Nitrogen removal by using sulfur-based carriers: a comparison of configurations for the denitrification process

Thi-Kim-Quyen Vo[a], Ahyoung Jeong[a], Jiyoung Song[a], Jeong-Jun Lee[b], Joon-Seok Kang[c], Seo-Gyeong Park[a], Han-Seung Kim[a,*]

[a]Department of Environmental Engineering and Energy, Myongji University, 116 Myongji-ro, Cheoin-gu, Yongin-si, Gyeonggi-do 17058, Korea, Tel. +82 31 330 6695; Fax: +82 31 336 6336; emails: kimhs210@mju.ac.kr (H.-S. Kim), vokimquyen77@gmail.com (T.-K.-Q. Vo), dkdud7163@naver.com (A. Jeong), twd_77@naver.com (J. Song), psk7026@naver.com (S.-G. Park)
[b]Future Strategy and Convergence Research Institute, Korea Institute of Civil Engineering and Building Technology, Daehwa-Dong 283, Goyangdae-ro, Ilsanseo-gu, Goyang-si, Gyeonggi-do 10223, Korea, email: dlwjdwns87@naver.com
[c]Department of Water Resource, Sungkyun Kwan University, 2066 Seobu-ro, Jangan-gu, Suwon, Gyeonggi-do 16419, Korea, email: joonseok0724@gmail.com

ABSTRACT

To enhance the nitrogen removal efficiency from wastewater, elemental sulfur-based carriers were utilized to create the occurrence of the autotrophic denitrification (ADN) process. Three processes including sole heterotrophic denitrification (HDN), simultaneous heterotrophic and ADN (mixotrophic denitrification (MDN)), sequential heterotrophic and ADN were evaluated to compare the nitrogen removal performance in this study. The total nitrogen removal efficiency was 43% ± 2% for the HDN due to the low C/N ratio of 2.8, and it was increased upto 56% ± 2% for the MDN, and the highest removal efficiency was 93% ± 1% for the sequential process. The ratios of sulfate generated to nitrate removed were 1.35 ± 0.15, 3.46 ± 0.14 mg SO_4^{2-}/mg NO_3^-–N for the mixotrophic and sequential denitrification processes, respectively, and lower than the theoretical ratio (7.54 mg SO_4^{2-}/mg NO_3^-–N). The chemical oxygen demand (COD) and total phosphorus (TP) removal efficiencies were obtained at 98% ± 1%, 37% ± 4%, and similar for three processes. The sulfur-based carriers can enhance the nitrogen removal without any adverse effect on COD and TP treatment.

Keywords: Sulfur-based autotrophic denitrification; Heterotrophic denitrification; Mixotrophic denitrification

1. Introduction

Eutrophication is a phenomenon that the water body is enriched with nutrients. This induces some adverse effects such as algae bloom, a decreasing of dissolved oxygen (DO) concentration in water, damages on aquatic ecosystems, deterioration of water quality, etc [1]. The main cause of this phenomenon is the excessive discharge of nitrogen and phosphorus substrates as known as nutrients into water bodies like lakes, rivers, oceans, etc. Additionally, most of the nitrogen compounds are harmful, such as ammonia is a poison effecting on aquatic organisms, nitrite and nitrate are cancer inducers for human [2,3]. On the other hand, the discharge standards of nutrient compounds will become stricter in the future. Therefore, the nitrogen elimination becomes important.

Nowadays, the biological treatment of nitrogen in wastewater is widely used and includes two sequential processes as nitrification and denitrification. The nitrification occurs in aerobic conditions in which NH_4^+ (ammonium) is sequentially

* Corresponding author.

