# EFFECT OF AERATION RATES ON SIMULTANEOUS NITRIFICATION AND DENITRIFICATION IN INTERMITTENT AERATED BIOREACTOR

# S.T.T. LE<sup>1, 2</sup>, W. KHANITCHAIDECHA<sup>1, 2\*</sup> AND A. NAKARUK<sup>3</sup>

<sup>1</sup>Department of Civil Engineering, Faculty of Engineering <sup>2</sup>Centre of Excellence for Innovation and Technology for Water Treatment <sup>3</sup>Department of Industrial Engineering, Faculty of Engineering Naresuan University, Thailand

(Received 10 January, 2015; accepted 12 April, 2015)

Key words : Nitrogen wastewater, Eutrophication, Intermittent aerated bioreactor, Nitrification and denitrification

**Abstract**– Eutrophication caused by high nitrogen wastewater has become a serious problem in many countries. One of the current common applied technology for nitrogen removal is biological process. In this work, an intermittent aerated bioreactor was developed to treat high nitrogen wastewater. The effect of different aeration rates on the reactor performance was also investigated. The results showed that at the low aeration of 0.5 L/min, the nitrogen removal efficiency reached the peak of 65%, while the nitrification and denitrification rates were approximately 0.2 and 1.8 mg/L-min, respectively. Despite of the higher nitrification rates at higher aeration rates, the denitrification rates were found to decrease because of competitive microorganisms growth and the lack of carbon source. This resulted in the drop of the overall nitrogen efficiency with the increasing aeration rates.

#### **INTRODUCTION**

Nitrogen and phosphorus are the essential nutrients for living things. However, when these nutrients are present at high concentrations in water bodies, they can cause the explosive growth of aquatic plants, typically algae, which leads to the oxygen depletion in water and the death of aquatic organisms as a result. This phenomenon is usually known as algae bloom or eutrophication. One of the main sources of nutrients discharge which results in eutrophication is the domestic wastewater (Gong et al., 2012; Wang et al., 2014). In the typical domestic wastewater, the concentration of nitrogen can be up to 44 mg/L of NH<sub>4</sub>-N (Nguyen et al., 2014a; Nguyen et al., 2014b). Meanwhile, less than 0.5 mg/L of NH<sub>4</sub>-N should be found in surface water (PCD, 2014). Eutrophication is a big and challenging problem in many countries all over the world. One of the reasons is the lack of a both highly effective and economical technology to remove nitrogen from the wastewater.

Among the most common used techniques these days, biological process has been considered to be able to obtain good performance and relatively cost effectiveness (Huang et al., 2013; Yao et al., 2013). Liu et al. (2013) revealed that the biological nitrogen removal can be enhanced by intermittent aeration mode, and the nitrification microbial process plays an important role. The growth of ammoniaoxidizing bacteria and nitrite oxidizing bacteria which are nitrifying microorganisms was reliable to increase under the intermittent aeration (Fan et al., 2013). However, the different intermittent periods can cause a shift of diverse microbial community (such as phylum Proteobacteria, Firmicutes and Bacteroidetes) in the nitrogen removal system (Guadie et al., 2014). Therefore, the aim of this work is to investigate the nitrogen removal efficiency of an intermittent aerated bioreactor under a typical intermittent period. The effects of different air supply rates on the bioreactor performance and microbial community are also examined.

#### METHODOLOGY

#### Synthetic Wastewater

In order to minimize variability in the

\*Corresponding author's email: wilawank1@gmail.com, wilawank@nu.ac.th

experiment, the synthetic wastewater was used in this study by mixing the following chemicals (g/L); NH<sub>4</sub>Cl 0.15 g, NaHCO<sub>3</sub> 0.48 g, KH<sub>2</sub>PO<sub>3</sub> 0.02 g, MgSO<sub>4</sub> 0.06 g, CaCl<sub>2</sub> 0.36 g, FeSO<sub>4</sub> 0.003 and trace element 0.5 mL(Guo *et al.*, 2013). The concentration of NH<sub>4</sub>-N was approximately 40 mg/L. In the meantime, other nitrogen forms, particularly NO<sub>2</sub>-N and NO<sub>3</sub>-N have the concentration of less than 1 mg/L.

