



## Effect of nitrogen loading on the nitrogen removal performance in membrane bioreactor coupled with elemental sulfur-based carriers

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Received 15 December 2019; Accepted 4 July 2020

### ABSTRACT

The elemental sulfur-based carrier is demonstrated as a useful selection to enhance nitrogen removal from water. A mixed anoxic-anaerobic-MBR system was able to remove 39%–45% of total nitrogen from wastewater, however, the removal efficiency was increased up to 51%–65% by adding elemental sulfur-based carriers. Besides, nitrogen loading is one of the operational factors that affect nitrogen removal, the nitrogen removal efficiency fluctuated at  $54\% \pm 2\%$ ,  $61\% \pm 2\%$ , and  $57\% \pm 1\%$  when the nitrogen loading rate was changed at 0.06, 0.12, and 0.18 g N/L/d, respectively. Due to a simultaneous occurrence of heterotrophic and autotrophic denitrification, the mass ratio of generated sulfate to removed nitrate was  $1.9 \pm 0.2$  and lower than the stoichiometric ratio of 7.2. On the other hand, the results also indicated that elemental sulfur-based carrier carriers had no adverse effect on the biological treatment of organic matters and total phosphorus. The total phosphorus removal efficiency was obtained at  $44\% \pm 3\%$  and organic compounds were completely removed.

*Keywords:* Autotrophic denitrification; Elemental sulfur-based carrier; Heterotrophic denitrification; Nitrogen loading

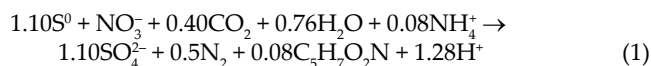
### 1. Introduction

The heterotrophic denitrification plays a major role in the nitrogen treatment by biological processes, and the denitrification process only occurs completely when the organic carbon source is provided adequately [1,2]. Ćurko et al. [3] also demonstrated that the specific denitrification rate achieved at 1.2 mgNO<sub>3</sub>-N/g MLSS/h by continuously adding glucose and the rate decreased down to 0.4 mgNO<sub>3</sub>-N/g MLSS/h without adding glucose (MLSS – mixed liquor suspended solids). Wastewater from livestock farming, landfill, industrial factories (e.g., leather, petrochemical, fertilizer, etc.) has a low carbon-to-nitrogen ratio [4,5], consequently, an

external organic carbon source is supplemented to achieve a full denitrification process. This leads to an increase in the treatment cost, moreover, when the organic carbon source is excessively added, the residual will cause secondary pollution. From these drawbacks, the sulfur-based autotrophic denitrification process has emerged as an alternative. In the autotrophic denitrification, autotrophs use inorganic sulfur compounds (e.g., sulfide, elemental sulfur, thiosulfate, tetrathionate, and sulfite) as electron donors and nitrate as an electron acceptor to conduct redox reactions [6,7]. The autotrophs get energy from redox reactions, while inorganic carbon compounds are utilized as carbon sources. Among inorganic sulfur compounds, elemental sulfur has been used

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widely due to its characteristics as available, cheap, non-toxic, stable under normal conditions [8]. Overall, the elemental sulfur-based autotrophic denitrification can be expressed as the below equation [9]:



Sahinkaya and Dursun [10] demonstrated the high efficiency of the autotrophic denitrification process by using a fixed-bed reactor filled with elemental sulfur, limestone, and activated carbon. The reactor was operated with the influent nitrate concentration of 50 mg/L and hydraulic retention time (HRT) of 12 h, the effluent concentration was lower than 0.1 mg/L. However, the sulfur-based autotrophic denitrification has a major disadvantage as sulfate formation. While nitrate is reduced to nitrogen gas, elemental sulfur is oxidized to sulfate, resulting in pH drop. According to Eq. (1), 7.2 g of  $SO_4^{2-}$  is produced when 1 g of  $NO_3^-$ -N is removed. To solve this problem, Sahinkaya et al. [11] showed a method to reduce the sulfate generation by stimulating the simultaneous occurrence of heterotrophic and autotrophic denitrification (mixotrophic denitrification). An external carbon source as methanol was supplemented in a packed-bed reactor that was filled with elemental sulfur and limestone, 75 mg/L of nitrate was completely removed within 11 h, the effluent sulfate concentration was 225 mg/L. In this case, the mass ratio of generated sulfate to removed nitrate was 3.0 and lower than the theoretical value of 7.2. This result is similar to the study of Oh et al. [12], the ratio was 3–4 mg $SO_4^{2-}$ /mg  $NO_3^-$ -N under the mixotrophic denitrification process.

