



## Removal of ammonium and organic carbon from leachate by the anammox process in a fixed bed bioreactor

Nadali Alavi<sup>a,b</sup>, Hajar Salamifar<sup>c</sup>, Mohammad Javad Mohammadi<sup>d,e</sup>,  
Mohammad Almasian<sup>f</sup>, Amir Hesam Hassani<sup>g</sup>, Monireh Majlesi<sup>a,b</sup>, Seyyed Abbas Mirzaee<sup>h,\*</sup>

<sup>a</sup>Environmental and Occupational Hazards Control Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, emails: alavi@sbmu.ac.ir (N. Alavi), monireh\_majlessi@yahoo.com (M. Majlesi)

<sup>b</sup>Department of Environmental Health Engineering, School of Public Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>c</sup>Environmental Engineering, Islamic Azad University, Science and Research Branch-Khuzestan, Ahvaz, Iran, email: hajar\_salamifar@yahoo.com

<sup>d</sup>Asadabad School of Medical Sciences, Asadabad, Iran, email: javad.sam200@gmail.com

<sup>e</sup>Student Research Committee, Department of Environmental Health Engineering, School of Public Health and Environmental Technologies Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>f</sup>School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

<sup>g</sup>Department of Environmental Engineering, Islamic Azad University, Science and Research Branch-Tehran, Tehran, Iran, email: ahassani@srbiau.ac.ir

<sup>h</sup>Department of Environmental Health Engineering, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, Tel. +98989183459155; Fax: +986113361544; email: Mirzaee.seyyed@gmail.com

Received 26 April 2017; Accepted 17 November 2017

---

### ABSTRACT

One of the inevitable drawbacks of sanitary landfilling of municipal solid waste (MSW) is the production of leachate. This study aimed to assess the treatment of leachate for the purpose of removing ammonium and organic carbon using the anammox process. To grow autotrophic bacteria in the reactor, the carbon source was gradually decreased from 500 mg L<sup>-1</sup> to less than 10 mg L<sup>-1</sup>. NH<sub>4</sub> and nitrite concentrations from 5 and 6.6 to 300 and 396 mg L<sup>-1</sup>, respectively, were injected into the reactor and the removal was investigated. Finally, in order to assess carbon and nitrogen removal simultaneously, the concentrations of chemical oxygen demand, ammonia, and nitrite were increased to 250, 500, and 660 mg L<sup>-1</sup>, respectively. The highest efficiencies for ammonium and nitrite removal that were obtained were 76.69% and 91.12%, respectively, at the volumetric loading rate of 0.15 and 0.132 kg m<sup>-3</sup> d<sup>-1</sup> in the hydraulic retention time (HRT) of 24 h. As HRT decreased from 24 to 6 h and the loading rate increased, the highest efficiencies achieved for the removal of ammonia and NO<sub>2</sub> were 95.19% and 80.56%, respectively. Eventually, ammonium removal efficiency did not exceed 22.28% while the removal efficiency for organic matters was 72.63%. Contrary to nitrite, ammonium removal is dependent on the appropriate performance of the anammox process.

*Keywords:* Anammox process; Leachate treatment; Ammonium removal; Organic carbon; Municipal solid waste

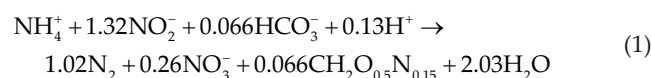
---

\* Corresponding author.

## 1. Introduction

Sanitary landfilling, in the integrated solid waste management system, is still the final and most common disposal option for municipal solid waste (MSW) in the world [1]. One important inevitable drawback in sanitary landfilling of MSW is the production of leachate, which is an important threat to receiving water bodies and other ecosystems [1,2]. Leachate is produced from sanitary landfills or composting facilities due mainly to the percolation of rainwater through the layers of waste materials, the moisture content of waste materials, and biochemical interactions in the waste [2,3]. Municipal landfill leachate is defined as a hazardous and complex wastewater that may contain high concentrations of organic and inorganic pollutants such as toxic and non-biodegradable, high ammonium nitrogen, dissolved organic matter (DOM) (humic and fulvic acids), heavy metals (HM), and xenobiotic organic (XO) compounds [1]. DOM, natural organic matter (NOM), HM, and XO are important threats to receiving water resource and other ecosystems [4–8]. Various physico-chemical and biological treatment methods including precipitation, adsorption and ion exchange [9], supercritical water partial oxidation [10], Fenton processes [11,12], membrane process [13], catalytic oxidation process [14], and different kinds of constructed wetland [15,16] have been used to treat leachate [3,17–20]. Biological process for high-strength wastewater such as landfill leachate that contain high level of recalcitrant organic compounds is preferred treatment because economic and ecological aspects including lower operational and maintenance costs, energy saving, and smaller space requirements. But due to high levels of ammonium and organic carbon in the leachate, any treatment method alone cannot be satisfactory [18,19]. A specific type of biological process such as anaerobic ammonium oxidation (anammox) could be effective for the removal of high levels of nitrogenous compounds along with organic matter [21–24]. The conventional process of biologically removing nitrogen includes two stages, namely, aerobic nitrification of ammonium to nitrate and anoxic denitrification of nitrate to  $N_2$  along with using organic compounds as electron acceptors [19,25]. Despite the advantages of conventional nitrification/denitrification such as high potential removal efficiency, relatively easy process control, low area required, and moderate costs, it is usually applied for treating wastewaters with total nitrogen concentrations less than  $100 \text{ mg N L}^{-1}$  [24]. Therefore, it is not an appropriate process for nitrogen removal from waste streams that contain a high nitrogen concentration such as landfill leachate [19]. The anammox process, which was initially discovered by Mulder and colleagues in a denitrifying fluidized bed reactor about 25 years ago, is a promising alternative method for the treatment of waste streams with high nitrogen concentrations. In this process, ammonium and nitrite are applied as the electron donor and the electron acceptor, respectively, then, the nitrogenous substance is converted into  $N_2$  gas [26–29]. In this autotrophic process, under anoxic conditions, ammonium is directly converted to  $N_2$  by autotrophic ammonium bacteria (such as the Planctomycete phylum bacteria including Brocadia, Kuenenia, Scalindua genera that have been found in wastewater and natural environments), which use nitrite as an electron acceptor. Compared with nitrification, anammox bacterial activity is 25-fold more intense, when using  $NO_2^-$  as the electron acceptor. By considering mass balances in different

chemostat experiments, overall, the most widely used stoichiometry for the anammox process is given in Eq. (1) [19,30,31]:



Anammox bacteria have a slow growth rate and a low biomass yield. Due to the fact that anammox bacteria are autotrophic, 100% conversion of ammonium to  $N_2$ , without the need for additional organic matter such as methanol, can take place and nitrate is produced from nitrite oxidation to provide the cells with enough reducing equivalents for carbon fixation through acetyl CoA pathways. Anammox bacteria can use carbon dioxide for their growth [18]. Researchers have reported two most likely pathways as the mechanism for the anammox process: (1) ammonium is oxidized by hydroxylamine to form hydrazine, and (2) the nitrite is reduced to nitric oxide, which then combines with ammonium to produce hydrazine via the hydrazine forming enzymes, with the uptake of one plus three low energy electrons [18,19].

As expected from their metabolism, ammonium bacteria consume ammonia and produce  $N_2$  gas with nitrite as the electron acceptor in the anammox process. The appropriate ratio between ammonium and nitrite is not 1:1, and the 1:1.32 ratio is used, it means that the excess 0.32 mol of nitrite is anaerobically oxidized to nitrate. The overall stoichiometry for the anammox process is the 1:1.32:0.26 ratio for the amounts of ammonium consumed, nitrite utilized, and nitrate formed, respectively [25,32].

The anammox process offers a variety of advantages such as higher nitrogen removal rates, no need for an organic carbon source, less oxygen requirements and reduced emission of  $N_2O$  during the oxidation of ammonia, lower operational costs, and smaller space requirements [19,31,33,34]. Furthermore, there are evidently lower volumes of bio-sludge production from the anammox process, compared with other previously applied methods. The anammox process, alone or in combination with other processes, has already been used to treat different types of industrial wastewaters such as wastewaters containing thiocyanate [35], and in the removal of  $17\beta$ -estradiol as an estrogen using the partial nitrification-anammox process [36], the removal of nitrogen in self-sustainable biofilm reactors [37], and the simultaneous removal of nitrate and low C/N domestic wastewater at low temperatures using the partial denitrification-anammox process [38]. As is reported in the literature, the anammox process has a versatility advantage in the treatment of wastewaters containing high levels of nitrogen. Therefore, in this study, in order to improve and overcome the limitations of the anammox process such as long start-up periods, a doubling time of about 10–14 d, and sensitivity to environmental conditions, granular sludge from the wastewater treatment plant of Pegah Tehran Pasteurized Milk was used. Then, the anammox bioreactor efficiency was evaluated for synthetic leachate treatment based on the removal of organic carbon, ammonium, and nitrite.

## 2. Materials and methods

### 2.1. Anammox reactor configuration

A lab-scale upflow anaerobic fixed-bed bioreactor in continuous mode was used for synthetic leachate treatment based on

the removal of ammonium and organic carbon by the anammox process. The details of the anammox reactor in this study have been described in our previous work [39]. In brief, the lab-scale bioreactor consisted of a double-walled Plexiglass cylindrical column (25 cm in height), including an inner cylinder (with internal and external diameters of 11 and 12 cm, respectively) and an outer cylinder as water jacket (with internal and external diameters of 14 and 15 cm, respectively). The anammox reactor was inoculated with 400 mL of granular sludge taken from upflow anaerobic sludge blanket (UASB) reactors in the wastewater treatment plant of Pegah Tehran Pasteurized Milk and inoculated in the anammox bioreactor. Initially, the mixed liquor volatile suspended solids (MLVSS) of the granular sludge was 12.77 g. The effective volume of the bioreactor was 1.8 L and the temperature was kept at  $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . Plastic media (Bee-Cell 2000) were used as biofilm support material due to their large surface area ( $650 \text{ m}^2 \text{ m}^{-3}$ ) and high porosity (pore volumes of up to 87%) and filled up to 50% of the total reactor volume. Synthetic wastewater flow to the bioreactor was regulated by decreasing COD/N ratio gradually from  $500 \text{ mg L}^{-1}$  to less than  $10 \text{ mg L}^{-1}$  in input in 130 d during the study. The composition of the synthetic leachate was based on a previous work [39]. During the whole study period (a total of 228 d), the anammox reactor received a synthetic wastewater continuous flow rate of  $1.8 \text{ L d}^{-1}$  and the HRT was gradually decreased from 24 to 6 h. pH was kept constant at the range of 7.5–8.0 during the study. In this stage, the aim of the study was to produce and adapt autotrophic bacteria for the anammox process and decrease heterotrophic bacterial growth rate in order to have anammox bio-sludge instead of sludge from UASB reactor. The C/N/P ratio of 100/5/1 was applied for the synthetic leachate (i.e., carbon, nitrogen, and phosphorous were provided as glucose, ammonium nitrate, and monopotassium phosphate, respectively).