# **Bioreactor Set-Up and Operation**

Active sludge was obtained from the wastewater treatment plant of Wongtong Hospital, Phitsanulok, Thailand and acclimatised in the synthetic wastewater for a month. A bioreactor with 24 cm in diameter and 40 cm in height was set up using 2 L of acclimatized sludge and 8 L of synthetic wastewater. During operation, the sludge was not disposed (SRT = 0), and the MLSS and MLVSS were approximately 4,600 and 3,200 mg/L. A cycle of intermittent aeration supply was 24 hours. During the aeration period, air was supplied for two hours at three different flow rates; low aeration (0.5 L/min), medium aeration (1.0 L/min) and high aeration (2.0 L/min). During the non-aeration period, no air was supplied to the bioreactor for two hours, thus the dissolved oxygen (DO) concentration was sharply dropped to 0.5 mg/L. The acetate solution was added in the first non-aeration period as a carbon source for the denitrification process. To minimize the operation cost, a low C/N ratio of 1.5 was controlled (Khanitchaidecha et al., 2010). After that the aeration and non-aeration periods of two hours were continued to finish 24 hours (Fig. 1). The nitrogen removal efficiency of the bioreactor was calculated using Eq. 1.

$$Efficiency = (1 - \frac{[NH_4 - N]_{eff} + [NO_3 - N]_{eff} + [NO_2 - N]_{eff}}{[NH_4 - N]_{inf}}) \times 100$$
...(1)

where,  $[NH_4-N]_{inf}$  = concentration of ammonium in the synthetic wastewater

 $[NH_4-N]_{eff'}$   $[NO_3-N]_{eff'}[NO_2-N]_{eff}$  = concentrations of ammonium, nitrate and nitrite in the treated water

After one cycle of treatment (24 hours) finished, the treated wastewater in the bioreactor was drained out and replaced by the new synthetic wastewater with the same constituents and volume. The bioreactor was operated for three months and the average efficiency of nitrogen removal was achieved.

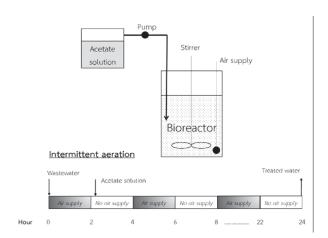


Fig. 1. Schematic diagram of bioreactor

# Nitrification and Denitrification Rates Determination

For the nitrification rate determination, the NH<sub>4</sub>-N wastewater of 40 mg/L was added in the bioreactor which continuously stirring and aerating. The water was sampled every 10 min and analysed for changing NH<sub>4</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N. The rate of NH<sub>4</sub>-N reduction can refer to nitrification rate. On the other hand, the denitrification rate was determined by adding the NO<sub>3</sub>-N wastewater of 40 mg/L into the bioreactor. The bioreactor was continuously stirring and no aerating. The acetate solution was added in the beginning to maintain the C/N ratio of 1.5. Then the water was sampled every 10 min and analysed for changing NO<sub>2</sub>-N and NO<sub>3</sub>-N.

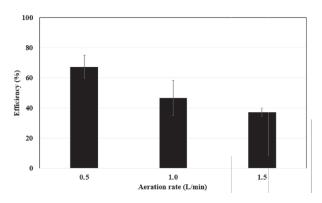
#### **RESULTS AND DISCUSSION**

# Performance of Intermittent Aerated Bioreactor and Nitrogen Removal Mechanisms

The bioreactor was operated under intermittent aeration at various aeration rates of 0.5 (low), 1.0 (medium) and 2.0 (high) L/min, respectively. At the low aeration rate, the NH<sub>4</sub>-N and NO<sub>2</sub>-N was no found in the treated water, however the NO<sub>3</sub>-N ranging of 10-20 mg/L was occurred (data not shown). The average efficiency was around 65% (Fig. 2). The water samples were taken to determine the mechanisms occurred in the bioreactor for nitrogen removal, and the results are shown in Figure 3a. In the first aeration period (hour 0-2), the NH<sub>4</sub>-N was sharply decreased from 40 to 27 mg/L,

while the  $NO_3$ -N was immediately increased to 16 mg/L. This indicated the occurrence of nitrification process and the sufficient oxygen supply to convert  $NH_4$ -N to  $NO_3$ -N with no intermediate form of  $NO_2$ -N. From Figure 3b and 3c, the reductions of DO and pH refer that large volume of oxygen was consumed for nitrification and H<sup>+</sup> was generated. The theoretical nitrification equation was present in Eq. 2 (Khanitchaidecha *et al.*, 2013).