oxidized to NO_2^- (nitrite) and NO_2^- to NO_3^- (nitrate). This process is carried out by autotrophic microorganisms called as nitrifiers. Nitrate produced from the nitrification is converted to nitrogen gas as known as the denitrification, resulted in the removal of nitrogen. Commonly, the denitrification process is carried out by heterotrophic microorganisms in the presence of organic carbon compounds such as methanol, ethanol and acetate [4]. Eq. (1) expressed the heterotrophic denitrification (HDN), with methanol as a carbon source [5]. Thus, for the wastewaters with insufficient carbon source, the complete denitrification requires an external carbon source, resulted with an increase in treatment cost. In recent decades, some studies have started to develop technologies to improve the nitrogen removal. Wang et al. [6] indicated a new method to improve nitrogen removal from wastewater with low carbon concentration. Coupling of an anaerobic-anoxic-oxic reactor and a nitrifying reactor removed about 65% total nitrogen (TN) from wastewater, and the TN removal efficiency increased up to 83% by treating sludge in the nitrifying reactor with 1.9 mg N/L free nitrous acid [6]. Another method that has been investigated to enhance the nitrogen removal efficiency from wastewater was the utilization of autotrophic denitrification (ADN) process. In contrast to HDN, ADN is carried out in the absence of organic carbon compounds. Autotrophic denitrifiers derive energy from oxidation-reduction reactions between nitrate (an electron acceptor) and elemental sulfur (an electron donor) (Eq. 2) and the carbon source is inorganic carbon compounds [5]. The ADN process has several advantages such as, no external carbon supplementation and less sludge generation which results in the minimization of treatment cost and less greenhouse gas (N_2O) production [7–11]. Nevertheless, according to Eq. (2), the sulfate generation is a noticeable issue for the ADN process, resulted in pH drop.

$$NO_3^- + 1.08\ CH_3OH + H^+ \rightarrow 0.47\ N_2 + 2.44\ H_2O + 0.76\ CO_2 + 0.065\ C_5H_7O_2N \tag{1}$$

$$NO_3^- + 1.05\ S + 0.74\ H_2O + 0.28\ CO_2 \rightarrow 0.47\ N_2 + 1.05\ SO_4^{2-} + 1.09\ H^+ + 0.060\ C_5H_7O_2N \tag{2}$$
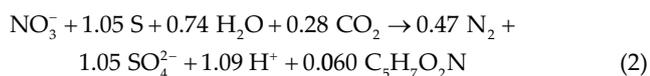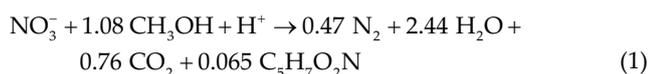
The nitrate treatment capacity of the ADN process has been investigated by several researches. A fixed-bed bioreactor filled with elemental sulfur, limestone and activated carbon was operated with the influent nitrate concentration of 75 mg/L, the efficiency of ADN obtained at 95% within hydraulic retention time (HRT) of 12 h [12]. Oh et al. [13] also indicated that, a complete ADN of 100 mgNO_3^-–N/L occurred in a sulfur packed column with HRT of 1.5 h. Most of the nitrifiers and denitrifiers are slow-growing microorganisms and it is difficult to achieve high biomass concentration in some conventional bioreactors. Therefore, the combining of biological activated sludge and the separation by membranes was known as membrane bioreactor (MBR) is considered as an effective process. MBR can be kept in a high mixed liquor suspended solid (MLSS) concentration due to its longer sludge retention time (SRT). Additionally, some advantages of MBR includes space saving, high water quality of permeates, stable operations, etc. [14]. Sahinkaya et al.

[15] reported that, a flat sheet of membrane with an external sulfur supplementation was operated in 5 h to remove completely of 50 mg NO_3^-–N/L in synthesis water. A completely elimination of nitrate (25 mg NO_3^-–N/L) was achieved within 160 min by a coupling of sulfur-based ADN and membrane separation [8].

Most of the studies on the sulfur-based ADN utilized granular sulfur, sulfur powder and an external alkalinity supplementation. Sahinkaya and Dursun [12] indicated that small-sized sulfur causes a clogging and produced gas entrapment in a reactor, resulting problems in operating full-scale treatment plants. Therefore, new carrier was considered in this study. The carriers were made from elemental sulfur, calcium carbonate (as an alkalinity for ADN process), powder activated carbon (PAC) (enhancing the hardness of carriers and decreasing the membrane fouling [16]). Additionally, MBR was also used as an oxic compartment for the nitrification performance and not only for the enriched nitrifiers but also for enhanced water quality. Overall, the aim of this study was to compare the nitrogen removal performance of HDN, mixotrophic denitrification (MDN) or simultaneous heterotrophic and ADN, and sequential HDN-ADN.

