Partial Nitrification by controlling Environmental Conditions in a Swim-bed Reactor

Xiumei Duan¹,* , Li Li², Shuping Deng¹, Haiyan Liu¹

¹YingKou Institute of Technology, Yingkou 115014, P.R. China.
²Puritek Environment Group Co., Ltd., Nanjing, Jiangsu 210048, China.

*Corresponding author e-mail: duanxiumeiabc@163.com

Abstract. The effects of ammonia concentration, hydraulic detention time and dissolved oxygen on the ammonia conversion rate and nitrite accumulation efficiency were studied in the swim-bed biofilm reactor with the constant temperature and pH value. The temperature was controlled by heating rod at 35±1 ℃, the pH value was automatically regulated at 7.5 by adding 2 mol/L NaOH, influent ammonia concentration increased from 200 mg N/L, the dissolved oxygen concentration was below 2.5 mg/L, a stable nitrite accumulation were achieved in a swim-bed reactor and nitrite oxidizing bacteria was washed out.

1. Introduction

After anammox process was discovered (Mulder et al., 1995), partial nitrification process had attracted wide attention with anammox process as an alternative nitrogen removal process (Kuai and Verstraete, 1998; Pynaert et al., 2003). Currently, about 40 full-scale partial nitrification/anammox plants are established, treating mostly sewage sludge reject water, landfill leachate or food processing digestate. Four of these are spatially separated with partial nitrification and anammox, while others are executed in single-stage process (Vlaeminck et al., 2012).

Partial nitrification is a pretreatment for anammox. For this, many researchers have done efforts to achieve partial nitrification. Partial nitrification can be obtained by selectively inhibiting nitrite oxidizing bacteria, such as DO concentration, sludge retention time, pH, temperature, substrate concentration and load, inhibitor and so on (Wang and Yang, 2004). Joo et al. (2000) achieved 2 kg NH₄⁻-N/m³/d, more than 95% of NH₄⁺-N transformed to NO₂⁻-N and NO₃⁻-N adopting a upflow biological aerated filter, and NO₂⁻-N was accumulated up to 60% of the total NOₓ⁻-N when oxygen was limited. Wang and Yang (2004) showed that partial nitrification was steadily obtained and the optimal operational parameters were pH 7.5, DO 1.5 mg/L, T 30℃ based on ammonia oxidation rate and nitrite accumulation rate. Blackburne et al. (2008) achieved stable nitritation under DO concentration of 0.3 mg/L, resulting in a nitritation capacity of 0.25 kg N/m³/day but only about 70% conversion of ammonium to nitrite.

In this paper, a stable nitrite accumulation were achieved by controlling environmental conditions in a swim-bed reactor, The temperature was controlled by temperature-controlled heating rod at 35±1℃, the pH value was automatically regulated at 7.5 by adding 2 mol/L NaOH during the period of start-up of the reactor, influent ammonia concentration increased gradually from 200 mg N/L to 500 mg N/L. At
the end of the phase, the dissolved oxygen concentration was monitored. Finally, the ammonia-oxidizing bacteria were enriched, and most of the other bacteria were washed out of the reactor.

2. Materials and methods

2.1. Influent water and inoculated sludge
A synthetic wastewater, prepared with tap water and containing NH4HCO3 (nitrogen source, carbon source and buffer) was fed. The composition of wastewater (g/L) was shown as the paper (Third et al., 2001). NH4HCO3 concentration and flow rate were adjusted depending on the TN removal capacity during the experiment, and to obtain the desired nitrogen load and hydraulic retention time (HRT).

The reactor was initially seeded using activated sludge from Yingkou East sewage treatment plant with mixed-liquor suspended solids (MLSS) concentration of 5000 mg/L.

2.2. Experimental setup and operational conditions for partial nitrification
A schematic diagram of the swim-bed reactor is shown in Fig. 1a. The reactor was rectangular, made of plexiglass, the height is about 770 mm, the length is about 80 mm, the width is 70 mm, the effective volume is 5.5 L.

Biofringe (BF) packing was made of hydrophilic polyester fibers, fiber wires in the center at intervals of 30 mm were connected by a rope in the vertical center connected. Two fiber wires remain point of view by reversing the up and down. The length of filament is 10 cm, diameter 3 mm. One piece of the biofill (acryl-fiber biomass carrier; NET, Hyogo) was used as filling material, as shown in Fig. 1b.

The temperature was controlled by temperature-controlled heating rods at 35±1℃, the pH value was automatically regulated at 7.5 by adding 2 mol/L NaOH during the period of start-up of the reactor.

The whole experiment is divided into two stages. In the first stage (day 1-99), partial nitrifying sludge formed. The ammonia-oxidizing bacteria were enriched, and most of the other bacteria were washed out of the reactor. Influent ammonia concentration increased gradually from 200 mg N/L to 500 mg N/L. At the end of the phase, the dissolved oxygen concentration was monitored. In the second stage (day 100-126), temperature, pH, ammonia concentration and hydraulic retention time were the same. The activated sludge in the sedimentation tank was sent back to reactor every three hours, the DO concentration variation was monitored during this time. The samples were taken every two days, and measured ammonia nitrogen, nitrite nitrogen and nitrate nitrogen concentrations.