Due to the slow growth rate of nitrifying bacteria and denitrifying bacteria, biomass should be retained longer in bioreactors to achieve a high nitrogen removal efficiency [13]. Consequently, the membrane bioreactor (MBR) technology which combines membrane separation and biological processes has been considered in recent decades. Biomass in MBR is prevented from washing out the bioreactor by membrane modules, this encourages the growth of nitrifying bacteria and denitrifying bacteria. Besides, MBR technology has many advantages such as space-saving, high-quality permeate, high MLSS concentration, and high sludge retention time (SRT), etc. [14]. The combination of MBR and autotrophic denitrification was investigated in several studies. Sahinkaya et al. [7] reported that 25 mg/L of nitrate was completely removed within 5 h by adding powdered sulfur to a bench-scale MBR once a week. In another study, an ultrafiltration membrane bioreactor coupled with elemental sulfur-based autotrophic denitrification was utilized to remove nitrate from groundwater. The denitrification process completely occurred with the influent nitrate concentration of 25 mg/L and HRT of 160 min [15].

Sulfur in the granular or powdered form was frequently utilized in many studies on the sulfur-based denitrification process. Nevertheless, Sahinkaya and Dursun [10] demonstrated that using small-sized sulfur caused some serious problems as clogging, entrapment in bioreactors due to gas generation. It is difficult to apply in full-scale wastewater treatment plants therefore, elemental sulfur-based carriers were used to stimulate the autotrophic denitrification

occurrence in this study. In addition, nitrogen loading is one of the operational factors that affect nitrogen removal, it decides the final product of the denitrification process as nitrogen gas ( $N_2$ ) or nitrite ( $NO_2^-$ ). Thus, the objective of this study was to evaluate the effect of the nitrogen loading on the nitrogen removal performance in a mixed anoxic-anaerobic-membrane bioreactor system coupled with elemental sulfur-based carriers.

## 2. Materials and methods

### 2.1. Lab-scale systems

Most nitrogen compounds exist in wastewater in the form of ammonia, and the conversion of ammonia to nitrogen gas involves two main processes as nitrification (occurring in the aerobic condition) and denitrification (occurring in the anoxic condition). Besides, phosphorus is removed by combining anaerobic and aerobic bioreactors. Consequently, to study the nitrogen and phosphorus removal performance, a system including anoxic, anaerobic, oxic compartments was set up in this study. The working volumes of three compartments were 2, 3, 6.9 L, respectively (Fig. 1). The oxic compartment was installed two membrane modules, and each module had a dimension of 120 mm × 120 mm. The flat sheet membranes made by chlorinated polyvinyl chloride (Pure Envitech, Republic of Korea) had 0.06 m<sup>2</sup> surface area and 0.4 μm pore size. The operational flux was 10 L/m<sup>2</sup>/h (LMH), and the permeate was controlled by a peristaltic pump with 9 min of suction and 1 min of standby. A digital pressure gauge was connected to the permeate for measuring the trans-membrane pressure (TMP), when TMP value exceeds 20 kPa, membrane modules will be cleaned by sodium hypochlorite (0.5%) within 6 h. A wastewater stream from the oxic compartment was recycled to the anoxic compartment to supply nitrate for the denitrification process. Dissolved oxygen (DO) values were controlled at 0.23 ± 0.07, 0.05 ± 0.02 mg/L in anoxic, anaerobic compartments by using stirring motors, respectively. Air diffusers were installed at the bottom of the oxic compartment to supply DO concentration of 3.53 ± 0.26 mg/L. In addition, two systems with the same configuration were parallelly operated in this study. One of the systems was daily added 1 g of elemental sulfur-based carriers in the anoxic compartment to stimulate the autotrophic denitrification, and the other was operated without adding carriers.

