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EFFECT OF HYDRAULIC RETENTION TIME ON NITROGEN REMOVAL IN DOMESTIC WASTEWATER BY PARTIAL NITRITATION AND ANAMMOX PROCESSES

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Abstract

The nitrogen treatment technology using the Anammox process is known to have advantages over conventional technology of nitrification - denitrification. For the purpose of evaluating the effect of hydraulic retention time to nitrogen removal in domestic wastewater by Anammox process, the authors conducted the study on partial nitritation and Anammox reactors, separately. Partial nitritation (PN) reactor used Felibendy plate material with *Nitrosomonas* bacteria while Anammox (AX) reactor used Felibendy cubes carrier material with strain *Candidatus Brocadia anammoxidans*. This study was implemented during 210 days. The nitrogen treatment efficiency of the system was evaluated with different hydraulic retention times (HRTs). The short HRT of 4.5 hours in the AX reactor affected to the total nitrogen treatment efficiency is low of 52.76 ± 1.29%. With the hydraulic retention times in PN + AX reactors of 9 and 6 hours, the effluent quality met the requirements of B-column according to QCVN 14:2008/BTNMT or QCVN 40:2011/BTNMT.

Keywords: Nitrosomonas; *Candidatus Brocadia anammoxidans*; partial nitritation process; Anammox process; nitrogen treatment.

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1. Introduction

With the socio-economic development, the amount of domestic wastewater discharged into water bodies is increasing and creating challenges to the environment. The main components of domestic wastewater are suspended solids, organic substances, nutrients and microorganisms. This untreated wastewater will cause secondary pollution for the receiving water source or water quality declination. Nitrogen compounds are some of quality control components in National Technical Regulations on natural water source, receiving source and discharge. According to QCVN 14:2008/BTNMT for domestic wastewater [1] or QCVN 40:2011/BTNMT for industry wastewater [2], before discharging into the receiving bodies, which serve as sources for domestic and none-domestic water supply purposes, total nitrogen concentrations must be less than 20 mg N/l and 40mg/l for A-column and B-column, respectively.

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For the treatment of nitrogen compounds in wastewater, centralized wastewater treatment plants use conventional biological treatment methods (aerobic); Advanced biological treatment (nitrogen compounds and phosphorus compounds treatment). With the wastewater treatment technologies being applied in Vietnam, some technologies can not fully handle nitrogen such as trickling biofilter (TF) or conventional activated sludge (CAS) technology. Besides, some other technologies require internal sludge recirculation, or require large amounts of oxygen, for example anoxic oxic (AO), anaerobic – anoxic – oxic (A2O), sequencing batch reactor (SBR) or additional carbon sources. Applying a different processing technology to overcome the above weaknesses is very necessary.

The discovery of anammox bacteria led to the development of a fully autotrophic process that does not required chemical and uses less energy for aeration or mixing, offering the plants [3]. The technology of nitrogen treatment by Anammox process need firstly, partial nitritation (partial oxidation of ammonium to nitrite, Eq. (1)) and secondly, the anammox process (anoxic combination of ammonium and nitrite to form dinitrogen gas, Eq. (2)) [4].

$$NH_4^+ + 0.83O_2 \rightarrow 0.45NH_4^+ + 0.55NO_2^- + 0.55H_2O + 1.1H^+ \tag{1}$$

$$NH_4^+ + 1.32NO_2 + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \tag{2}$$

The application of the partial nitritation and anammox process in municipal wastewater treatment can convert them from energy consuming into energy producing process. Compared to conventional biological nitrogen removal processes, the application of the partial nitriation and anammox process can reduce the operation expenses by 60%, eliminates the need for external carbon sources and the waste activated sludge is much lower [5]. Furthermore, the process reduces the greenhouse gas emissions by 90% since CO_2 is consumed and there are no N_2O emissions [6]. Hydraulic retention time is one of influencing factors for the anammox process [7, 8]. A practical purpose while applying anammox is to pursue a shorter HRT for higher nitrogen loading rate. So in this study, the authors used PN and AX reactor to evaluate the effect of nitrogen treatment on domestic wastewater to meet the requirements of the receiving source. The main purpose of the study was (i) to evaluate the effect of nitrogen treatment on domestic effluent of the model system, (ii) to determine the appropriate water retention time of the system.

