ± 0.02. These ratios were very close to the anammox stoichiometry reported by Dosta et al. [18], Strous et al. [4], and Çelen-Erdem et al. [19].
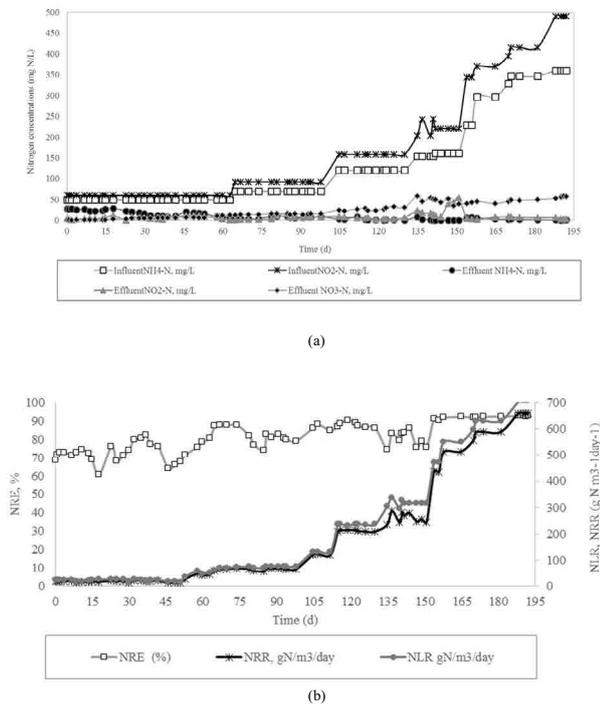
Fig. 3. Performance of R2. (a) Influent concentrations of ammonium and nitrite, effluent concentrations of ammonium, nitrite, and nitrate and (b) nitrogen loading rates (NLRs) and nitrogen removal rates (NRRs) and nitrogen removal efficiencies (NREs).
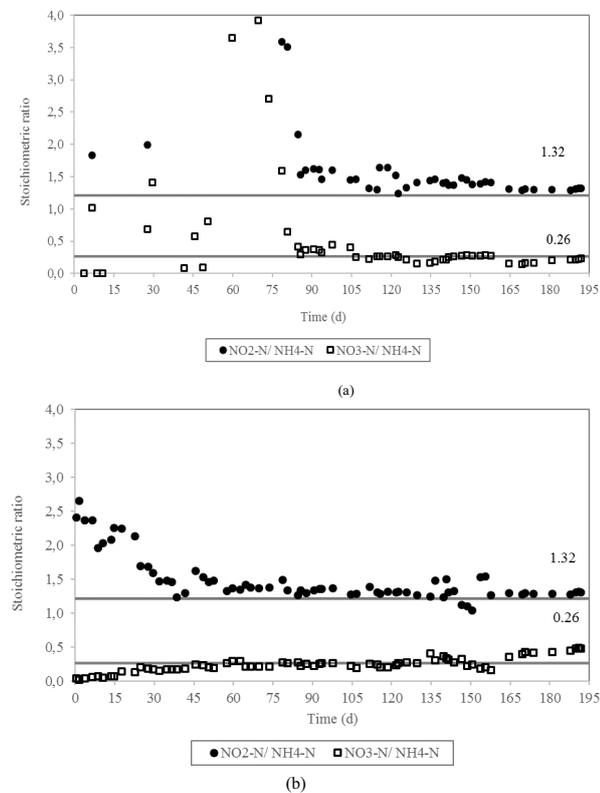
Fig. 4. Evolution of the stoichiometric ratios in the effluent of (a) R1 and (b) R2.

4. Discussion

To investigate whether the pretreatment of the activated sludge as inoculum is feasible in the rapid enrichment of anammox bacteria, two identical laboratory-scale upflow packed bed anammox reactors (R1 and R2) were compared. The pretreatment of the activated sludge by aeration and washing with tap water to shorten the period for the removal of unwanted organisms and their cell lysis phase on anammox start-up was not reported by any researcher before.

Chen et al. [9] operated an upflow anaerobic sludge blanket, which was seeded with denitrifying sludge to start-up the anammox process. They reported that the effluent ammonium was higher than the influent ammonia in the first 16 d due to cell lysis. Similarly, [8] concluded that the cell lysis phase lasted 13 d when the UASB reactor was seeded with denitrifying granular sludge and anammox bacteria. In this study, where only activated sludge seed was used, the cell lysis and washing out of unwanted organisms have been completed in 4 d under fully aerobic conditions. Anammox bacteria could survive in this environment. This is probably the anammox that was covered by a layer of nitrifying organisms which consumed oxygen and producing a suitable amount of nitrite for anammox bacteria. Ammonium was also produced during the cell lysis phase and used by anammox bacteria. When the pretreated activated sludge was used as inoculum, the time required for anammox enrichment is reduced compared to the enrichment experiment without pretreatment that was performed in a laboratory-scale reactor (R1).

Anammox reaction stoichiometry of R1 and R2 reactors were observed in 112 (NRR of 30 gN/m³ d) and 58 d (NRR of 48.5 gN/m³ d), respectively. The start-up time in R2 (58 d) was much less than the other results which might range from about 100–200 d reported in other works [7,20,21]. In this study, anammox biomass was successfully enriched from municipal activated sludge and the existence of anammox activity was confirmed by the stoichiometric ratio in both R1 and R2.

It is concluded that the activated sludge pretreatment is a successful method for faster anammox enrichment.

Acknowledgments

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