## 2.2. Experimental setup

In this study, experiments were conducted in three stages, including stage 1: the assessment of the performance of the anammox process in the removal of ammonium and nitrite; stage 2: the assessment of the effects of HRT on process performance; and stage 3: the evaluation of the performance of the simultaneous removal of ammonium and organic carbon (glucose) in the anammox reactor. In stage 1, lasting approximately 1–130 d, autotrophic organisms were adapted by gradually increasing the concentration of nitrogen ( $\text{NH}_4$  and  $\text{NO}_2$ ) in the synthetic leachate inside the reactor as well as adjusting the  $\text{NH}_4/\text{NO}_2$  ratio to 1:1.32, for example, ammonium and nitrite concentrations were set to 300 and  $396 \text{ mg L}^{-1}$ , respectively. The constant temperature and pH of  $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and 7.5–8 were applied in the study, respectively. In stage 2, the effects of HRT on the performance of the reactor were assessed in the three HRTs of 6, 12, and 24 h. It should be mentioned that, in the end, the appropriate HRT of 6 h was achieved after 42 d of bioreactor activity. In stage 3, by considering the  $\text{NO}_2/\text{NH}_4$  ratio of 1.32 and organic carbon/ $\text{NH}_4$  ratio of 0.5, the synthetic leachate was prepared and injected into the reactor. The concentrations of ammonium and nitrite were increased from 100 and  $132 \text{ mg L}^{-1}$  to 500 and  $660 \text{ mg L}^{-1}$ , respectively, and the COD concentration was increased from 50 to  $250 \text{ mg L}^{-1}$ . This period lasted approximately 56 d. Sampling was conducted daily in order to carefully check the possible changes of the intended parameters.

Furthermore, nitrate was also measured as another important parameter of this stage.

## 2.3. Analytical methods

Ammonium and nitrite were analyzed by the colorimetric method and nitrate was measured by the spectrophotometric method based on the standard methods [40]. The soluble COD was measured through colorimetric method by closed reflux method [40]. pH and dissolved oxygen (DO) were measured by a portable pH meter (Metrohm, model 826) and a DO meter (EU-tech, model 1500). The biomass of the ammonium oxidizing bacteria (AOB) culture was measured using the standard methods [40]. The measurement was done in triplicate and the mean value was reported.

## 3. Results and discussion

In the present study, the removal of organic carbon and ammonium in a fixed-bed bioreactor pilot plant by the anammox process was assessed. In the start-up period, granular sludge was obtained from the UASB reactor of the wastewater plant of Pegah Tehran Pasteurized Milk. The concentrations of the sludge biomass and MLVSS in the bioreactor in the first and final stages of the operation were measured as 12.77 and  $24.55 \text{ g VSS L}^{-1}$ , respectively. The growth rate of the biomass in the bioreactor was  $11.78 \text{ g VSS L}^{-1}$ . Based on our experiments, the biomass growth rate had a twofold increase. The adaptation of granular sludge to the anammox process was carried out by gradually decreasing the carbon source in the synthetic leachate and consequently raising the competitive condition for the carbon source among the heterotrophic bacteria (Fig. 1).

This condition led to the start of the endogenous phase and continued until the death of the heterotrophic bacteria, which eventually disappeared in the bioreactor. Then, the autotrophic bacteria become predominant by gradually increasing the nitrogen source of the synthetic leachate. In this step, the adaptation and growth of the autotrophic bacteria were intended and there is no observation of significant ammonium nitrogen removal [41,42]. The summary of main steps, characteristics, and performance of the anammox process during the whole study is presented in Table 1.

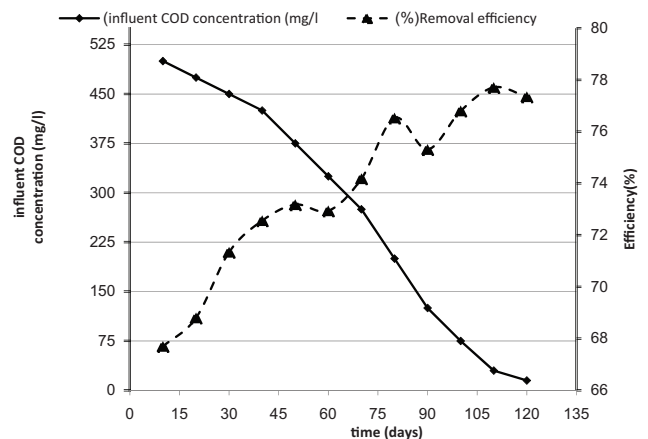


Fig. 1. Experiments of organic carbon removal during anammox process.

Table 1  
The main steps, characteristics, and performance of the anammox process in this study

Period	Operation day	Type wastewater	Influent COD (mg L <sup>-1</sup> )**	Influent NO <sub>2</sub> (mg L <sup>-1</sup> )**	Influent NH <sub>4</sub> (mg L <sup>-1</sup> )**	COD removal (%)	NO <sub>2</sub> removal (%)	NH <sub>4</sub> removal (%)	Performance
1	1–130	Synthetic leachate (C/N/P: 100/5/1)	500 to fewer than 10	6.6–396	5–300	–	91.12	76.69	Reduce heterotroph bacteria and increase AOBs and adaptation
2	130–172*	Synthetic leachate	–	396	300	–	95.16	80.56	HRT optimization (6 h)
3	172–228	Synthetic leachate	50–250	132–660	100–500	72.63	78.14	22.28	Stable condition*

\*Optimum condition: HRT = 6 h, volumetric loading rate: 0.15 kg m<sup>-3</sup> d<sup>-1</sup>  
\*\*Increasing or decreasing gradually.

3.1. Performance of anammox process in removing ammonium and nitrite

In this stage, in order to establish appropriate sludge and to accelerate the growth of AOB, the lowest ammonium and nitrite concentrations in the influent bioreactor leachate were used, that is, 5 and 6.6 mg L<sup>-1</sup>, respectively. In this period, lasting for 130–170 d, a fluctuation between influent and effluent ammonium concentrations ranging from 5 to 30 mg NH<sub>4</sub> L<sup>-1</sup> was observed, in which the ammonium concentration in the effluent was more than that of the influent. The fluctuations in the ammonium concentration range can be attributed to the changing growth conditions of the sludge from low carbon concentrations to high nitrogen concentrations. In other words, during this period, most of the main bacteria, including the nitrifying bacteria, were destroyed, consequently, effluent ammonium concentrations increased. This change in the influent composition may have been the cause of the disintegration of the nitrifying bacteria and, consequently, increased ammonium concentrations in the effluent [43]. In addition, due to the low concentration of ammonium in the biomass, the organic carbon residue from the previous stage was consumed as the carbon source and the electron donor under anaerobic conditions. This resulted in an increase in ammonium concentrations and also decreased the efficiency of ammonium removal [44]. Therefore, it can be concluded that adapting autotrophic bacteria by increasing their nitrogen source in the anammox process is not a suitable method. However, compared with ammonium concentrations, variations in nitrite concentrations during the operation were completely different. In fact, nitrite was completely consumed along with the organic carbon residue in the denitrification process. It should be noted that nitrogen gas bubbles could be seen inside the sludge as an indicator of the denitrification process. The results are in accordance with previously conducted studies in this field [45,46]. After adapting the bacterial mass and gradually increasing the volumetric load of nitrogen, ammonia started to decrease in the effluent. Fig. 2 shows the efficiency of ammonium removal during

