the farm and then introduced to the top of the bedding material in
which the cloth covered in 3 layers. Thus, most coarse solids and gooey contents in milking center wastewater were taken out. The air-diffuser was placed on one-third of the reactor from the bottom and air was supplied continuously with an aeration rate of 0.12 m^3 h^{-1}, whereby the aerobic and anoxic phases were formed at upper and lower zone, respectively. The effluent was discharged from the bottom of the reactor without pumps by the elongated line to the surface level of the reactor. The reactor also had 2 sampling ports on the side for sampling from both different zones (aerobic and anoxic zones).

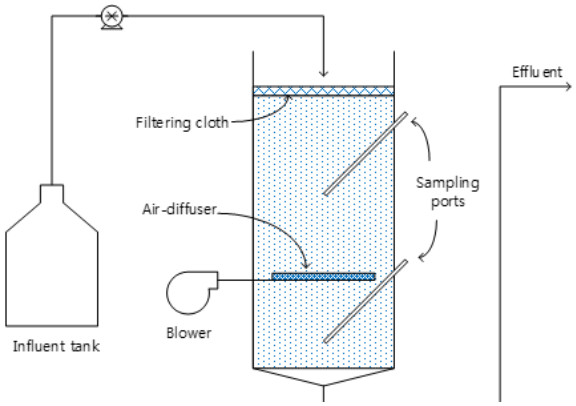

Fig. 1. Schematic layout of biofilm filtration system per set

2.2 Milking center wastewater

At the beginning of the experiment, return activated sludge prepared from public municipal sewage treatment facilities located in Chuncheon, South Korea was inoculated in the reactor. The reactor was adapted with diluted milking center wastewater (1:10) for two months. Activated sludge grew up on the surface of bedding materials during the adaptation period for a month and the photographs were taken by a camera (DIXI eXcope X3, Dixi optics, Korea) equipped on a microscope (Olymus AX70, Olympus, Japan) (Fig. 2).

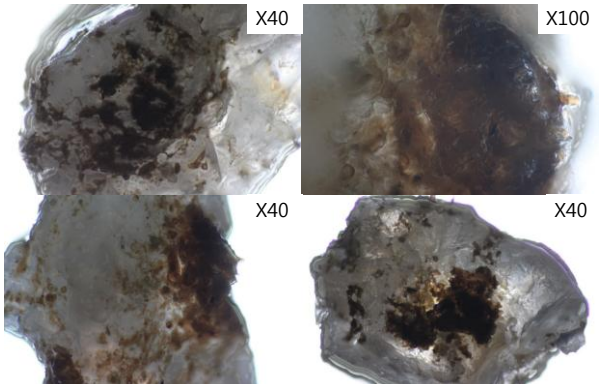

Fig. 2. Photographs of biofilm on sand as a bedding material at the beginning of the experiments

Milking center wastewater was collected from the research & development center in Seoul Dairy Co. every 2nd week throughout the experiment. The characteristics of wastewater filtered through cloth were very fluctuated during the experimental period of 8 months and summarized in Table 1. Milking center wastewater had a higher fraction of inert solids. The fraction of total volatile solids (TVS) to total solids (TS) was only 0.5 and further, volatile fraction in suspended solids (SS) was only 0.1. The SS in milking center wastewater was relatively low, but very variable as seen by 707.7±499.8 mg L^{-1}.

Table 1. Characteristics of milking center wastewater after filtering through cloth

Parameters	Concentration (mg L^{-1})	
SBOD*	2,175.3	± 1,075.5
SCOD	2,713.9	± 1,261.2
T-N	1,010.8	± 446.6
NH_4-N	198.3	± 77.6
NO_X-N	2.5	± 5.6
T-P	153.6	± 67.5
O-P	52.3	± 10.0
TS	2,993.1	± 1,123.6
TVS	1,497.3	± 691.9
SS	707.7	± 499.8
VSS	72.8	± 57.4

*SBOD, soluble biochemical oxygen demand; SCOD, soluble chemical oxygen demand; T-N, total nitrogen; T-P, total phosphate; O-P, ortho phosphate; TS, total solids; TVS, total volatile solids; SS, suspended solids; VSS, volatile suspended solids

2.3 Experimental procedures

In order to find an optimal NLR, four biofilm reactors were operated with the different flow rate of influent which led to 4 different NLRs in each reactor (Table 2). Four different flow rates were maintained by a peristaltic pump and the influent was introduced into the reactor for 1 minute in every hour. In order to maintain NLR designated in R1~4, the influent flow rates were separately adjusted, which resulted in the wide ranges of HRT under the four different NLR conditions as shown in Table 2. The effluent was naturally discharged via the outlet by atmospheric pressure. All the influent and effluent were collected every 2nd day and analyzed as quickly as possible and stored at 4^{o}C.