2. Material and method

2.1. Partial nitritation (PN) and Anammoxreactor (AX) system

The PN + AX reactor system consists of Partial nitritation (PN) reactor and Anammox (AX) reactor as shown in Fig. 1. The PN reactor [9] is rectangular in the bottom size of 10×20 (cm), height 31 cm, total volume $V = 6.2L$. Inside the PN reactor, there is a Felibendy material plate (16 cm × 22 cm) implanted with *Nitrosomonas* bacteria contributed by Institute of Tropical Biology, Vietnam.

The AX reactor is a circular cylinder with an inner diameter of 7.1 cm, a height of 41 cm, a useful volume of 1.62 liters [10, 11]. Within the reaction column using 1 × 1 × 0.8 cm Felibendy cubes, anammox bacteria were cultured by the Meidensa company (Japan), using the Anammox strain *Candidatus Brocadia anammoxidans*.

2.2. Wastewater and operating parameters

The study used the domestic wastewater from the three-compartment septic tank at the National University of Civil Engineering. In order to simulate wastewater from the combined sewerage and

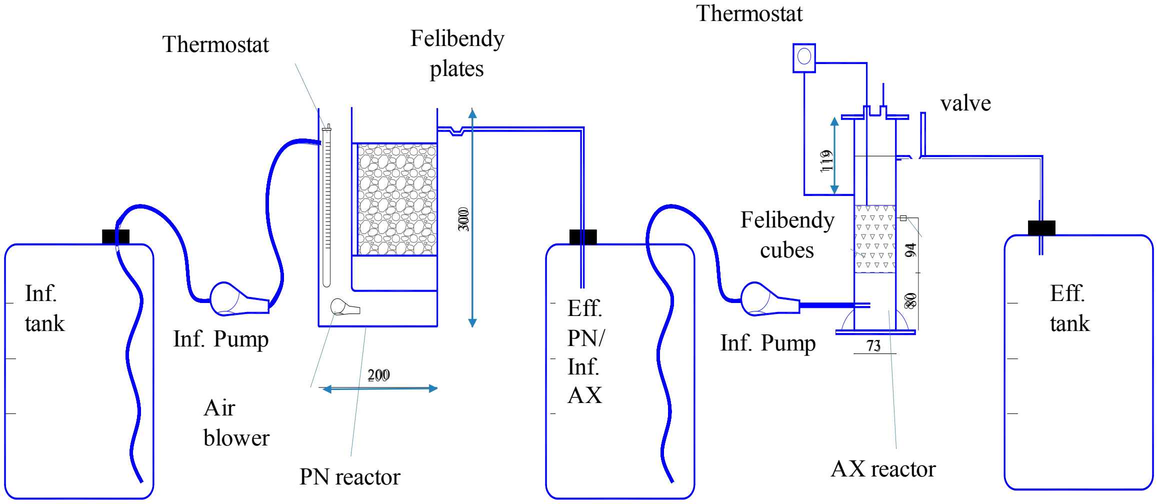


Figure 1. Schematic diagram of Partial Nitritation and Anammox reactor system

drainage system in the rainy season and dry season and the separated sewerage system, wastewater was diluted with gray water in the ratio 1 : 3 (period 1) and 1 : 2 (period 2). The non-diluted wastewater was used in period 3 to simulate separated sewerage system. Partial nitritation (PN) reactor was operated under aerobic conditions (DO ≈ 2 mg/l) and Anammox (AX) reactor under anaerobic conditions (DO < 0.5 mg/l).

The PN reactor is responsible for the conversion of part of ammonium to nitrite to produce nitrite/ammonium suitable ratio for the Anammox process. In order to take place the partial nitritation by *Nitrosomonas* bacteria, the HRT should not be prolonged due to that ammonium will be able to transform to nitrate, but also should not be too short because of insufficient time for transformation process. Therefore, the study will conduct experiments with the HRT in the first period (start-up period) is 18h, then will gradually decrease to 12h and 9h. Composition of nitrogen compounds in wastewater and operating parameters of the PN + AX reactors system is shown in Table 1.