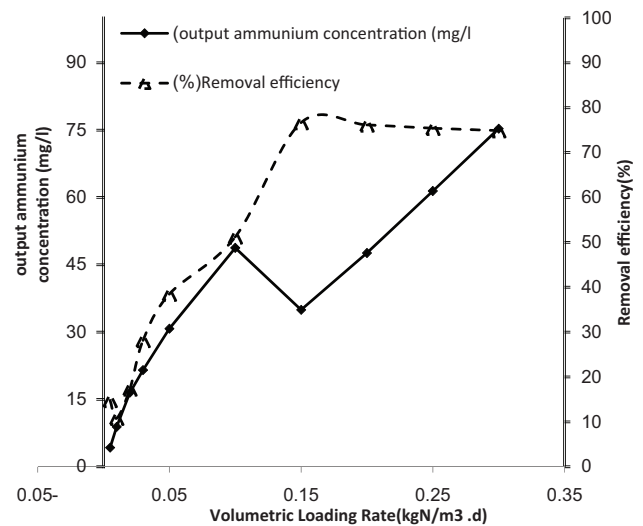


Fig. 2. Ammonium removal efficiency (%) with gradually increasing VLR (kg N m<sup>-3</sup> d<sup>-1</sup>).

the operation time along with the increased VLR of nitrogen. Based on the results obtained from the present study, the maximum efficiency of ammonium removal of 76.69% was achieved at a VLR of 0.15 kg m<sup>-3</sup> d<sup>-1</sup>. Furthermore, it was seen that the reactor is at a stable condition, and increasing the ammonium VLR from 0.15 to 0.3 kg m<sup>-3</sup> d<sup>-1</sup> could not significantly reduce ammonium concentrations. As can be seen in Fig. 2, the average and maximum removal efficiencies at a VLR of 0.2 kg m<sup>-3</sup> d<sup>-1</sup> were 75.5% and 76.18%, respectively. Similar results have been reported by other studies too [44].

In addition to tracking changes in ammonium concentrations during the operation, changes in nitrite concentrations were also recorded as another parameter of the present study. Due to the adaptation which occurred at the system, and also lack of organic compounds which resulted in the death of the bacterial mass responsible for nitrification, a significant decrease in nitrite nitrogen removal was recorded. Based on

the experiments, the abovementioned conditions resulted in a gradual predomination of the bacterial mass of the anammox process in the reactor. The maximum removal of nitrite after 70 d of operation and at a VLR of  $0.132 \text{ kg m}^{-3} \text{ d}^{-1}$  was 91.12%. It should be mentioned that changes in nitrite concentrations during the stabilized condition were similar to those of ammonium; in other words, increasing the nitrogen VLR from 0.264 to  $0.396 \text{ kg m}^{-3} \text{ d}^{-1}$  had no significant effect on nitrite removal, which is shown in Fig. 3. The average and maximum nitrite removal efficiencies at a VLR of  $0.264 \text{ kg m}^{-3} \text{ d}^{-1}$  were 82.85% and 83.16%, respectively. Other studies have also reported similar results [47]. It is a considerable point that the removal efficiency of ammonium and nitrite decreases when nitrogen VLR increases in the stable condition of the reactor. It is mainly due to the fact that high concentrations of residual nitrite in the reactor is known as a strong biotoxic inhibitor to the anammox process [19,31,48]. In the present study, the residual nitrite concentration was between 45 and  $70 \text{ mg L}^{-1}$  when the VLR was between 0.264 and  $0.396 \text{ kg m}^{-3} \text{ d}^{-1}$ . In this regard, Strous et al. [25] reported that the biotoxic effects of nitrite are in the range of  $70\text{--}180 \text{ mg NO}_2 \text{ L}^{-1}$ , which could strongly inhibit the biomass of the anammox reactor. In another study, authors reported that the toxic and inhibitory effect of nitrite concentration in anammox process was  $224 \pm 10 \text{ mg NO}_2 \text{ L}^{-1}$  [53]. As can be seen, different ranges have been reported for the inhibitory concentrations of nitrite in previous studies, and there is no agreement among scientists on the concentration of nitrite in terms of toxicity and inhibitory effects. However, it is clear that increasing the VLR of nitrogen can lead to a decrease in the activity of the AOBs responsible for the anammox process, due to the presence of residual  $\text{NO}_2$ . Therefore, it can be concluded that, compared with the amount of nitrogen, nitrogen VLR affects ammonium removal efficiency more significantly [49]. In addition, residual concentrations of nitrite inside the bioreactor should be carefully monitored in order to prevent the inhibition of the anammox process. In the present study, the removal efficiencies of total nitrogen (TN) and  $\text{NH}_4$  were 79.2% and 75.5%, respectively, which indicates the higher removal of TN, compared with  $\text{NH}_4$ . This could be due to the occurrence of denitrification inside the bioreactor, which was the dominant process at this stage, compared with the anammox process.

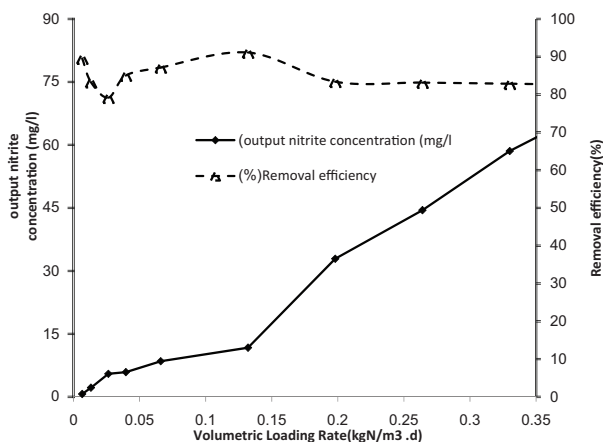


Fig. 3. Nitrite removal efficiency (%) with gradually increasing VLR ( $\text{kg N m}^{-3} \text{ d}^{-1}$ ).