2. Materials and methods

2.1. Experimental set-up

All experiments were conducted in lab-scale reactors. Two systems with similar configuration were operated in parallel. Each system contained anoxic, anaerobic and oxic compartments with working volume of 2, 3 and 6.9 L, respectively (Fig. 1). Two flat sheet submerged membrane modules made by chlorinated polyvinyl chloride (C-PVC) from Pure Envitech (South Korea) were installed in the oxic compartment. The module characteristics include 0.03 m² membrane surface, 0.4 μm pore size and a dimension of 120 × 120 mm. To reduce the membrane fouling, the permeate pump was operated intermittently with 9 min suction, 1 min standby and a flux of 8 LMH (L/m²/h). The recycle stream from oxic to anoxic compartments and the feed rate were controlled by peristaltic pumps. DO concentrations were controlled at 0.27 ± 0.06, 0.05 ± 0.01 mg/L in anoxic and anaerobic compartments by using eletric stirrers with rates of 60 and 15 rpm, respectively. Besides, DO in the oxic compartment was maintained at 3.34 ± 0.18 mg/L by a blower with an air supply rate of 60 L/min. Trans-membrane pressure (TMP) was daily measured via digital pressure gauges. During operating period, when TMP increased up to 20 kPa, membrane modules were chemically cleaned (NaOCl 0.5%) within 6 h prior to use.

For the MDN process, elemental sulfur-based carriers were added into the anoxic compartment of the first system with a rate of 5 g/d to create the mixotrophic condition. The second system was operated without elemental sulfur-based carriers for the occurrence of the HDN process. For the HDN-ADN process, a cylindrical filter column ($D × H$ = 130 × 300 mm) was filled with an elemental sulfur-based carriers which connected to the effluent of the oxic compartment of the second system. The carriers were added into the filter column with a volume of 2 L (50% v/v) and the HRT was 4 h. The major components of carriers were S
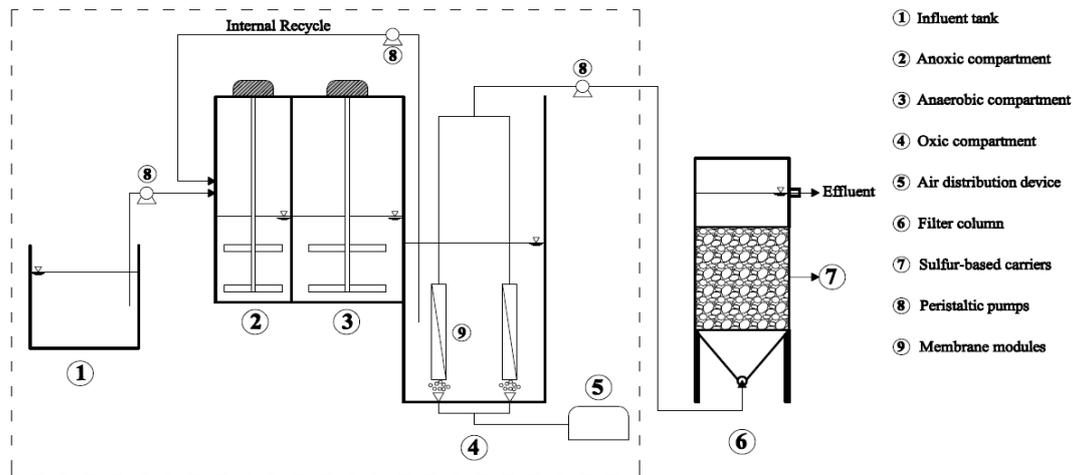
Fig. 1. Schematic diagram of a lab-scale system.

(elemental sulfur), $CaCO_3$ (calcium carbonate), PAC with the volume percentage as 50%, 40%, and 10%, respectively. Carriers were dried at temperature of 105°C within 24 h, and then broken into small pieces.

The feed was synthetic wastewater made by $C_6H_{12}O_6$ (137.5 mg/L), NH_4HCO_3 (282.14 mg/L), KH_2PO_4 (21.94 mg/L), $MgSO_4·7H_2O$ (15 mg/L), $MnSO_4·H_2O$ (0.09 mg/L) $ZnSO_4·7H_2O$ (0.3 mg/L), $CaCl_2·2H_2O$ (55 mg/L), $FeCl_2·2H_2O$ (3 mg/L), and $NaHCO_3$ (300 mg/L). The activated sludge was taken from a wastewater treatment plant located in Yongin city, South Korea, then cultured in reactors with a MLSS concentration of 4,468 ± 108 mg/L.