### 2.2. Characterization of materials

The systems were fed with synthetic wastewater made by  $C_6H_{12}O_6$  (137.5 mg/L),  $KH_2PO_4$  (21.94 mg/L),  $MgSO_4 \cdot 7H_2O$  (15 mg/L),  $MnSO_4 \cdot H_2O$  (0.09 mg/L),  $ZnSO_4 \cdot 7H_2O$  (0.3 mg/L),  $CaCl_2 \cdot 2H_2O$  (55 mg/L),  $FeCl_2 \cdot 2H_2O$  (3 mg/L),  $NaHCO_3$  (300 mg/L). The influent chemical oxygen demand (COD), total phosphorus (TP) concentrations were 150 ± 1, 5.0 ± 0.1 mg/L, respectively. The influent total nitrogen (TN) was changed at 50, 100, and 150 mg/L by changing the concentration of  $NH_4HCO_3$  (282.14, 579.28, and 923.33 mg/L) corresponding to the nitrogen loading rate of 0.06, 0.12, and 0.18 gN/L/d.

The activated sludge with an initial MLSS concentration of 5,000 mg/L was collected from a bioreactor of a

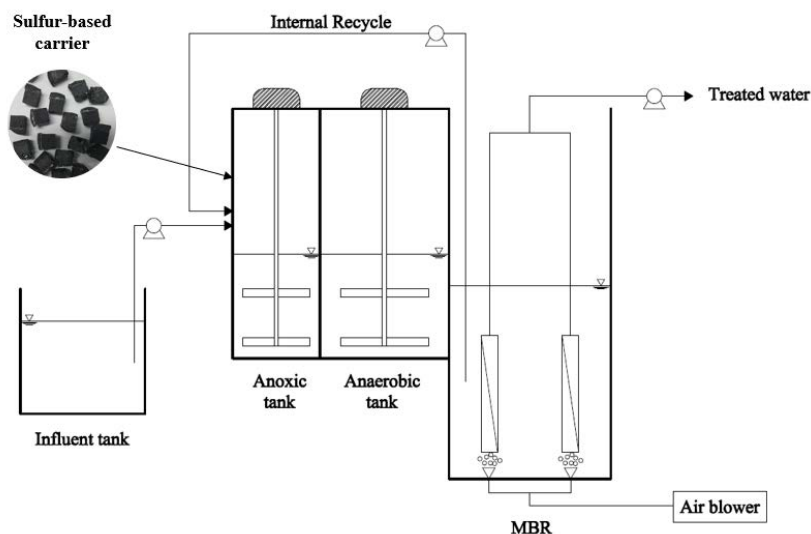


Fig. 1. Schematic of a lab-scale system.

wastewater treatment plant (Yongin city, Republic of Korea). The seed sludge was stabilized in anoxic condition for 24 h, then inoculated in two systems. Besides, elemental sulfur-based carriers were added to the second system to stimulate the autotrophic denitrification process. Both systems were acclimatized for 15 d, the MLSS concentration and SRT were maintained at  $4,461 \pm 298$  mg/L and 40 d during operational periods.

Elemental sulfur-based carriers were made by elemental sulfur ( $S^0$  – serving as an electron donor for the autotrophic denitrification process), calcium carbonate ( $CaCO_3$  – serving as an alkalinity supplement), and powdered activated carbon (PAC – for enhancing the biofilm formation on carriers). All components in powdered form were mixed with a mass ratio of 5:4:1 corresponding to elemental sulfur, calcium carbonate, activated carbon. Water glass (sodium silicate) was supplemented into the mixture at a ratio of 1:1 for sticking components together. The carriers were shaped into cubes ( $10\text{ mm} \times 10\text{ mm} \times 10\text{ mm}$ ) and dried for 10 h at a temperature of  $105^\circ\text{C}$ .

### 2.3. Analytical methods

COD,  $NH_4^+-N$ ,  $NO_2^- -N$ ,  $NO_3^- -N$ , TN, TP, and  $SO_4^{2-}$  were determined by HACH test kits and a spectrophotometer (Loveland, Colorado, United States of America (USA)). pH and DO concentration were daily recorded by Orion 4-star (Thermo Fisher Scientific, Waltham, Massachusetts, United States of America (USA)). MLSS and mixed liquor volatile suspended solid (MLVSS) concentrations were measured according to APHA standard methods [16].