It should be noted that the removal efficiencies of TN and  $\text{NH}_4$  are not significantly different, which could be due to the short-term predominance of the denitrification process in the bioreactor. This has also been reported by other studies [47]. Based on our results, the maximum removal efficiencies of ammonium and nitrite at a VLR of  $0.15 \text{ kg m}^{-3} \text{ d}^{-1}$  were 76.69% and 91.12%, respectively. Therefore, the maximum removal of nitrogen occurs at a VLR of  $0.15 \text{ kg m}^{-3} \text{ d}^{-1}$ . In other studies, a value of  $0.12 \text{ kg m}^{-3} \text{ d}^{-1}$  has been reported [30], which is somewhat consistent with the results of the present study. Additionally, in another study, nitrite was reported to be the main inhibitor, at a VLR of more than  $0.16 \text{ kg m}^{-3} \text{ d}^{-1}$ , decreasing nitrogen removal efficiency [50]. In the present study, increasing the VLR from 0.15 to  $0.3 \text{ kg m}^{-3} \text{ d}^{-1}$  decreased the removal efficiency of ammonium and nitrite, mainly due to the inhibitory effects of nitrite. Moreover, applying the SHARON process (partial denitrification) as pretreatment could definitely increase the removal of nitrogen compounds.

### 3.2. Effects of HRT on the performance of anammox process

In this stage, after achieving a stable condition in the bioreactor (an influent ammonia concentration of  $300 \text{ mg L}^{-1}$ , with a constant nitrite concentration of  $396 \text{ mg L}^{-1}$ ), HRT was gradually reduced from 24 to 12 and then to 6 h. As can be seen in Fig. 4, the maximum removal efficiencies of ammonia were 83.8%, 78.87%, and 80.56% corresponding to HRTs of 24, 12, and 6 h, respectively. Accordingly, the maximum removal efficiencies of nitrite were 75.35%, 89.93%, and 95.16%, respectively. In the present study, maximum removal occurred at the lowest HRT. Other studies have also presented similar trends [51]. This could be due to lack of the inhibitory effects of nitrite at low HRTs, and the washing out of the by-products resulting from the anammox process at low HRTs [52]. In a study conducted by Egli et al. [53], by returning the effluent to the reactor, they were able to achieve the maximum removal of ammonium and nitrite at the lowest possible HRT at a molar ratio of  $1.32 \text{ NO}_2/\text{NH}_4$ . Therefore, HRT and the molar ratio of  $\text{NO}_2/\text{NH}_4$  can be taken as the two main factors in meeting the maximum removal of nitrogen [52]. In contrast, in this study, gradually decreasing HRT

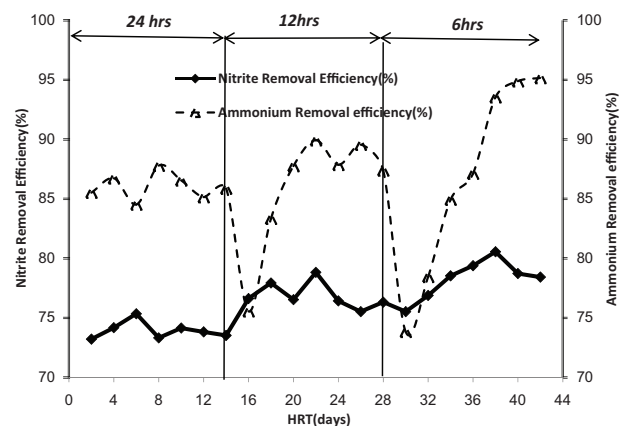


Fig. 4. Variation of ammonium and nitrite concentration with gradually decreasing HRTs.

resulted in produced nitrate concentrations of 29.86, 29.74, and 28.56 mg L<sup>-1</sup>, which did not differ significantly.

### 3.3. Simultaneous removal of ammonium and organic carbon in anammox process

In stage 3, the simultaneous removals of ammonium and organic carbon, and nitrogen and organic carbon were performed at seven regular steps. As shown in Fig. 5, the maximum and minimum COD removal efficiencies achieved were 72.63% and 33.35%, respectively. The maximum removal was recorded at the final stage of the operation, while the minimum removal efficiency occurred at the beginning of the operation. At the beginning, the heterotrophic bacteria faced some problems in developing and growing inside the bioreactor due mainly to the dominance of anammox bacteria. It was expected that the minimum removal efficiency of organic carbon would be achieved at this condition. However, a gradual increase in organic compounds, which negatively affect the anammox process, made the condition suitable for heterotrophic bacteria, which contribute to the denitrification process. It should be mentioned that organic carbon removal is indeed a function of the leachate's age. In other words, compared with old leachate, fresh leachate has higher concentrations of biodegradable organic carbon, which could contribute to a higher removal efficiency for fresh leachates [44].