In the first non-aeration(hour 2-4), the NH<sub>4</sub>-N continuous to decrease to 18 mg/L. Although air supply was stopped, however the remaining oxygen can oxidise some NH<sub>4</sub>-N to NO<sub>3</sub>-N. Due to the low oxygen and existence of organic carbon, the occurred NO<sub>2</sub>-N was simultaneously removed by denitrification process, as supported by increasing pH from generated OH<sup>-</sup>. The theoretical nitrification equation was present in Eq. 3 (Khanitchaidecha et al., 2011). The results reveal that the nitrification and denitrification was occurred simultaneously in this period. The NH<sub>4</sub>-N was continuously oxidised in the second aeration (hour 4-6), until its concentration became zero. However, due to insufficient organic carbon (~5 mg COD /L remained), the NO<sub>3</sub>-N still remained in the treated



**Fig. 2.** Average efficiency of intermittent aerated bioreactor at various aeration rates.

water (data not shown).

$$\begin{split} & \mathsf{NH}_4^+ + 1.86\mathrm{O}_2 + 0.10\mathrm{CO}_2 {\rightarrow} 0.98\mathrm{NO}_3^- + 0.09\mathrm{H}_2\mathrm{O} + 1.98\mathrm{H}^+ + \\ & 0.02\mathrm{C}_5\mathrm{H}_7\mathrm{NO}_2 & \dots & (2) \\ & \mathrm{NO}_3^- + 1.08\mathrm{CH}_3\mathrm{OH} + \mathrm{H}^+ {\rightarrow} 0.47\mathrm{N}_2 + 2.44\mathrm{H}_2\mathrm{O} + 0.76\mathrm{CO}_2 + \\ & 0.065\mathrm{C}_5\mathrm{H}_7\mathrm{NO}_2 & \dots & (3) \end{split}$$

#### **Performance at Various Aeration Rates**

Figure 2 presents the average efficiency of nitrogen removal during three months of operation under different aeration rates. It can be seen that the highest efficiency was obtained at the low aeration rate of 0.5 L/min. The nitrogen removal efficiency decreased from 65% to 47% and 38% with the increase of aeration rates from 0.5 L/min to 1.0 and 2.0 L/min, respectively. These results have proved that the aeration rate affected to the nitrogen removal efficiency of the intermittent aerated bioreactor. The observed decrease of nitrogen removal efficiency can be explained based on the results of NH<sub>4</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N concentration analysis (Table 1). Although the nitrogen removal efficiency in this study was fairly low, the lower air supply and carbon addition than other studies were significant reasons (as summarised in Table 2). In addition, the use of supporting media such as polyurethane foam for increasing microorganisms and enhancing reactor performance was found in the previous studies.

The nitrogen removal mechanisms occurred in this bioreactor can be divided into two stages that are nitrification and denitrification. Nitrification is the process in which  $NH_4$ -N is transferred into  $NO_2$ -N and then  $NO_3$ -N in the presence of oxygen. Meanwhile, in the denitrification process, in the presence of carbon source and in the absence of oxygen,  $NO_3$ -N is transferred into  $NO_2$ -N and then nitrogen gas which is finally released into the air. Table 1 shows the removal rates of  $NH_4$ -N,  $NO_2$ -N and  $NO_3$ -N as the indicators of nitrification and denitrification processes at different aeration rates.