## 3. Results and discussion

### 3.1. Effect of nitrogen loading on nitrogen removal performance

In this study, the influent TN concentration was sequentially changed at 50, 100, and 150 mg/L corresponding to period 1, 2, and 3. The variation of nitrogen species

during operational periods was presented in Fig. 2. The influent  $NH_4^+-N$  concentrations of three periods were  $50.18 \pm 0.58$ ,  $95.82 \pm 7.67$ , and  $146.62 \pm 1.91$  mg/L, respectively. Ammonia was almost completely oxidized in period 1 and 2, the effluent concentrations of both systems were lower than 4 mg/L. However, when the nitrogen loading was increased to 0.18 g N/L/d in period 3, the effluent  $NH_4^+-N$  concentration increased to  $12.32 \pm 3.04$  mg/L for the system with adding carriers and  $28.74 \pm 4.50$  mg/L for the system without adding carriers. The system with adding carriers showed a slightly higher ammonia removal rate ( $0.049 \pm 0.003$ ,  $0.083 \pm 0.007$ , and  $0.125 \pm 0.002$  mgN- $H_4^+-N$ /mg MLVSS/d) to compare with the system without adding carriers ( $0.040 \pm 0.002$ ,  $0.070 \pm 0.006$ , and  $0.109 \pm 0.002$  mgN- $H_4^+-N$ /mg MLVSS/d) during period 1, 2, and 3. The nitrification process consumes alkalinity, and  $CaCO_3$  in carriers made alkalinity of wastewater increase, this led to more ammonium removed. Especially, when the influent  $NH_4^+-N$  was quite high in period 3, the effectiveness of carriers was clearly showed. Besides, a small portion of ammonia is involved in autotrophic denitrification according to Eq. (1), consequently, the system with adding carriers showed a better ammonium removal.

The influent nitrate concentration was maintained at  $1.8 \pm 0.4$  mg/L during operational periods. Nitrate in the system was produced by the nitrification process that occurred in the oxic compartment, then nitrate was recycled to the anoxic compartment to participate in the denitrification process. The system with adding elemental sulfur-based carriers had a simultaneous heterotrophic and autotrophic denitrification occurrence, consequently, thus the effluent nitrate concentration was almost lower than that of the system without carriers (Fig. 2). Additionally, the results also indicated that the specific denitrification rate (SDNR) increased with the increase of nitrogen loading. SDNR of the mixotrophic denitrification achieved at  $0.027 \pm 0.001$ ,  $0.052 \pm 0.007$ , and  $0.081 \pm 0.002$  mg $NO_3^- -N$ /mg MLVSS/d in period 1, 2, 3, respectively. With respect to the heterotrophic