In addition, gradually increasing the carbon source resulted in higher removal of NO<sub>2</sub>. As shown in Fig. 6, the weekly average of the maximum removal efficiency of NO<sub>2</sub> was 78.14%. However, the weekly average of ammonium removal efficiency was 22.28% (Fig. 6), which is considerably lower than the corresponding amount for NO<sub>2</sub>. This phenomenon can be due to the presence of organic compounds along with high concentrations of nitrite inside the bioreactor. In other words, high concentrations of NO<sub>2</sub> have a catalyzing effect on the performance of the anammox process. It should be noted that in natural environments, the anaerobic oxidants of ammonium and heterotrophic biomass responsible for denitrification are always in competition for taking NO<sub>2</sub>. However, due to the difference in their growth rates and at the presence of organic compounds in the natural environment, anammox bacteria are not capable of competing with the other group for a long period. Hence, the autotrophic bacteria of AOBs cannot continue their existence and denitrifying bacteria become the dominant ones and denitrification takes place [32]. Based on the results obtained from the present study, the removal efficiencies of ammonium and nitrite are conversely related to COD concentrations; in other words, increasing the concentration of COD results in a decrease in ammonium removal efficiency (Fig. 6). As can be seen in Fig. 5 and Table 1, the maximum COD, NO<sub>2</sub>, and NH<sub>4</sub> removal achieved under stable conditions (i.e., HRT 6 h, VLR 0.15 kg m<sup>-3</sup> d<sup>-1</sup>) were 72.63%, 78.14%, and 22.28%, respectively. Due to the consumption of nitrite by organic compounds, the removal efficiency of nitrite nitrogen increases, while ammonium removal is dependent on the appropriate performance of the anammox process. One approach to overcoming this problem is the application of the SHARON process or denitrification as the pretreatment and post-treatment of the anammox process. Therefore, partial oxidation as pretreatment could improve the removal efficiency of nitrogen compounds

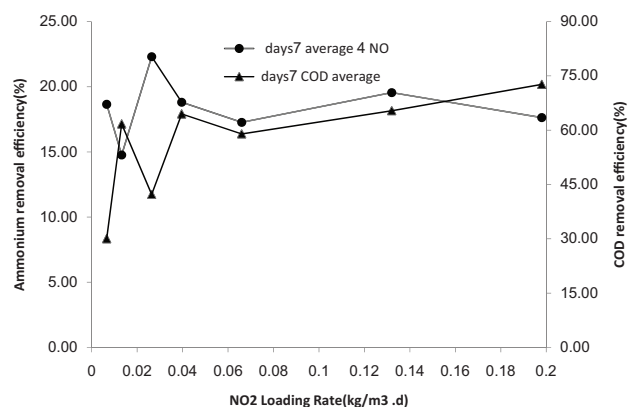


Fig. 5. Seven days average variation removal efficiency of NH<sub>4</sub> and COD with gradually increasing NO<sub>2</sub> loading rate.

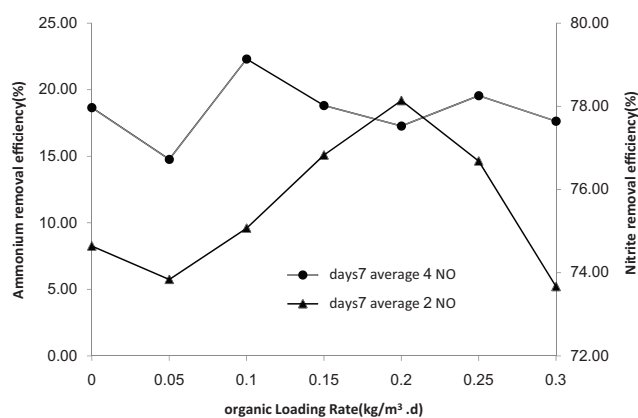


Fig. 6. Seven days average variation removal efficiency of NH<sub>4</sub> and NO<sub>2</sub> with gradually increasing organic loading rate.

at high loads of organic compounds [19,32]. Thus, conducting the abovementioned processes contributes to an increase in nitrogen removal efficiency. This also increases the ability of the process against high concentrations of organic compounds (load shocks) and their negative effects. It should be noticed that the anammox process plays an important role in such combinations of processes, and that the removal efficiency of the pollutants from the leachate mostly depends on the performance of the anammox process [19,53].

## 4. Conclusions

In the present study, after establishing appropriate operational conditions in the reactor, high efficiencies for ammonium and nitrite removal were achieved. The gradual decrease of HRT led to the maximum removal of ammonium and nitrite at the lowest HRT (6 h). However, in the simultaneous removal of ammonium and organic carbon stage, the removal efficiency of ammonium was low (22.28%). This phenomenon can be attributed to the presence of organic compounds together with high concentrations of nitrite inside the bioreactor. In other words, high concentrations of NO<sub>2</sub> have an accelerating effect on the performance of the anammox process. It can be concluded that the removal efficiencies of ammonium and nitrite are conversely related to

COD concentrations, that is, increasing the concentration of COD results in a decrease in ammonium removal efficiency. Due to the consumption of nitrite by organic compounds, the removal efficiency of nitrite nitrogen increases, while ammonium removal is dependent on the appropriate performance of the anammox process. One approach to overcoming this problem is the application of the SHARON process or denitrification as the pretreatment and post-treatment of the anammox process. Therefore, partial oxidation as pretreatment could improve the removal efficiency of nitrogen compounds at high loads of organic compounds.

### Conflict of interests

Authors have no conflict of interests.