Table 2. Comparison of the reactor performance and operating condition of this study and previous studies

Intermittent hours (on/off)	Influent NH <sub>4</sub> -N (mg/L)	C/N ratio	Air supply	Efficiency (%) (L/min)	Reference
0.75/0.25	40-60	1.5	8	82	Guadieet al., 2014
1/1	50	1.0	$N/A^*$	57	Lim <i>et al.</i> , 2012
1/2	25	1.2	4	80	Cho <i>et al.</i> , 2007
1/5	40	1.7	2	80	Fan <i>et al.</i> , 2013
2/1	25	3.2	1.4	82	Mouraet al., 2012
2/2	40	1.5	0.5	65	This study

Remark: \*DO 6-7 mg/L

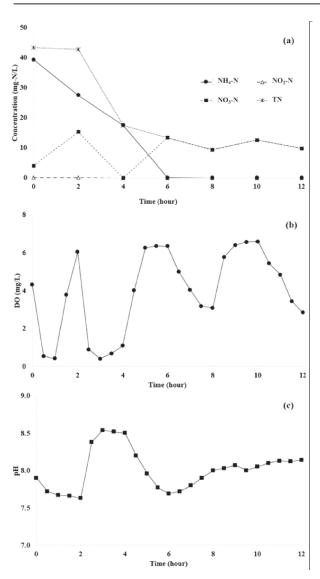


Fig. 3. Profiles of (a) nitrogen, (b) DO and (c) pH at low aeration rate.

It can be seen that the nitrification rate increased with the increasing aeration rates. This is because the activity of the microorganism driving nitrification process was enhanced under high oxygen conditions (Jia *et al.*, 2013). In the next stage of denitrification, although there was no aeration, the excess of oxygenwas still remained in the bioreactor. This amount of oxygen not only inhibited the activity of denitrification microorganisms but also created conditions for the growth of competitive microorganisms, typically heterotroph. Heterotroph consume both oxygen and carbon source, which led to the shortage of carbon for denitrification microorganisms. Therefore, denitrification process was inhibited, leading to the drop of denitrification rates. This explanation is also supported by the change of the sludge colour from dark brown to light brown during the operation process, which refers to the change in dominant microorganism's community in the bioreactor. The SVI became to increase from 54 mL/g at the low aeration to 72 mL/g at the high aeration.

Furthermore, it has to be noted that in addition to the growth of heterotroph, nitrogencould not be completely removed from the wastewater also because of the insufficient carbon source. The bioreactor fed on acetate solution as carbon source only one time when the bioreactor was set up. This carbon source was sufficient for the first nonaeration period (hour 2-4). However, in the next periods, acetate was consumed and became insufficient for the denitrification process to occur effectively. Thus the NO<sub>3</sub>-N still remained while the NH<sub>4</sub>-N was completely removed after treatment.

#### CONCLUSION

In the present work, the intermittent aerated bioreactor for nitrogen removal has successfully developed. The results showed that the set-up bioreactor was able to remove 65% of nitrogen at the aeration rate of 0.5 L/min. However, the efficiency decreased with the increasing aeration rates due to the growth of competitive microorganisms. Additionally, the lack of carbon source was found to be one of reasons of the incomplete nitrogen removal. In conclusion, this work has proved that the nitrogen removal process consisted of two stages, nitrification and denitrification, which were affected due to the aeration rates.

### ACKNOWLEDGEMENT

The authors would like to thank to OpaTangpitukkul's scholarship and Naresuan University's research funding for the financial support.

#### REFERENCES

- Cho, E.S., Zhu, J. and Yang, P.Y. 2007. Intermittently aerated EMMC-biobarrel (entrapped mixed microbial cell with bio-barrel) process for concurrent organic and nitrogen removal. *Journal of Environmental Management* 84 : 257-265.
- Fan, J., Zhang, B., Zhang, J., Ngo, H.H., Guo, W., Liu, F., Guo, Y. and Wu, H. 2013. Intermittent aeration

strategy to enhance organics and nitrogen removal in subsurface flow constructed wetlands. *Bioresource Technology.* 141: 117-122.