2.2. Analytical methods

Parameters such as chemical oxygen demand (COD), total phosphorus (TP), TN, NH_4^+–N, NO_2^-–N, NO_3^-–N, SO_4^{2-} were measured according to HACH (Loveland, Colorado, United States of America (USA)) test kits by using a spectrophotometer. Two digital pressure gauges were used to determine TMP value of membrane modules. pH and DO were measured by Thermo Scientific Orion 4-star (Waltham, Massachusetts, United States of America (USA)). MLSS was determined corresponding to APHA standard method [17]. For the analysis of the microbial community structure, sludge samples were sent to ChunLab, Inc. (Seoul, Republic of Korea), (www.chunlab.com).

3. Results and discussion

3.1. Nitrogen removal performance

The fluctuation of nitrogen concentrations for the HDN, MDN, and HDN-ADN processes are illustrated in Fig. 2. While the influent ammonia concentration was maintained at 49.56 ± 0.28 mg/L, there was no ammonia detection in three effluents during experiments. This indicated that, ammonia was completely oxidized to nitrite and nitrate. In addition, the specific nitrification rate was around 0.018 mg NH_4^+–N/ mg MLSS/d and same in three processes; it means that the use of elemental sulfur-based carriers did not affect the nitrification.

Then, nitrate produced from the nitrification process was converted to nitrogen gas by heterotrophs and/or autotrophs by denitrification process. The result of nitrification and denitrification is the removal of TN in wastewater.

The TN influent concentration was maintained at 50 ± 2 mg/L. The TN effluent concentration of the HDN, MDN, HDN-ADN processes were 30 ± 1, 23 ± 1, 4 ± 1 mg/L, corresponding to the removal efficiencies of 42% ± 2%, 56% ± 2%, and 93% ± 1%, respectively (Fig. 3). In the HDN process, only heterotrophic denitrifying bacteria were responsible for the nitrate conversion and affected by organic substrates that expressed as the COD/N ratio. Fu et al. [18] reported that the theoretical COD/N ratio of 2.86 is required for the complete denitrification occurrence; however, the practical ratio is higher. The TN removal efficiencies of a MBR system were 69% and 91% at the COD/N ratio of 7.0 and 9.3, respectively. Choi et al. [19] was also demonstrated the effect of the COD/N ratio on the nitrogen removal in an intermittently aerated MBR operated with a HRT of 12 h and an influent NH_4^+–N concentration of 40 mg/L. The results showed that the TN removal efficiencies were 62%, 89% and 93% corresponding to the COD/N ratio of 4.5, 7.0 and 10, respectively. In this study, the COD/N ratio was only 2.8, resulted in the TN removal efficiency of 43% ± 2% and the TN effluent concentration was 30 ± 1 mg/L.