### References

- Z.G. Rahmat, M.V. Niri, N. Alavi, G. Goudarzi, A.A. Babaei, Z. Baboli, M. Hosseinzadeh, Landfill site selection using GIS and AHP: a case study: Behbahan, Iran, *KSCE J. Civil. Eng.*, 21 (2017) 111–118.
- P. Ghosh, I.S. Thakur, Enhanced removal of COD and color from landfill leachate in a sequential bioreactor, *Bioresour. Technol.*, 170 (2014) 10–19.
- S. Renou, J.G. Givaudan, S. Poulain, F. Dirassouyan, P. Moulin, Landfill leachate treatment: review and opportunity, *J. Hazard. Mater.*, 150 (2008) 468–493.
- G. Hassani, A. Babaei, A. Takdastan, M. Shirmardi, F. Yousefian, M. Mohammadi, Occurrence and fate of 17 $\beta$ -estradiol in water resources and wastewater in Ahvaz, Iran, *Glob. NEST. J.*, 18 (2016) 855–866.
- M. Keshtkar, S. Dobaradaran, F. Soleimani, V.N. Karbasdehi, M.J. Mohammadi, R. Mirahmadi, F.F. Ghasemi, Data on heavy metals and selected anions in the Persian popular herbal distillates, *Data Brief*, 8 (2016) 21–25.
- M. Mohammadi, J. Salari, A. Takdastan, M. Farhadi, P. Javanmardi, A. Yari, S. Dobaradaran, H. Almasi, S. Rahimi, Removal of turbidity and organic matter from car wash wastewater by electrocoagulation process, *Desal. Wat. Treat.*, 68 (2017) 122–128.
- M. Mohammadi, A. Takdastan, S. Jorfi, A. Neisi, M. Farhadi, A. Yari, S. Dobaradaran, Y. Khaniabadi, Electrocoagulation process to chemical and biological oxygen demand treatment from carwash grey water in Ahvaz megacity, Iran, *Data Brief*, 11 (2017) 634–639.
- M.V. Niri, A.H. Mahvi, M. Alimohammadi, M. Shirmardi, H. Golastanifar, M.J. Mohammadi, A. Naeimabadi, M. Khishdost, Removal of natural organic matter (NOM) from an aqueous solution by NaCl and surfactant-modified clinoptilolite, *J. Water Health*, 13 (2015) 394–405.
- N. Reynier, L. Coudert, J.-F. Blais, G. Mercier, S. Besner, Treatment of contaminated soil leachate by precipitation, adsorption and ion exchange, *J. Environ. Chem. Eng.*, 3 (2015) 977–985.
- Y. Gong, S. Wang, H. Xu, Y. Guo, X. Tang, Partial oxidation of landfill leachate in supercritical water: optimization by response surface methodology, *Waste Manage.*, 43 (2015) 343–352.
- K. Klein, A. Kivi, N. Dulova, I. Zekker, E. Mölder, T. Tenno, M. Trapido, T. Tenno, A pilot study of three-stage biological-chemical treatment of landfill leachate applying continuous ferric sludge reuse in Fenton-like process, *Clean Technol. Environ. Policy*, 19 (2017) 541–551.
- K. Klein, E. Kattel, A. Goi, A. Kivi, N. Dulova, A. Saluste, I. Zekker, M. Trapido, T. Tenno, Combined treatment of pyrogenic wastewater from oil shale retorting, *Oil Shale*, 34 (2017) 82.
- R. He, B.-H. Tian, Q.-Q. Zhang, H.-T. Zhang, Effect of Fenton oxidation on biodegradability, biotoxicity and dissolved organic matter distribution of concentrated landfill leachate derived from a membrane process, *Waste Manage.*, 38 (2015) 232–239.
- Y. Yu, Z. Chen, Z. Guo, Z. Liao, L. Yang, J. Wang, Z. Chen, Removal of refractory contaminants in municipal landfill leachate by hydrogen, oxygen and palladium: a novel approach of hydroxyl radical production, *J. Hazard. Mater.*, 287 (2015) 349–355.
- R. Bakhshoodeh, N. Alavi, A.S. Mohammadi, H. Ghanavati, Removing heavy metals from Isfahan composting leachate by horizontal subsurface flow constructed wetland, *Environ. Sci. Pollut. Res.*, 23 (2016) 12384–12391.
- R. Bakhshoodeh, N. Alavi, M. Majlesi, P. Paydary, Compost leachate treatment by a pilot-scale subsurface horizontal flow constructed wetland, *Ecol. Eng.*, 105 (2017) 7–14.
- M. Cotman, A.Ž. Gotvajn, Comparison of different physico-chemical methods for the removal of toxicants from landfill leachate, *J. Hazard. Mater.*, 178 (2010) 298–305.
- S.S. Shalini, K. Joseph, Nitrogen management in landfill leachate: application of SHARON, ANAMMOX and combined SHARON-ANAMMOX process, *Waste Manage.*, 32 (2012) 2385–2400.
- S.W. Van Hulle, H.J. Vandeweyer, B.D. Meesschaert, P.A. Vanrolleghem, P. Dejans, A. Dumoulin, Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams, *Chem. Eng. J.*, 162 (2010) 1–20.
- L.-k. Wang, G.-m. Zeng, Z.-h. Yang, L.-l. Luo, H.-y. Xu, J. Huang, Operation of partial nitrification to nitrite of landfill leachate and its performance with respect to different oxygen conditions, *Biochem. Eng. J.*, 87 (2014) 62–68.
- I. Zekker, E. Rikmann, K. Kroon, A. Mandel, J. Mihkelson, T. Tenno, Ameliorating nitrite inhibition in a low-temperature nitrification-anammox MBBR using bacterial intermediate nitric oxide, *Int. J. Environ. Sci. Technol.*, 14 (2017) 2343–2356.
- T. Tenno, K. Uiga, A. Mashirin, I. Zekker, E. Rikmann, Modeling closed equilibrium systems of H<sub>2</sub>O–dissolved CO<sub>2</sub>–solid CaCO<sub>3</sub>, *J. Phys. Chem. A*, 121 (2017) 3094–3100.
- M. Raudkivi, I. Zekker, E. Rikmann, P. Vabamäe, K. Kroon, T. Tenno, Nitrite inhibition and limitation—the effect of nitrite spiking on anammox biofilm, suspended and granular biomass, *Water. Sci. Technol.*, 75 (2017) 313–321.
- L. Daija, A. Selberg, E. Rikmann, I. Zekker, T. Tenno, T. Tenno, The influence of lower temperature, influent fluctuations and long retention time on the performance of an upflow mode laboratory-scale septic tank, *Desal. Wat. Treat.*, 57 (2016) 18679–18687.
- M. Strous, J. Heijnen, J. Kuunen, M. Jetten, The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms, *Appl. Microbiol. Biotechnol.*, 50 (1998) 589–596.
- A. Mulder, A.A. van de Graaf, L. Robertson, J. Kuunen, Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor, *FEMS Microbiol. Ecol.*, 16 (1995) 177–183.
- T. Tenno, E. Rikmann, I. Zekker, T. Tenno, L. Daija, A. Mashirin, Modelling Equilibrium Distribution of Carbonaceous Ions and Molecules in a Heterogeneous System of CaCO<sub>3</sub>-Water-Gas, *Proc. Estonian Academy of Sciences*, Vol. 65, 2016, pp. 68–77.
- I. Zekker, E. Rikmann, A. Mandel, K. Kroon, A. Seiman, J. Mihkelson, T. Tenno, T. Tenno, Step-wise temperature decreasing cultivates a biofilm with high nitrogen removal rates at 9 C in short-term anammox biofilm tests, *Environ. Technol.*, 37 (2016) 1933–1946.
- E. Rikmann, I. Zekker, M. Tomingas, T. Tenno, L. Loorits, P. Vabamäe, A. Mandel, M. Raudkivi, L. Daija, K. Kroon, Sulfate-reducing anammox for sulfate and nitrogen containing wastewaters, *Desal. Wat. Treat.*, 57 (2016) 3132–3141.
- Y.-H. Ahn, Sustainable nitrogen elimination biotechnologies: a review, *Process Biochem.*, 41 (2006) 1709–1721.
- 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.
- B. Molinuevo, M.C. García, D. Karakashev, I. Angelidaki, Anammox for ammonia removal from pig manure effluents: effect of organic matter content on process performance, *Bioresour. Technol.*, 100 (2009) 2171–2175.
- U. van Dongen, M. Jetten, M. Van Loosdrecht, The SHARON-Anammox process for treatment of ammonium rich wastewater, *Water. Sci. Technol.*, (2001) 153–160.