- Gong, L., Jun, L., Yang, Q., Wang, S., Ma, B. and Peng, Y. 2012. Biomass characteristics and simultaneous nitrification-denitrification under long sludge retention time in an integrated reactor treating rural domestic sewage. *Bioresource Technology*. 119 : 277-284.
- Guadie, A., Xia, S., Zhang, Z., Zeleke, J., Guo, W., Ngo, H.H. and Hermanowicz, S.W. 2014. Effect of intermittent aeration cycle on nutrient removal and microbial community in a fludized bed-reactormembrane bioreactor combo system. *Bioresource Technology*. 156 : 195-205.
- Guo, J., Zhange, L., Chem, W., Ma, F., Liu, H. and Tian, Y. 2013. The regulation and control strategies of a sequencing batch reactor for simultaneous nitrification and denitrification at different temperatures. *Bioresource Technology*. 133 : 59-67.
- Huang, X., Li, W., Zhang, D. and Qin W. 2013. Ammonium removal by a novel oligotrophic *Acinetobacter sp.* Y16 capable of heterotrophic nitrification-aerobic denitrification at low temperature. *Bioresource Technology* 146 : 44-50.
- Jia W., Liang, S., Ngo, H.H., Guo, W., Zhang, J., Wang, R. and Zou, Y. 2013. Effect of phosphorus load on nutrients removal and N2O emission during lowoxygen simultaneous nitrification and denitrification process. *Bioresource Technology.* 141 : 123-130.
- Khanitchaidecha, W., Koshy, P., Kamei, T., Shakya, M. and Kazama, F. 2013. Investigation of the effects of hydrogenotrophic denitrification and anammox on the improvement of the quality of the drinking water supply system. *Journal of Environmental Science and Health: Part A.* 48 : 1533-1542.
- Khanitchaidecha, W., Nakamura, T., Sumino, T. and F.Kazama 2010. Performance of intermittent aeration reactor on NH4-N removal from groundwater resources. *Water Science and Technology*. 61 : 3061-3069.

- Khanitchaidecha, W., Tanaka, Y., Sumino, T. and Kazama, F. 2011. Modelling of an immobilized sludge reactor with polyethylene glycol (PEG)-pellet to remove NH4-N from groundwater. *Water Science and Technology: Water Supply.* 11 : 534-544.
- Lim, J., Lim, P. and Seng, C. 2012. Enhancement of nitrogen removal in moving bed sequencing batch reactor with intermittent aeration during REACT period. *Chemical Engineering Journal.* 197 : 199-203
- Liu, L., Zhao, X., Zhao, N., Shen, Z., Wang, M., Guo, Y. and Xu, Y. 2013. Effect of aeration modes and influent COD/N ratios on the nitrogen removal performance of vertical flow constructed wetland. *Ecological Engineering*. 57 : 10-16.
- Moura, R.B., Damianovic, M.H.R.Z. and Foresti, E. 2012. Nitrogen and carbon removal from synthetic wastewater in a vertical structured-bed reactor under intermittent aeration. *Journal of Environmental Management.* 98 : 163-167.
- Nguyen, D.D., Ngo, H.H., Kima, S.D. and Yoon, Y.S. 2014a. A specific pilot-scale membrane hybrid treatment system for municipal wastewater treatment. *Bioresource Technology.* 169 : 52-61.
- Nguyen, D.D., Ngo, H.H., Yoon, Y.S. 2014b. A new hybrid treatment system of bioreactors and electrocoagulation for superior removal of organic and nutrient pollutants from municipal wastewater. *Bioresource Technology*. 153 : 116-125.
- Pollution Control Department (PCD), Ministry of Natural Resource and Environment, Thailand. URL: http:// www.pcd.go.th. Access September 2014.
- Wang, C., Sample, D.J. and Bell, C. 2014. Vegetation effects on floating treatment wetland nutrient removal and harvesting strategies in urban stormwater ponds. *Science of the Total Environment*. 499 : 384-393.
- Yao, S., Ni, J., Ma, T. and Li, C. 2013. Heterotrophic nitrication and aerobic denitrication at low temperature by a newly isolated bacterium, *Acinetobacter sp.* HA2. *Bioresource Technology*. 139:80-86.