Table 1. Operating parameters of PN + AX reactor system

Period	Day to day	Inf. NH_4^+-N (mg/l)	Inf. NO_2^--N (mg/l)	Inf. NO_3^--N (mg/l)	HRT (h)	
					PN	AX
1a 1b	0-30 31-60	39.67±1.72	3.69 ± 0.29	1.18±0.53	18 12	12 9
2a 2b 2c	61-90 91-120 121-150	81.03 ± 1.38	4.95 ± 0.58	2.99±0.69	12 9 9	9 6 4.5
3a 3b	151-180 181-210	115.06±1.74	7.3±0.56	3.91±0.53	9 9	6 6

2.3. Chemical analyses

The experiment was conducted in the laboratory of Water Supply and Sanitation Division, Faculty of Environmental Engineering, National University of Civil Engineering. Parameters of influent and effluent flow were measured 3 times per week. Ammonium concentrations were measured by colorimetic method with Nessler reagent at wavelength of 420 nm. In accordance with Standard Methods [12], nitrite and nitrate concentrations were estimated by the colorimetric method (4500-NO_2-B) and the UV spectrophotometric screening method (4500-NO_3-B), respectively. Nitrite was known to have an interfering response in the nitrate UV screening method of 25% of the nitrate response on a nitrogen weight basis, thus the results were corrected by calculation. Levels of pH were measured by using a Mettler Toledo-320 pH meter and DO was measured by using a DO meter (D-55, Horiba).

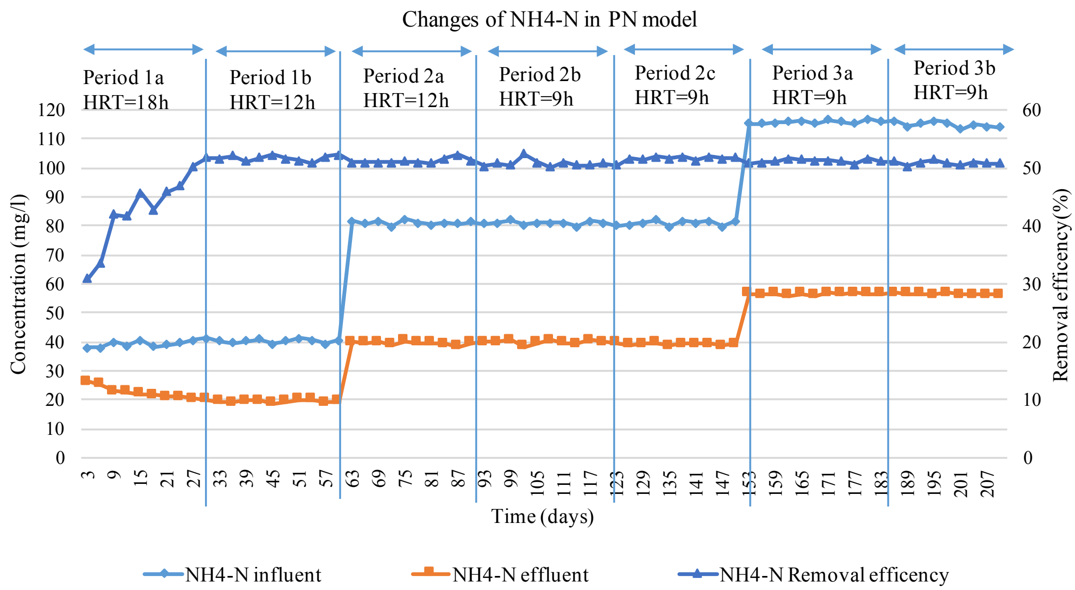
3. Result and discussion

3.1. Changes of ammonium (NH_4^+-N), nitrite (NO_2^--N) and total nitrogen (TN) concentrations in the partial nitritation reactor