However, in the MDN and HDN-ADN processes, the nitrate conversion was carried out by heterotrophs and autotrophs. For the MDN, heterotrophic and autotrophic denitrifying bacteria were in the anoxic compartment, so the HDN and ADN occurred simultaneously. Consequently, the TN removal efficiency increased up to 56% ± 2% and the TN effluent concentration decreased to 23 ± 1 mg/L. The high nitrogen removal efficiency of the MDN process was also indicated in the study of Sahinkaya and Dursun [12]. This study showed that a fixed-bed reactor was filled with elemental sulfur (3.0–5.0 mm), limestone (1.0–3.0 mm), activated carbon (1.5–2.0 mm) and methanol was added with a rate of 0.72 g/L d to create the MDN process. Consequently, the feed nitrate concentration of 75 mg/L was completely removed within 12 h [12]. To compare with this study, the high nitrate removal capacity of the study of Sahinkaya and Dursun [12]
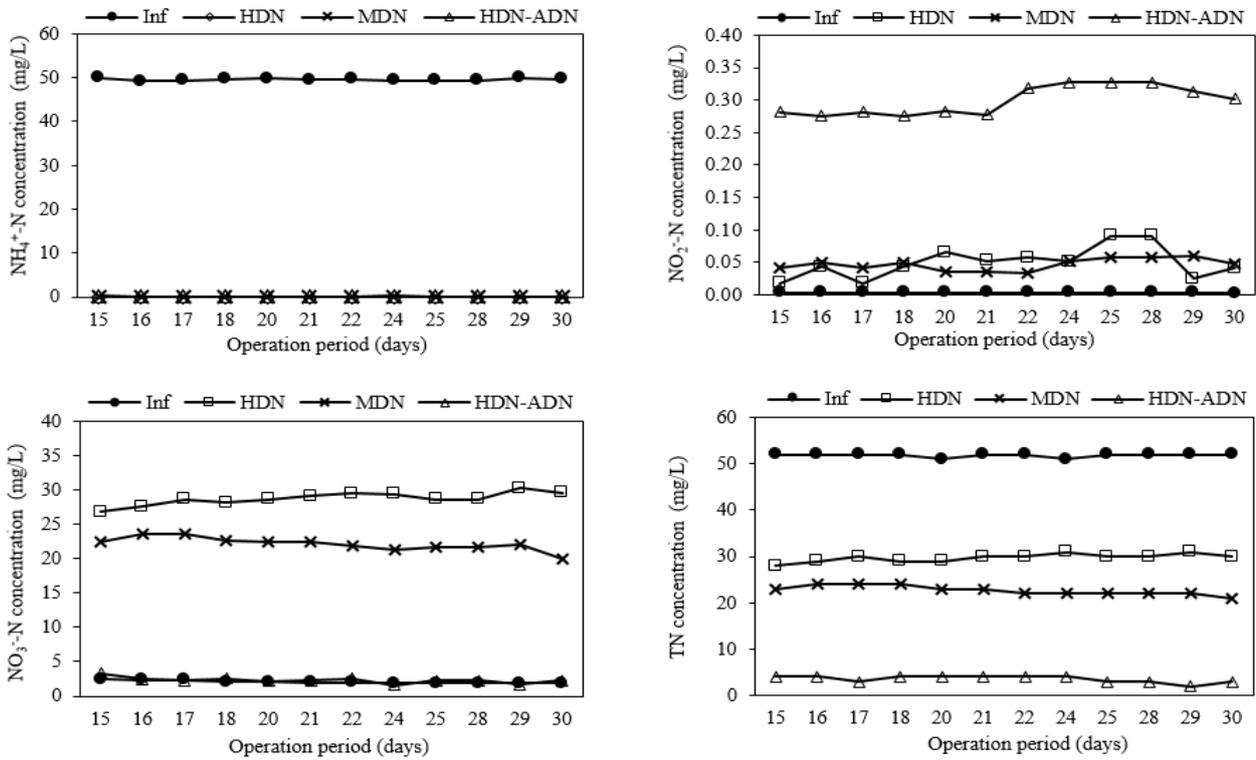
Fig. 2. Variation of concentration of species of nitrogen during operation period.
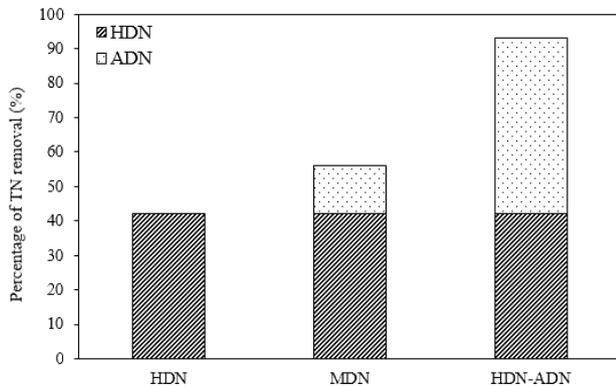
Fig. 3. Proportion of TN removal for denitrification processes.
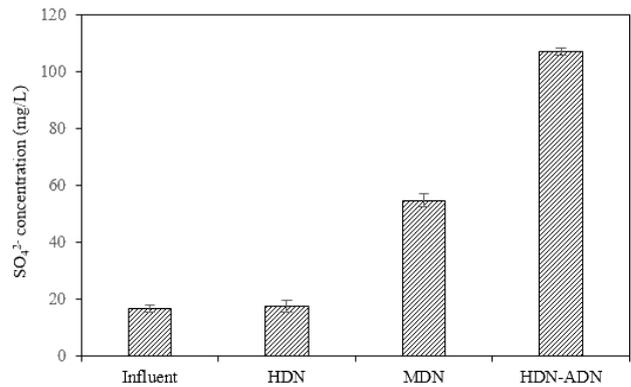
Fig. 4. Sulfate generation of each denitrification process.