Table 2. Nitrogen loading rate in four reactors according to varying hydraulic retention time

	R1	R2	R3	R4
HRT* (day)	0.8-2.1	1.1-2.8	1.7-4.2	3.3-8.3
NLR (kg T-N m^{-3} d^{-1})	0.14±0.05	0.29±0.10	0.44±0.16	0.58±0.21

*HRT, hydraulic retention time; NLR, nitrogen loading rate; R1-4, labelling for four reactors

After the optimal NLR was determined, the position of air-diffuser was compared in between the one third and bottom of the reactor. Since the air-diffuser position resulted in the formation of aerobic or anoxic zone, the performance of nitrogen removal through SND might be compared to the separated reactions of nitrification and denitrification. Thus, two biofilm reactors had the air-diffuser at one-third of the reactor and the other two reactors had the air-diffuser at the bottom of the reactor. It might be expected that aerobic and anoxic zones formed separately when the air-diffuser was placed at one third of the reactor. The samples for comparison between upper and lower the air-diffuser were taken once a week using sampling ports.

Finally, the aeration rates of 0.06, 0.12, and 0.24 m^3 h^{-1} were also tested for two months. Thus, the performance of the biofilm reactor for nitrogen removal was evaluated with consideration of DO level.

2.3 Analytical methods

During the experimental period, the DO concentration was measured by DO meter (YSI 58, YSI Inc., USA). Once samples were collected, TS, TVS, SS, and volatile suspended solids (VSS) were firstly measured. The rests of samples were centrifuged at 3000 rpm for 10 min and the supernatant was analyzed for soluble biochemical oxygen demand (SBOD) and soluble chemical oxygen demand (SCOD) according to Standard methods (APHA, 1995).

Auto water analyzer (QuikChem 8500 series 2, Lachat, USA) was used for the analysis of nitrogen and phosphorus; i.e., NH_4-N, NO_x-N, and ortho phosphate (O-P) were measured using the analyzer and T-N and T-P were also analyzed after digestion with a block digester (BD 46, Lachat, USA).

3. RESULTS

3.1 Optimization of nitrogen loading rate

Firstly, the air-diffuser was placed at one third from the bottom and operated with the constant aeration rate of 0.12 m^3 h^{-1} in all four reactors. As described in Table 1, NLR was ranged at 0.14, 0.28, 0.43, and 0.57 kg TN m^{-3} d^{-1}. As result of NH_4-N removal efficiency, it was thought that the aeration rate of 0.12 m^3 h^{-1} might provide sufficient oxygen for nitrification since NH_4-N removal efficiency was over 90% except R4 (the highest NLR 0.57 kg m^{-3} d^{-1}).

Seyfried et al. (2001) described that the external carbon sources were required to achieve sufficient denitrification when C/N ratio was below 2.5. In this regard, the influent of this study contained insufficient carbon sources and C/N ratio was only 2.15 based on the SBOD as a readily available carbon source (Table 1). The high T-N removal efficiency was presumably due to simultaneous nitrification and denitrification (SND) without the addition of carbon sources. The NOx-N concentration was maintained at relatively low levels, which may support SND occurred in the biofilm reactor. In addition, DO level in all the biofilm reactor during the experimental period was 0.37±0.02 mg DO L^{-1} which was the appropriate level of 0.2-0.5 mg DO/L for SND (Moriyama et al., 1990).

The Fig. 3 displays T-N concentrations in effluent and removal efficiencies

according to the different NLR from 0.14 to 0.57 kg TN m^{-3} d^{-1}. Total nitrogen (T-N) removal efficiency was relatively high over 83% except R4 (the highest NLR of 0.58 kg m^{-3} d^{-1}) and NLR 0.45 kg TN m^{-3} d^{-1} was an optimal level to obtain higher nitrogen removal efficiency.

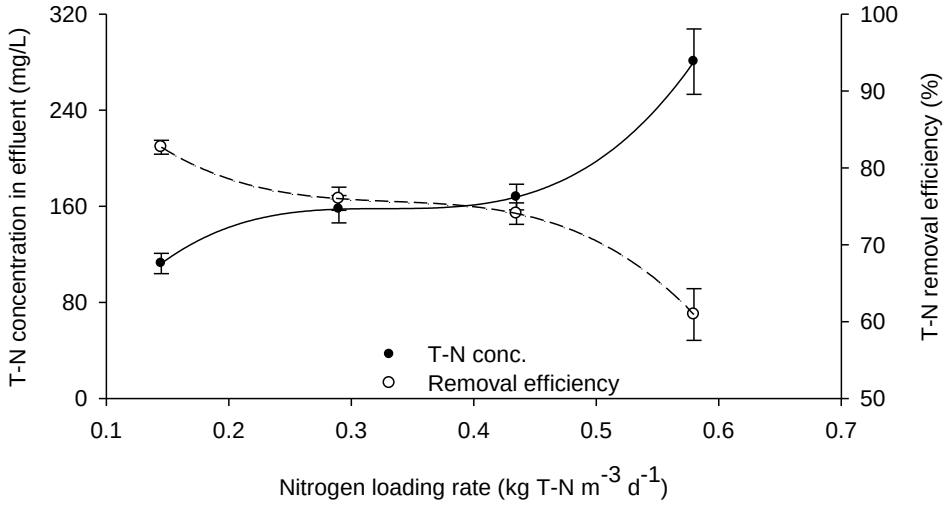

Fig. 3. Total nitrogen concentration in effluent and removal efficiencies according to nitrogen loading rate