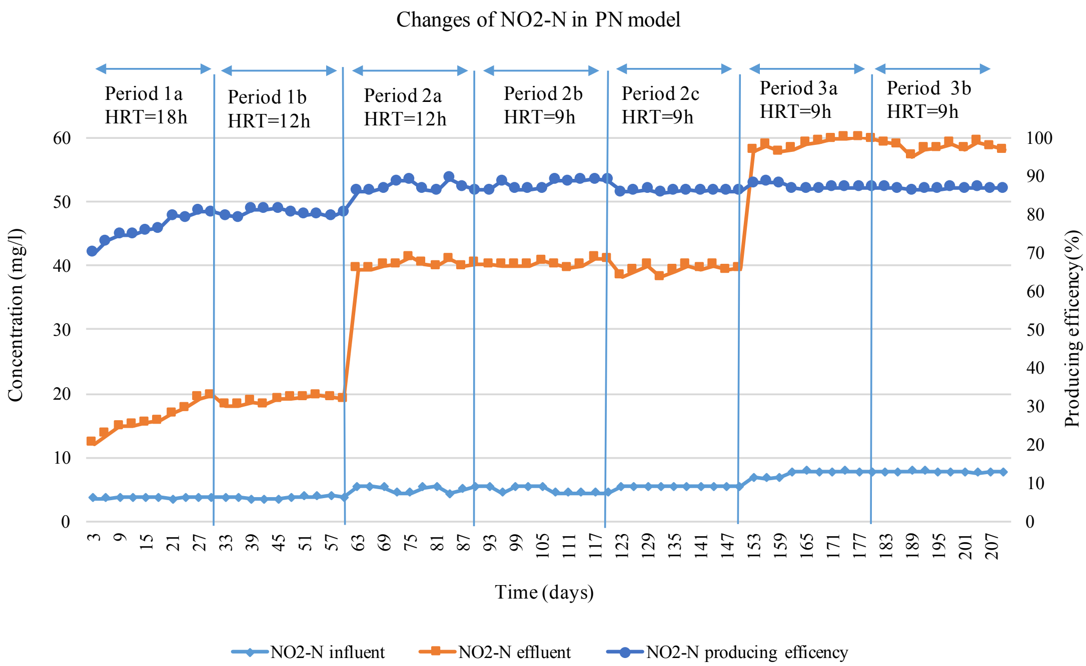
As shown in Fig. 2, the first period was operated with diluted wastewater with an ammonium NH_4^+-N concentration of 39.67±1.72 mg/l. In the first days of operation, *Nitrosomonas* bacteria was not adapted to operating conditions, while competing with other microorganisms in domestic wastewater, the efficiency of NH_4^+-N conversion to NO_2^--N is low. Ammonium concentration in wastewater after the first 3 days of partial nitritation was only reduced from 38 mg/l to 26.25 mg/l, reaching a conversion rate of 30.92%. However, in the following days, when the bacteria adhered, adapted and promoted the role of converting ammonium to nitrite, the efficiency was significantly improved to reach 51.79%. In addition, nitrite concentration formed in PN reactor was also increased, respectively, from 12.07 mg/l (after the first day) to 19.62 mg/l (after day 30). As a result, the ratio of NO_2^--N: NH_4^+-N was also increased from 0.46 to 0.98. HRT in period 1b is reduced from 18h to 12h and substrate concentration was kept as stage 1a. Results showed that the average conversion efficiency of ammonium to nitrite was 51.48 ± 0.75% and after partial nitritation, the ratio of NO_2^--N: NH_4^+-N averaged 0.97 ± 0.05.

In the second period, the concentration of ammonium was increased from 39.67 ± 1.72 mg/l to 81.03 ± 1.38 mg/l but the HRT was 12 hours as the first stage. Because of the increasing in substrate concentration, the efficiency of the process was slightly reduced from 51.7% to 50.83%, then stabilized toward the end of period 2a reaching 51.26%. The ammonium concentration after the PN reactor was 39.66 ± 1.17 mg/l, the ammonium conversion efficiency of the partial nitritation process during this period was 51.54 ± 0.71%. The ratio of NO_2^--N: NH_4^+-N in wastewater after the PN reactor was 1.02 ± 0.03. In the next 30 days of 2b and 2c period, the experiment was continued running with the same substrate concentration but the HRT was reduced from 12h to 9h. With a HRT of 9h, the ammonium removal efficiency of the PN model was 51.25 ± 1.13%, corresponding to the ammonium concentration of 39.43 ± 1.12 mg/l in period 2a. Similar in period 2b, the ammonium concentration of the outlet was 39.1 ± 0.45 mg/l, the average ammonium removal efficiency was 51.24 ± 0.71%. As a result, the efficiency of ammonium to nitrite conversion has decreased but not significantly, so it can be confirmed that the 9h of HRT is appropriate for *Nitrosomonas* bacteria to perform partial nitritation.