is higher due to methanol was adding as an external carbon source and a longer HRT. However, without adding an external carbon source, the TN removal efficiency of 56% ± 2% that mentioned above is also remarkable. In the HDN-ADN process, nitrate was converted in the anoxic compartment by heterotrophs and further converted by autotrophs in the filter column filled with the sulfur-based carriers. The TN removal efficiency was highest among the three processes (93% ± 1%), and the HDN and ADN contributed 43% ± 2% and 51% ± 3%, respectively. A similar result was observed in the study of Kim et al. [5], the sequential HDN and ADN occurred in two column reactors, one reactor with polyethylene sponges and the other with sulfur particle were used for the HDN and ADN, respectively. The nitrate removal was achieved at 96% ± 5%, in which HDN was 49% ± 12% and ADN was 46% ± 12%. Liu et al. [20] conducted a combined process of HDN and ADN for the nitrate removal in drinking water treatment. The system was operated with a C/N ratio of 2.0 and a nitrate concentration of 30 mg NO_3^-–N/L. The completely removal of nitrate was achieved within 40 min in which HDN contributed 80% of removal efficiency.

3.2. Sulfate generation and bacterial communities

In the sulfur-based denitrification, nitrate is converted into nitrogen gas and elemental sulfur is oxidized to sulfate (Eq. (2)). This is a major disadvantage of the MDN and HDN-ADN processes. Obviously, a high sulfate concentration

in the effluent showed that, more nitrate was converted to nitrogen gas and it means more TN was removed from the wastewater. The influent sulfate concentration was around 17 ± 1 mg/L and the effluent concentrations were 18 ± 2, 55 ± 2, 107 ± 1 mg/L for the HDN, MDN, HDN-ADN processes (Fig. 4). According to Eq. (2), 7.45 mg of sulfate is produced when 1 mg of nitrate is removed. However, the $SO4_2^-/NO_3^-$–N ratios in this study were 1.35 ± 0.15 and 3.46 ± 0.14 for the MDN and HDN-ADN, respectively. These ratios were lower than the theoretical value due to a coupling of heterotrophic and ADN. Oh et al. [13] indicated a decrease of SO_4^{2-}/NO_3^-–N ratio, the range of 3–4 mg sulfate was created corresponding to 1 mg nitrate removed under mixotrophic conditions. Liu et al. [20] showed that, 6.90 mg SO_4^{2-}/L was generated when 1 mg NO_3^-–N/L was consumed by the HDN-ADN process. The low ratio also shows that elemental sulfur in carriers was utilized effectively, resulted in a decrease of an expend cost.

Next generation sequencing) results confirmed microbial community structure in reactors. *Proteobacteria* is phylum characterizing chemolithotrophic denitrification [21,22], and *Thiobacillus*, one species of the *Proteobacteria* phylum, is the most commonly sulfur-based autotrophic denitrifiers [23,24]. In this study, *Thiobacillus* accounted for 0.02%, 0.5% and 21.0% of the community in samples taken from the HDN, MDN, and HDN-ADN processes, respectively. This result indicated that the sulfur-based carriers enhanced the number of autotrophic denitrifiers in microbial community.

3.3. Organic matters and phosphor removal and membrane fouling

To obtain the COD/N ratio of 2.8, the COD concentration of the influent was fixed at 140 ± 2 mg/L during experiments. The COD concentrations measured at reactors of both system were not significantly different and they were 12 ± 2, 9 ± 2, 8 ± 1 mg/L in anoxic, anaerobic, oxic compartments, respectively (Fig. 5). The results showed that, COD decreased rapidly in the anoxic compartment, this indicated that heterotrophic denitrifiers used COD as a carbon source for the denitrification process. The COD concentrations of the effluents were lower than 5 mg/L, corresponding to the removal efficiency and specific removal rate of 98% ± 1% and 0.049 ± 0.003 mg COD/mg MLSS d, respectively. In addition, the COD effluent of the HDN-ADN was same to that of the HDN and the COD removal performance was similar for HDN, MDN, and HDN-ADN processes. Therefore, the sulfur-based carriers have no effect on the organic substrates treatment. The similar results were showed in another studies. Nguyen et al. [25] also indicated that the COD removal efficiency of 84% ± 10% was obtained in a MBR, the effluent COD was 11–16 mg/L.