3.2 Selection of air-diffuser position

In order to determine if the nitrification and denitrification occurs in upper and lower zones of air-diffuser corresponded with aerobic and anaerobic zones, respectively, nitrogen contents in liquid phase at the biofilm reactor were collected using the side ports as drawn in Fig. 1.

With higher NLR of 0.57 kg m^{-3} d^{-1} (corresponding to R4), difference of NOx-N concentration at aerobic and anoxic zone was around 6.2 mg NOx-N L^{-1} which was much higher than the others of 0.6-0.8 mg NOx-N L^{-1} in R1, 2, and 3 (Fig. 4). Since the characteristics of the influent was all the same for each reactor but the difference was only the flow rate of the influent, HRT and NLR were governed by the flow rate and SND might not be affected only by DO level but also NLR. Except R4, it was concluded that nitrification and denitrification simultaneously occurred regardless of aerobic and anoxic condition in this operational conditions.

Hence, the position of air-diffuser was changed to the bottom of the reactor (presenting “bottom”) and the performance of nitrogen removal was compared to the reactor in which air-diffuser was placed at the one third from the bottom of the reactor (presenting “middle”) under the same NLR condition of 0.1 kg m^{-3} d^{-1} with the constant aeration rate of 0.12 m^3 h^{-1}.

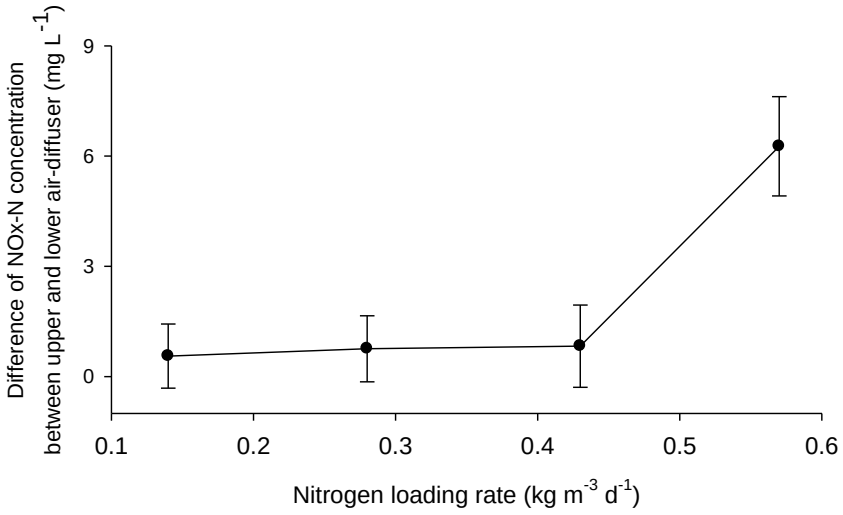

Fig. 4. Difference of NOx-N concentration between above and below air-diffuser in each reactor

There were no big differences in NH_4-N removal efficiency according to the changes of air-diffuser position as seen in Fig. 5. However, the better performance of denitrification, based on the result of NOx-N concentration, was found at the reactor with middle over bottom air-diffuser, whereby T-N concentration operated with middle air-diffuser showed the lower value than that with bottom air-diffuser. Though SND occurred in all reactors with both position of air diffuser, the one third position of air-diffuser might help the performance of denitrification below the air-diffuser. In other words, the residual NOx-N from the upper zone was further reduced in the lower zone, which may be caused by aeration rate. Thus, the one-third from the bottom of the reactor was selected as a better position of air-diffuser to obtain higher nitrogen removal efficiency.