Thus, in the third period, the wastewater was collected after the septic tank (not diluted with gray water) was used but experiments will conduct with HRT of 9h. The influent of the ammonium concentration was 115.06 ± 1.74 mg/l and the effluent was collected at 56.51 ± 0.46 mg/l. Corresponding to it, the nitrite input and output are respectively 7.3 ± 0.56 mg/l and 58.55 ± 1.44 mg/l. The nitrite/ammonium ratio in wastewater after PN was 1.03 ± 0.02.



(a) Changes of NH_4-N in PN model

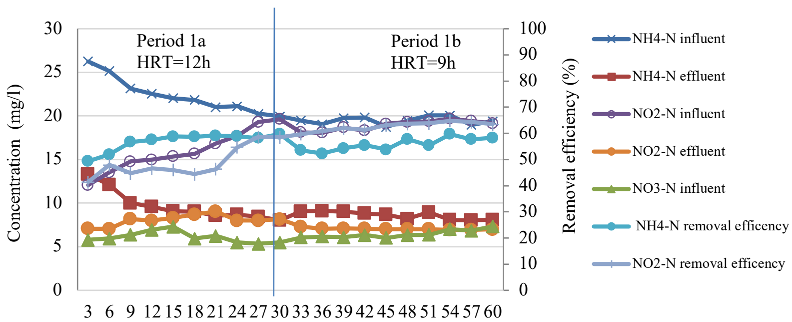


(b) Changes of NO_2-N in PN model

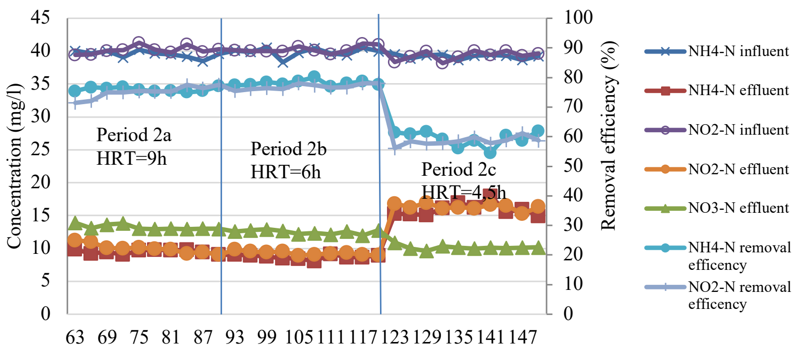
Figure 2. Changes of ammonium and nitrite in PN reactor

3.2. Changes of ammonium (NH_4^+-N), nitrite ($NO_2^- -N$) and total nitrogen (TN) concentrations in AX reactor

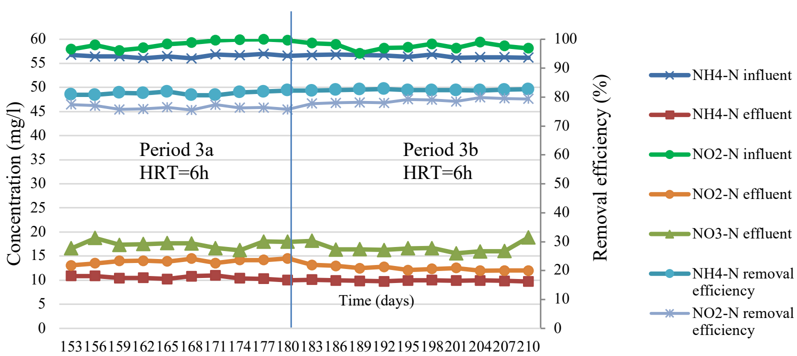
The effluent wastewater from the PN reactor was influent flow for the AX reactor. In period 1a, the partial nitritation process was effective in the early days of operation, so that wastewater from the PN reactor had an NH_4^+-N concentration of 26.25 mg/l and nitrite concentration is 12.07 mg/l. The effluent of the AX reactor has an initial NH_4^+-N concentration of 13.3 mg/l as shown in Fig. 3. The explanation for this is that although ammonium levels are not high, ammonium removal efficiency is low, reaching only 49.33% due to nitrite to ammonium ratio has not yet met the ammonium oxidation requirements of the Anammox process.