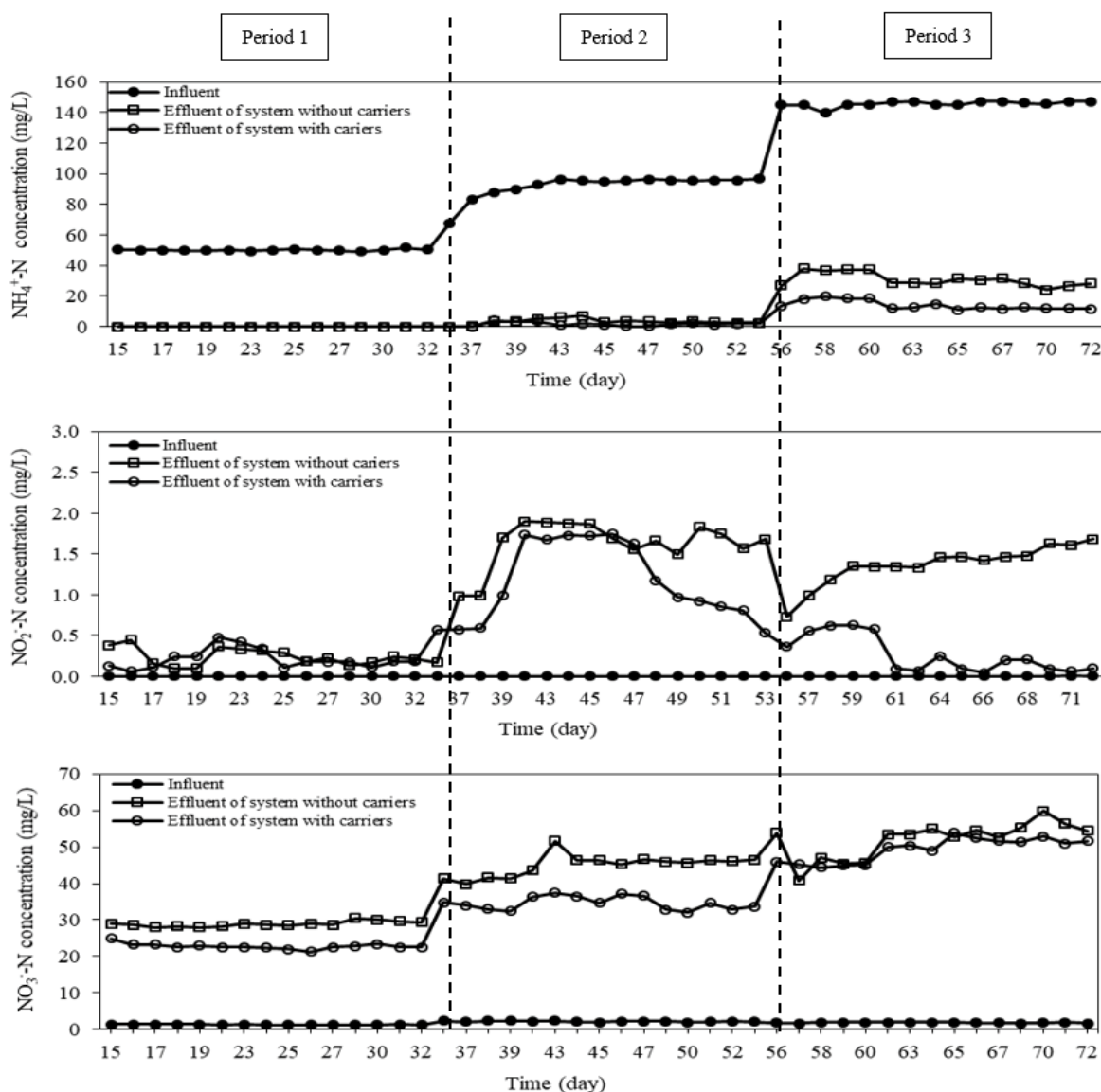


Fig. 2. Variation of  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and  $\text{NO}_3^-\text{-N}$  concentrations during operational periods.

denitrification, SDNR of three periods decreased at  $0.017 \pm 0.001$ ,  $0.035 \pm 0.005$ , and  $0.068 \pm 0.003$   $\text{mgNO}_3^-\text{-N}/\text{mg MLVSS}/\text{d}$ , respectively.

There was a significant difference in nitrogen removal between the two systems. The data from Table 1 indicated that the average TN removal efficiency of the system without carriers was  $42\% \pm 3\%$ , and it increased up to  $57\% \pm 4\%$  by adding carriers. Apparently, elemental sulfur-based carriers are effective for nitrogen treatment. Besides, the results also indicated that the effluent TN concentration increased with the increase in nitrogen loading. In period 2 and 3, the effluent concentration was almost higher than 30 mg/L, thus a post-treatment should be supplemented in order to meet the discharge standard. The similar results were observed in the study of Vo et al. [17], elemental sulfur-based carriers were supplemented in a mixed anoxic-anaerobic-membrane bioreactor system. The mixotrophic denitrification process eliminated  $63\% \pm 3\%$  of TN from the influent concentration

of 50 mg/L. In another study, a system including a bioreactor with sponge/fiber media and a column filled with sulfur particles was set up for the sequential heterotrophic and autotrophic denitrification process, the nitrate removal efficiency achieved  $96\% \pm 5\%$ , of which the sulfur-based autotrophic denitrification accounted for  $46\% \pm 12\%$  [18].

### 3.2. Sulfate generation and pH variation

In the sulfur-based autotrophic denitrification process, elemental sulfur is an electron donor and oxidized to sulfate, resulting in the sulfate generation. From Fig. 3, it is apparent that only the system with adding carriers produced sulfate, in contrast, the effluent sulfate concentration and in the other system was nearly equal to that of the influent. This indicated that the autotrophic denitrification occurs by using elemental sulfur-based carriers. The mass ratios of produced sulfate to removed nitrate ( $\text{SO}_4^{2-}/\text{NO}_3^-\text{-N}$