The TP influent concentration was kept at 5.0 ± 0.1 mg/L and the average effluent concentration decreased at 3.1 ± 0.3 mg/L and maintained stability during the operation period (Fig. 6). In this study, the TP removal capacity was increased by installing an anaerobic compartment in the system. In an anaerobic condition, the hydrolysis of polyphosphate occurs
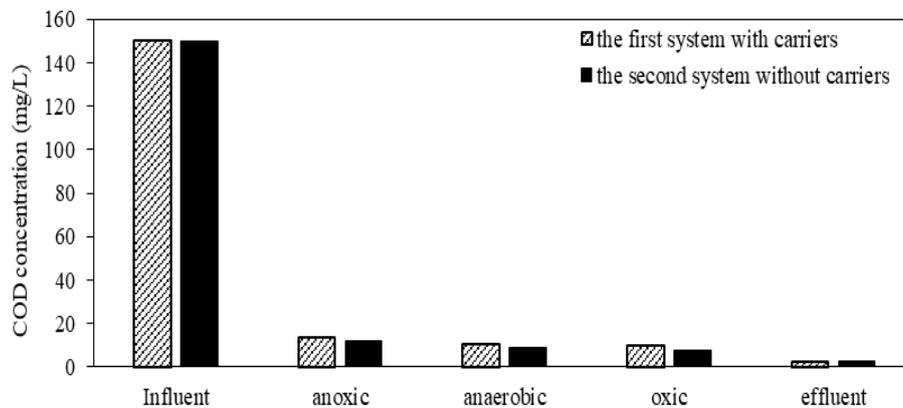
Fig. 5. Variation of COD concentration in each compartment.
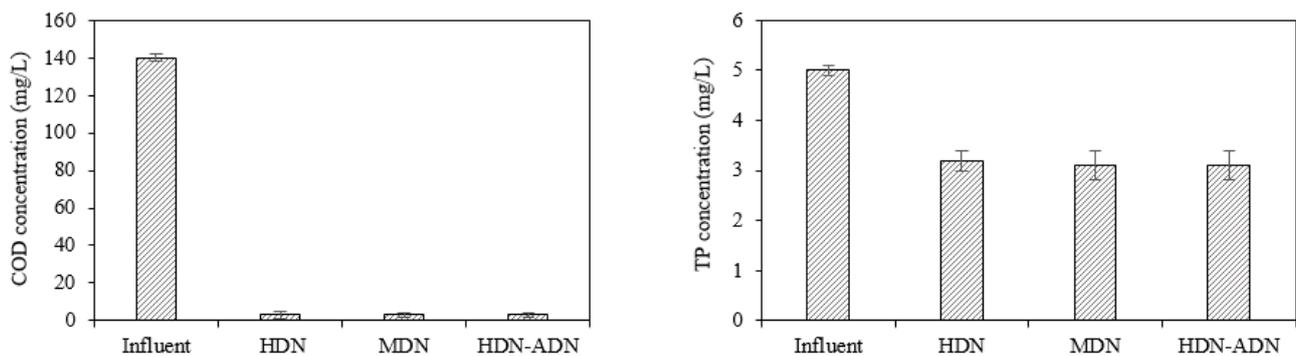
Fig. 6. Effluent COD and TP concentrations of each process.

and polyphosphate is accumulated in bacterial cells in a following oxic condition, and by discharging waste sludge, TP was released out of wastewater. The TP removal efficiency achieved at around 37% ± 4% and the specific removal rate was 0.0004 mg TP/mg MLSS/d. The results also showed that, it is insignificant in the difference between systems in term of TP removal, so the sulfur-based carriers cannot enhance TP treatment. Nguyen et al. [25] indicates that, a conventional MBR operated at a flux of 6 LMH could removed 20% ± 15% of TP concentration within 8 h, and the removal efficiency increased up to 26% ± 11% with adding sponges made by polyethylene into MBR.

4. Conclusions

The results of this study indicate some remarkable points as follows:

- Sulfur-based carriers can enhance the nitrogen removal capacity in wastewater treatment due to creating ADN process. The TN removal efficiency of a combination of HDN and ADN increased by 13%–50% to compare with the sole HDN process. Among three processes, the sequential HDN-ADN process was achieved a highest TN removal efficiency.
- Sulfur-based carriers are suitable to apply for the nitrogen treatment of wastewater contaminated by high nitrogen concentration and low organic carbon compounds.
- The actual ratio of sulfate generated to nitrate removed was lower than the theoretical value due to a coupling of heterotrophic and ADN. This reduces some risks for receiving water sources.
- Due to a combination of anoxic-anaerobic-oxic condition, the high removal efficiencies of COD and TP were achieved in both systems.

Based on the above conclusions, the further study should investigate on real wastewater.

Acknowledgements

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