(a) Changes in Period 1



(b) Changes in Period 2



(c) Changes in Period 3

Figure 3. Changes of nitrogen-containing compounds in AX reactor in each period

During the first 9 days of period 1a, NH_4^+-N ammonium concentration in effluent was still higher than 10 mg/l. However, in the following days, the ratio of NO_2^--N: NH_4^+-N in the effluent of the anammox process was improved gradually. Ammonium concentrations were reduced to less than 10 mg/l and remained stable at 8.78 ± 0.5 mg/l.

Simultaneously with the changes in ammonia concentration of the anammox process, the nitrite concentration was also reduced. Nitrite removal efficiency increased from 41.43% to 58.46% after 30 days of experiment. Total nitrogen removal efficiency increased from 38.38% to 52.69%, respectively.

During the next 30 days (period 1b), the reduction of the HRT in the AX reactor from 12h to 9h. By reducing the HRT, the nitrogen removal rate was increased from 0.13 gN/m^3.d to 0.18 gN/m^3.d, ammonium removal efficiency and averaged total nitrogen removal efficiency were 55.98 ± 3.72% and 51.33 ± 1.4%, respectively.

In the second period, ammonium concentration into the PN reactor has increased to 81.03 ± 1.38 mg/l and hence wastewater into the AX reactor has an ammonium concentration 39.43 ± 1.12 mg/l and the author conducted experiments with 3 periods of 9h, 6h and 4.5h. The results showed that, with the HRT of 4.5h, the ammonium concentration after the AX reactor was 16.45 ± 1.5 mg/l higher than the allowable level of the receiving source. Meanwhile, with 9h and 6h of HRT, the ammonium concentrations were 9.45 ± 0.4 mg/l and 8.6 ± 0.55 mg/l, respectively. It can be seen that the time of 4.5h is too short for Anammox bacteria to process the metabolism. Therefore, it is necessary to increase the HRT to 6h in the next experiment.

In period 3, real domestic wastewater was taken from the septic tank with the properties as shown in Table 1. After the partial nitritation process, wastewater with average ammonium, nitrite, nitrate concentrations were 56.51 ± 0.46 mg/l, 58.55 ± 1.44 mg/l and 6.37 ± 0.69 mg/l. With increasing inlet concentration, after 30 days, the effluent ammonium concentration was still higher than the standard allowed, variation from 10.89 mg/l to 10.08 mg/l due to the recovering of the Anammox bacteria activity. Besides, the substrate concentration in the effluent is also one of the factors influencing on the treatment efficiency of the model. After 30 days of period 3b, the effluent ammonium concentration was reduced to below 10 mg/l and remained stable at the range of 9.96 ± 0.14 mg/l. The effluent total nitrogen also fluctuates in the range of 38.51 ± 0.91 mg/l.

The relationship between TN removal rate, nitrite removal rate, nitrate production rate versus ammonium removal rate were established and shown in Fig. 4.