Table 1  
TN removal of two systems at different nitrogen loadings

	The influent TN concentration					
	50 mg/L		100 mg/L		150 mg/L	
	S1	S2	S1	S2	S1	S2
Effluent TN concentration (mg/L)	31 ± 1	23 ± 1	52 ± 3	38 ± 2	85 ± 1	64 ± 1
TN removal efficiency (%)	39 ± 2	54 ± 2	45 ± 2	61 ± 2	43 ± 1	57 ± 1
Specific TN removal rate (mgN/mgMLVSS/d)	0.016 ± 0.001	0.026 ± 0.001	0.033 ± 0.004	0.053 ± 0.003	0.048 ± 0.001	0.073 ± 0.001

S1: system without carriers; S2: system with carriers.

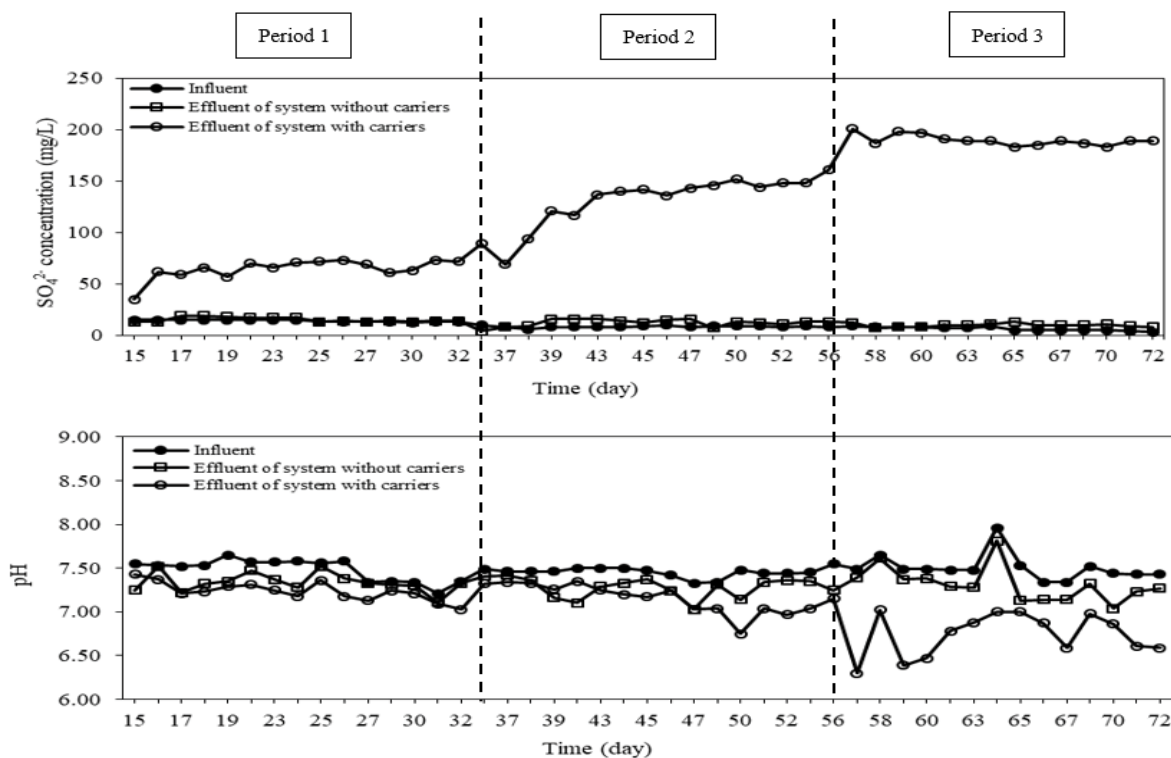


Fig. 3. Variation of sulfate concentration and pH during operational periods.

ratio) were  $1.95 \pm 0.12$ ,  $2.15 \pm 0.04$ , and  $1.86 \pm 0.01$  in period 1, 2, and 3, respectively. These ratios were lower than the stoichiometric ratio ( $7.2 \text{ mgSO}_4^{2-}/\text{mgNO}_3^-$ -N) calculated from Eq. (1) due to the simultaneous heterotrophic and autotrophic denitrification process. Oh et al. [12] also indicated that the mixotrophic denitrification reduced the sulfate production, the mass ratio was observed at  $3\text{--}4 \text{ mgSO}_4^{2-}/\text{mgNO}_3^-$ -N in sulfur packed columns with methanol supplementation.