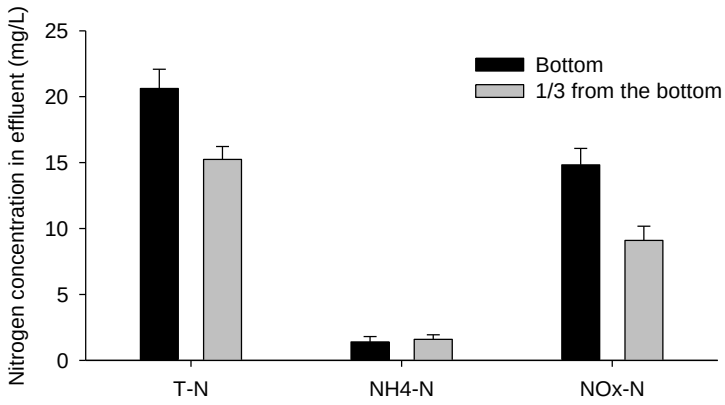

Fig. 5. The concentration of nitrogen contents derived from two different positions of air-diffuser

3.3 Evaluation of aeration rate

Under the same conditions of NLR 0.21 kg m^{-3} d^{-1} and the position of air-diffuser at one third from the bottom of the reactor, aeration rates of 0.06, 0.12, and 0.24 m^3 h^{-1} were given to three reactors separately.

Once air were supplied, the nitrification was relatively well achieved on NH_4-N removal efficiency of 93.9, 98.3, and 98.9% in each reactor with aeration rate of 0.06, 0.12, and 0.24 m^3 h^{-1}, respectively. With aeration rate of 0.12 and 0.24 m^3 h^{-1}, NH_4-N in influent was almost completely oxidized. However, aeration rate of 0.06 m^3 h^{-1} might be insufficient to achieve the complete oxidation of NH_4-N when compared to the other higher aeration rate, which resulted in the highest T-N amount remained in the effluent (Fig. 6). During this test, DO level with aeration rate of 0.12 m^3 h^{-1} was 0.47±0.19 mg DO L^{-1}, while aeration of 0.24 m^3 h^{-1} led to 2.14±0.95 mg DO L^{-1}. Thus, the SND might be inhibited with higher aeration of 4 L min^{-1} since oxygen gradient was not reached zero at the bottom layer of biofilm. In addition, since SND can be achieved with the partial oxidation of ammonium to nitrite (Surmacz-Gòrska et al., 1997; Yoo et al., 1999), then reduced to N_2 gas, nitrogen removal might be accomplished by the separated zones, formally aerobic and anoxic zones.

Accordingly, compared the aeration rate of between 0.12 and 0.24 m^3 h^{-1}, the slightly better performance of nitrogen removal could be obtained with aeration rate of 0.12 m^3 h^{-1} achieved by SND and the optimal aeration rate was 0.12 m^3 h^{-1} in the present study which resulted in an appropriate SND condition of DO range of 0.2-0.5 mg DO L^{-1} (0.47±0.19 mg DO L^{-1}).

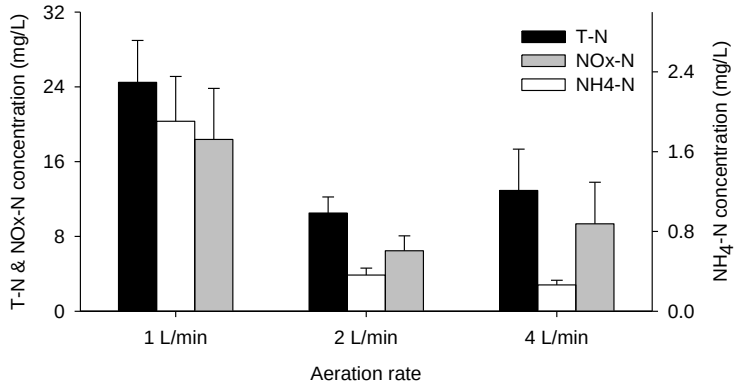

Fig. 6. Nitrogen concentration according to aeration rate of 0.06, 0.12, and 0.24 m^3 h^{-1}

4. CONCLUSIONS

In the present study, nitrogen removal from milking center wastewater was investigated using the biofilm filtration reactor of which the operational conditions (NLR, the position of air-diffuser, and an aeration rate) were optimized. Nitrogen removal was successfully achieved via SND in biofilm filtration reactor packed with sand as a bedding material without the addition of external carbon sources. Biofilm formed by attached growth microorganisms has an oxygen gradient with the thickness of biofilm. Thus, the inner zone of biofilm has an oxygen zero condition, which facilitates to

perform the denitrification.

As result of the evaluation of the operational parameters, the higher nitrogen removal was obtained when NLR was 0.45 kg m^{-3} d^{-1} and aeration rate of 0.12 m^3 h^{-1} was selected to maintain the optimal DO level for SND. The active nitrogen removal with higher aeration rate of 0.24 m^3 h^{-1} was presumably achieved by the conventional anoxic condition below air-diffuser. There was no big difference in the performance of nitrogen removal due to the air-diffuser position between the bottom and the on third from the bottom of the reactor but one third position could have a benefit to failure of SND. In order to investigate SND with the oxygen gradient according to the thickness of biofilm on a bedding material, the study of nitrite to nitrate ratio will be required in the future.

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