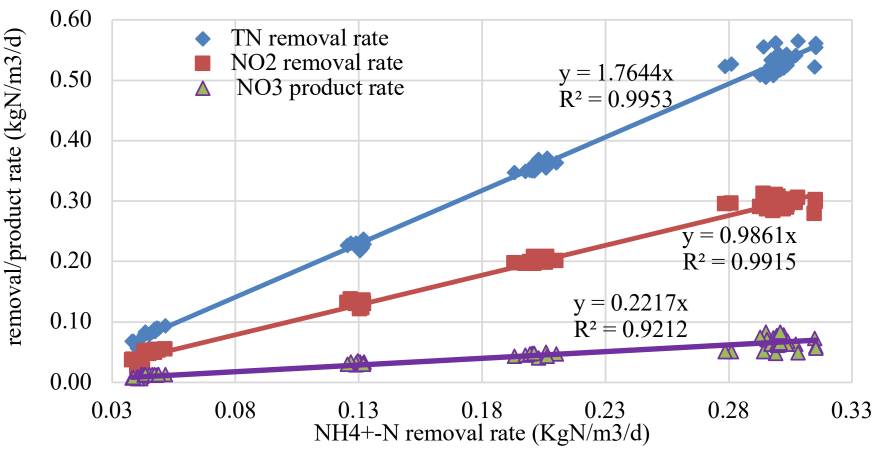


Figure 4. Ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates

Ratios of T-N removal, NO_2-N removal and NO_3-N production rates to NH_4-N removal rates for Anammox reactor were 1.76 : 0.98 : 0.22, which are very similar to the theoretical reaction ratios for the anammox reaction (Eq. (2)). In Hoa's research [11], ratios of T-N removal, NO_2-N removal and NO_3-N production rates to NH_4-N removal rates for reactor 1 (MC 3-5mm diameter pieces) were 1.98 : 1.15 : 0.17, for reactor 2 (MC 10-15mm diameter pieces) were 2.03 : 1.2 : 0.17, The differences between theoretical ratio and the actual ratios for NO_3-N production may be due to the biological denitrification process occur by existing of the anoxic heterotrophic bacteria which nitrate was reduced to nitrogen gas.

3.3. The efficiency of nitrogen treatment in PN + AX reactor system

The study was carried out on PN + AX reactor system with 3 periods corresponding to different wastewater types of three sewage and drainage systems (combined sewage and drainage system in rainy season, combined sewage and drainage system in dry season, separated sewage and drainage system). The nitrogen treatment efficiency of the system depends on the retention time of each reaction model. With a HRT of 12h in the PN reactor, the nitrite/ammonium content of the effluent will not be suitable for *Nitrosomonas* bacteria in the PN reactor, hence the treatment efficiency is very low, only 45.54 ± 7,16% as shown in Fig. 5. At the same time, the storage time of 4.5h in the AX reactor is considered to be insufficient for Anammox process to take place, so the total nitrogen treatment efficiency of the system is low at only 52.76 ± 1.29%. For the storage time in PN + AX reactor system of 9 and 6h, the effluent water quality is ensured according to the requirements of the column B (none water supply purpose) according to QCVN 14:2008/BTNMT [1].