A further problem emerging from the sulfate generation was a pH decline. The difference in pH values between the two systems is represented in Fig. 3. The influent pH was maintained at  $7.48 \pm 0.12$ , due to the sulfate generation, the effluent pH of the system with adding elemental sulfur-based carriers slightly decreased at  $7.23 \pm 0.11$ ,  $7.16 \pm 0.17$  in period 1 and 2, respectively. Especially in period 3, the sulfate was produced too much, thus the pH value decreased at  $6.77 \pm 0.26$ . By contrast, no sulfate generation was observed

in the system without adding carriers, and the effluent pH was average  $7.31 \pm 0.14$ . The interesting aspect of this study is that the effluent pH value is maintained at a neutral level despite the high sulfate concentration. This is explained by the presence of calcium carbonate ( $\text{CaCO}_3$ ) in carriers.

### 3.3. Organic matters and total phosphorus removal

The influent COD concentration was maintained at  $150 \pm 1 \text{ mg/L}$  during operational periods. Interestingly, two systems in this study included anoxic, anaerobic, and oxic conditions, thus the COD removal efficiency was quite high. Most organic matters were completely treated, the effluent COD concentrations of both systems were around  $3 \pm 1 \text{ mg/L}$  during operational periods. From the data in Fig. 4, it is apparent that organic matters were mainly biodegraded in the anoxic compartment because heterotrophic

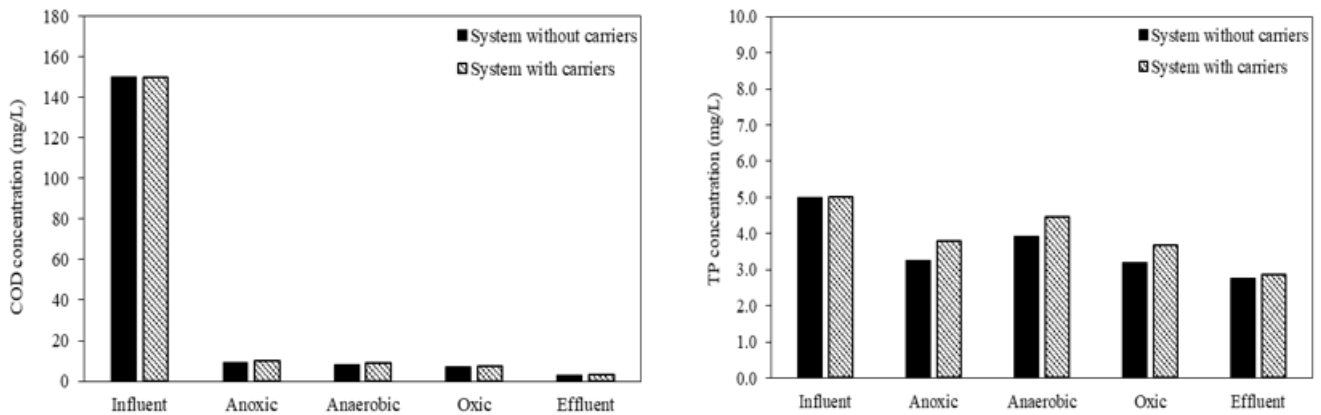


Fig. 4. Variation of COD and TP in compartments.

denitrifying bacteria used organic matters for biological metabolism. No significant difference between the two systems was observed in terms of COD treatment, and this showed that elemental sulfur-based carriers do not affect the organic matter removal. Besides, the high COD removal efficiency was also observed in the study of Nguyen et al. [19], a conventional MBR was operated at a flux of 6 LMH for the hospital wastewater treatment, the COD concentration of the permeate was around 11–16 mg/L, and the removal efficiency was obtained at  $84\% \pm 10\%$ .