- [34] C. Fux, H. Siegrist, Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitritation/anammox: environmental and economical considerations, *Water. Sci. Technol.*, 50 (2004) 19–26.
- [35] Q.-Q. Chen, H. Chen, Z.-Z. Zhang, L.-X. Guo, R.-C. Jin, Effects of thiocyanate on granule-based anammox process and implications for regulation, *J. Hazard. Mater.*, 321 (2017) 81–91.
- [36] P. Huang, S.T. Mukherji, S. Wu, J. Muller, R. Goel, Fate of 17 $\beta$ -estradiol as a model estrogen in source separated urine during integrated chemical P recovery and treatment using partial nitritation-anammox process, *Water. Res.*, 103 (2016) 500–509.
- [37] P. Chatterjee, M. Ghangrekar, S. Rao, Development of anammox process for removal of nitrogen from wastewater in a novel self-sustainable biofilm reactor, *Bioresour. Technol.*, 218 (2016) 723–730.
- [38] R. Du, S. Cao, S. Wang, M. Niu, Y. Peng, Performance of partial denitrification (PD)-ANAMMOX process in simultaneously treating nitrate and low C/N domestic wastewater at low temperature, *Bioresour. Technol.*, 219 (2016) 420–429.
- [39] A.A. Babaei, R. Azadi, N. Jaafarzadeh, N. Alavi, Application and kinetic evaluation of upflow anaerobic biofilm reactor for nitrogen removal from wastewater by Anammox process, Iran. *J. Environ. Health Sci. Eng.*, 10 (2013) 2–7.
- [40] W.E. Federation, A.P.H. Association, Standard Methods for the Examination of Water and Wastewater, American Public Health Association (APHA), Washington, DC, USA, 2005.
- [41] H.-g. Zhang, S.-q. Zhou, Treating leachate mixture with anaerobic ammonium oxidation technology, *J. Central South Univ. Technol.*, 13 (2006) 663–667.
- [42] A. Dapena-Mora, J. Campos, A. Mosquera-Corral, M. Jetten, R. Méndez, Stability of the ANAMMOX process in a gas-lift reactor and a SBR, *J. Biotechnol.*, 110 (2004) 159–170.
- [43] A.A. Van de Graaf, P. de Bruijn, L.A. Robertson, M.S. Jetten, J.G. Kuenen, Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor, *Microbiology*, 142 (1996) 2187–2196.
- [44] C.-C. Wang, P.-H. Lee, M. Kumar, Y.-T. Huang, S. Sung, J.-G. Lin, Simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) in a full-scale landfill-leachate treatment plant, *J. Hazard. Mater.*, 175 (2010) 622–628.
- [45] H. Chen, S. Liu, F. Yang, Y. Xue, T. Wang, The development of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal, *Bioresour. Technol.*, 100 (2009) 1548–1554.
- [46] G. Cema, J. Surmacz-Górska, K. Miksch, Implementation of Anammox Process in the Membrane Assisted Bioreactor, Integration and Optimization of Urban Sanitation Systems, Joint Polish-Swedish Seminars, 2004, pp. 81–92.
- [47] S. Cho, Y. Takahashi, N. Fujii, Y. Yamada, H. Satoh, S. Okabe, Nitrogen removal performance and microbial community analysis of an anaerobic up-flow granular bed anammox reactor, *Chemosphere*, 78 (2010) 1129–1135.
- [48] I. Tsushima, Y. Ogasawara, T. Kindaichi, H. Satoh, S. Okabe, Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors, *Water. Res.*, 41 (2007) 1623–1634.
- [49] E.S. Chian, F.B. DeWalle, Characterization of soluble organic matter in leachate, *Environ. Sci. Technol.*, 11 (1977) 158–163.
- [50] J. Liu, J.e. Zuo, Y. Yang, S. Zhu, S. Kuang, K. Wang, An autotrophic nitrogen removal process: short-cut nitrification combined with ANAMMOX for treating diluted effluent from an UASB reactor fed by landfill leachate, *J. Environ. Sci.*, 22 (2010) 777–783.
- [51] Y. He, G. Zhou, Y. Zhao, Nitrification with high nitrite accumulation for the treatment of “old” landfill leachates, *Environ. Eng. Sci.*, 24 (2007) 1084–1094.
- [52] Z. Liang, J. Liu, Landfill leachate treatment with a novel process: anaerobic ammonium oxidation (anammox) combined with soil infiltration system, *J. Hazard. Mater.*, 151 (2008) 202–212.
- [53] K. Egli, U. Fanger, P.J. Alvarez, H. Siegrist, J.R. van der Meer, A.J. Zehnder, Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate, *Arch. Microbiol.*, 175 (2001) 198–207.