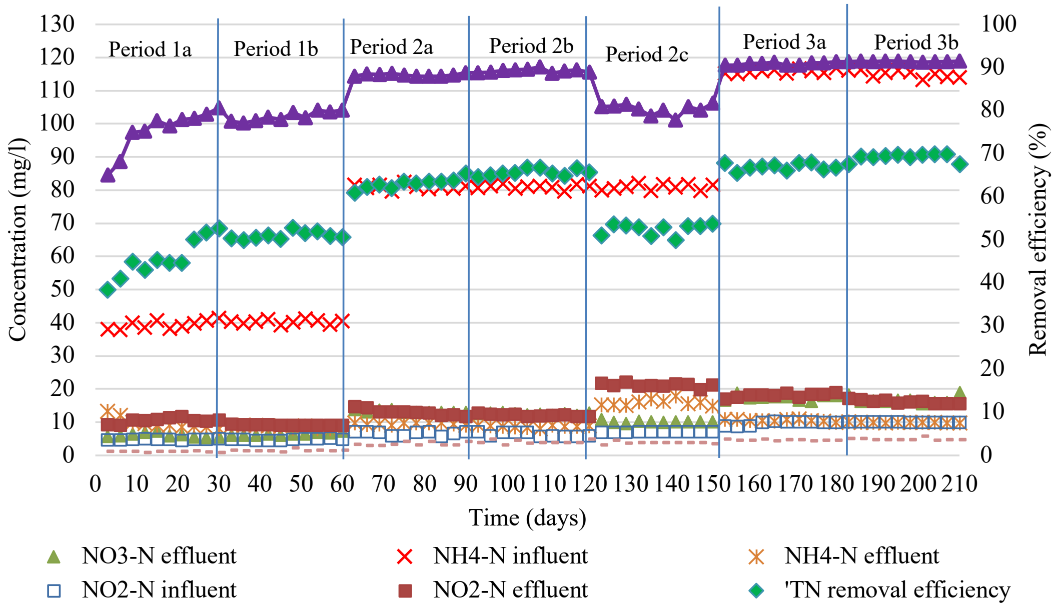


Figure 5. Changes of nitrogen compounds in PN + AX reactor system

3.4. The result of the gene sequence of bacteria on carrier materials

Molecular biology techniques for bacteria identifying are carried out at the Center for Biotechnology Research and Development, Institute of Biotechnology and Food Technology, Hanoi University of Science and Technology and shown in Fig. 6. The sequence includes designing bacteria-specific primers using Polymerase chain reaction (PCR); Collected nucleotide sequence data were included in the Multalin comparison tool and used Fast PCR software to reconcile the 16S rDNA gene segment on the mold separated from the original sample (root) and from biomass material after use for real wastewater treatment. To conduct bait design, 10 16S rDNA sequences of the strain *Candidatus Brocadia anammoxidans* were collected from NCBI data bank. The obtained nucleotide sequence data were included in the Multalin comparison tool to identify conservative regions. From the results, about 30 nucleotides of head 5' and 3' of the gene were used to carry out the design of the forward primer and the corresponding reverse primer. The selected sequence is included in the FastPCR software to calculate the parameters and select the sequence that satisfies the requirements: there are 20-25 nucleotide sequences, no additional pairing, no additional pairing. Primer sequence together, the temperature attached to the primer is about 55-62°C.

After obtaining the 16S rDNA-specific primer pair of *Candidatus Brocadia anammoxidans*, PCR was performed to amplify the 16S rDNA gene segment using DNA mold separated from the previous sample (root) and after use (from carrier material). The results are shown in Fig. 6. The results obtained DNA band about 500 bp in both samples before and after use. The size obtained was consistent with the theoretical size according to the design of the 16S rDNA gene fragment of the strain *Candidatus Brocadia anammoxidans*. From the results, it can be said that *Candidatus Brocadia anammoxidans* strain still exists in carrier materials after use to treat actual domestic wastewater. This also proves that Anammox bacteria are completely suitable for the actual domestic wastewater environment and play a role in the treatment of nitrogen in urban domestic wastewater.

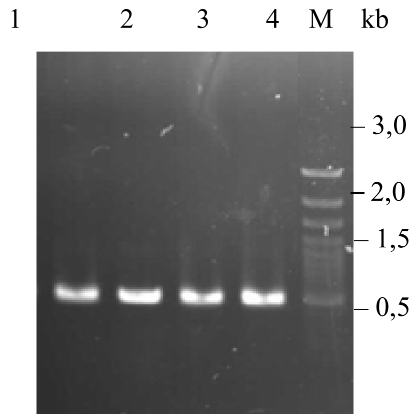


Figure 6. DNA electrophoresis of PCR products amplify the 16S rDNA gene segment from DNA obtained from the original sample (running lines 1,2) and samples after being used for water treatment (running lines 3,4); M path, standard DNA ladder

4. Conclusions

The study used the Partial nitritation and Anammox reactor system to evaluate the removal efficiency depend on the hydraulic rention time of nitrogen in dormitory's wastewater from the National University of Civil Engineering during 210 days. The partial nitritation reactor using Felibendy plate with *Nitrosomonas* bacteria and Anammox reactor using Felibendy cubes with presence of strains *Candidatus Brocadia anammoxidans* achieved the stable treatment efficiencies over the time. The research concluded that the short HRT of 4.5 hours in the AX reactor affected the Anammox process negatively. The optimal hydraulic retention times for PN and AX reactors are 9h and 6h, respectively.

Acknowledgments

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