There was no significant difference in the phosphorus removal efficiency between the two systems. The influent TP concentration was controlled at  $5.0 \pm 0.1$  mg/L, and the average effluent concentration and the specific TP removal rate of two systems were around  $2.8 \pm 0.1$  mg/L and  $0.002 \pm 0.001$  mgTP/mgMLVSS/d during operational periods, respectively. These results indicated that the TP treatment was not enhanced by using elemental sulfur-based carriers. In this study, the combination of anaerobic and oxic compartments facilitated the enrichment of phosphate accumulating organisms (PAOs) that can accumulate phosphate within cells, then biomass was removed from the system by withdrawing sludge frequently [20]. This combination is called as enhanced

biological phosphorus removal (EBPR) in which phosphorus is released under anaerobic condition and uptaken under aerobic condition, thus the TP concentration in the anaerobic compartment was  $3.9 \pm 0.9$  mg/L and higher than those of anoxic compartment ( $3.3 \pm 0.5$  mg/L) and oxic compartment ( $3.2 \pm 0.4$  mg/L) (Fig. 4). To compare with some other studies about MBR, the TP removal efficiency in this study ( $44\% \pm 3\%$ ) was slightly higher. For instance, Nguyen et al. [19] indicated that the TP removal efficiency of MBR that operated with a flux of 6 LMH was observed at  $20\% \pm 15\%$  and increased at  $26\% \pm 11\%$  by adding sponges.

### 3.4. Membrane fouling

According to the recommendation of the manufacturer, the membrane is cleaned when TMP is over 20 kPa to ensure the service life. The TMP values of two systems were lower than 15 kPa during operational periods, thus membrane modules were not externally cleaned in this study. Fig. 5 below illustrated that the fouling rate of the system with carriers rapidly increased to compare with that of the system without carriers. The fouling rates achieved at 0.03, 0.13, and 0.64 kPa/d for the system with carriers in period 1, 2, and 3 whereas those of the system without

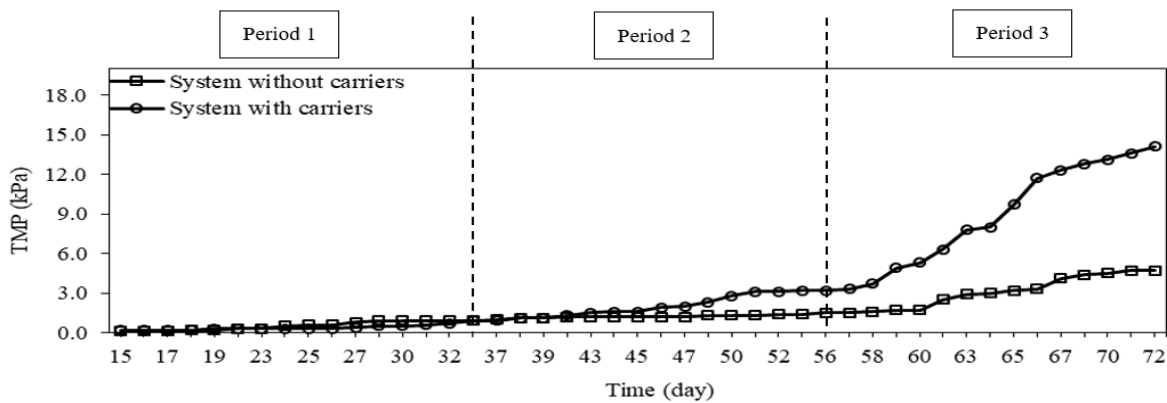


Fig. 5. Evolution of TMP in two systems at different operational periods.

carriers were 0.03, 0.04, and 0.19 kPa/d, respectively. The higher fouling rate in the system with carriers was caused by the attachment of carrier debris on membrane surfaces. The amount of debris attached to membrane surfaces gradually increased, moreover, membrane modules were not cleaned during operational periods, thus the high fouling rate was observed in Period 3.

#### 4. Conclusions

The autotrophic denitrification process is suitable to apply for the nitrogen treatment of wastewater contaminated by high nitrogen concentration and low organic carbon compounds. By adding elemental sulfur-based carriers into a conventional bioreactor will stimulate the simultaneous heterotrophic and autotrophic denitrification process to enhance the nitrogen removal. Besides, nitrogen loading affects nitrogen removal performance. When the nitrogen loading increased, the effluent TN concentration did not meet the discharge standard, thus a post-treatment is suggested. The actual mass ratio of generated sulfate to removed nitrate was lower than that of the theoretical ratio. By using elemental sulfur-based carriers, the pH drop was reduced.

#### Acknowledgments

This research was supported by a grant (NRF-2019 R1F1A1059078) from Korea Research Foundation funded by Ministry of Education, Korea.

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