2.3. Analytical methods
The concentration of NO2⁻-N was determined using N-1-naphthyl ethylenediamine reagent colorimetric method, and the concentration of NO3⁻-N was determined using H2SO4-salicylic acid reagent.
colorimetric method, NH\textsubscript{4}\textsuperscript{+}-N was measured using Nessler's reagent colorimetric method, MLVSS concentration was measured according to the standard methods (APHA, 1995). DO concentration was measured by a portable dissolved oxygen meter (FG4, Mettler-toledo, Switzerland).

2.4. Fluorescence in situ hybridization (FISH)
Molecular analysis allows insights into the distribution and amount of ammonia oxidizing bacteria and other bacteria (Schramm et al., 2000), potentially revealing a basis for nitrite accumulation. The procedure for FISH analysis was as described by (Zhou et al., 2007).

The enriched partial nitrifying sludge were fixed according to the paper (Egli et al., 2003), four oligonucleotide FISH probes labeled with fluorochrome FAM were used for hybridization: Nso1225, NEU, Nsv443, Nmv, which target the majority of ammonia oxidizing bacteria. And DAPI combined with nucleic acid for staining total bacteria in experiment. The hybridization procedure followed (Egli et al., 2003). Images were captured with a laser con-focal fluorescence microscopy (FV1000, Olympus, Japan). Image Pro-Plus software was utilized to analyze FISH images.

3. Results and discussion

3.1. Reactor startup and nitrite accumulation
In this study, a stable nitrite accumulation was realized by controlling temperature, pH value, DO concentration, and the results are shown in Figs. 2a and b.

In the first stage (day 1-99), the pH value and temperature of wastewater in reactor was controlled about 7.5 and 35°C. The aeration amount was adjusted to cycle in the reactor, the sludge in sedimentation tank was refluxed once every 12 h. During this time, the influent NH\textsubscript{4}\textsuperscript{+}-N average concentration is 502.7 mg N/L, effluent NO\textsubscript{2}\textsuperscript{-}-N and NO\textsubscript{3}\textsuperscript{-}-N concentrations are 247.4, 92.5 mg/L. the NO\textsubscript{2}\textsuperscript{-}-N accumulation rate reached 72.6%.

No ammonium was added during the first several days, but the NH\textsubscript{4}\textsuperscript{+}-N concentration was high, maybe that is because of bacterial digestion due to sufficient aeration. After starting the operation with 200 mg/L of NH\textsubscript{4}\textsuperscript{+}-N concentration, NO\textsubscript{2}\textsuperscript{-}-N production was observed immediately. As the influent NH\textsubscript{4}\textsuperscript{+}-N concentration increased stepwise to 300 mg/L and 500 mg/L, NO\textsubscript{2}\textsuperscript{-}-N production consistently followed a similar trend. Production of NO\textsubscript{3}\textsuperscript{-}-N was below 100 mg/L. The nitrite was accumulated rapidly by controlling temperature and pH value. The effluent NO\textsubscript{2}\textsuperscript{-}-N/TN increased to about 60% from 10%, and the effluent NO\textsubscript{3}\textsuperscript{-}-N/TN decreased to about 12% from 40%, and the NO\textsubscript{2}\textsuperscript{-}-N accumulation efficiency increased to about 82% from 68%.

In the phase (day 32-100), influent NH\textsubscript{4}\textsuperscript{+}-N concentration is 500-700 N mg/L, and the NH\textsubscript{4}\textsuperscript{+}-N conversion efficiency declined sharply to 25% from about 80% along the sludge concentration decrease, but the NO\textsubscript{2}\textsuperscript{-}-N accumulation efficiency increased to about 90%. In the phase (day 100-126), sludge reflow times increased, and activated sludge concentration in the reactor increased, and DO concentration in the reactor dropped. Effluent NO\textsubscript{2}\textsuperscript{-}-N concentration increased, and NO\textsubscript{3}\textsuperscript{-}-N concentration decreased slightly. The maximum NO\textsubscript{2}\textsuperscript{-}-N accumulation efficiency reached 86.4% on day 126.

In the second stage (100-126), the pH value and temperature of wastewater in reactor are constant, the difference is increasing the sludge reflow cycles, and the sludge concentration increased, DO concentration in the reactor dropped to below 2.5 mg/L. During this time, the influent NH\textsubscript{4}\textsuperscript{+}-N average concentration is 557.1 mg N/L, effluent NO\textsubscript{2}\textsuperscript{-}-N and NO\textsubscript{3}\textsuperscript{-}-N concentrations are 408.8, 89.3 mg/L. the NO\textsubscript{2}\textsuperscript{-}-N accumulation rate reached 82.1%.
Fig. 2 Time courses of nitrogen concentrations and sludge concentration (a), nitrogen percentages and nitrite accumulation rate (b) in the partial nitrification reactor.

3.2. The abundance of ammonia oxidizing bacteria
The abundance of ammonia oxidizing bacteria in the reactor was investigated by FISH and CLSM. Representative confocal images from reactor sampled on day 100 were shown in Figs. 3B. Ammonia
oxidizing bacteria in the reactor would be hybridized with AOBmix (green). Total bacteria would be combined with DAPI, so they show a blue color in presented confocal images.

On day 100 (after the startup of the reactor), the abundance of ammonia oxidizing bacteria was detected. Two groups of bacteria was analyzed, ammonia oxidizing bacteria and the total bacteria. Ammonia oxidizing bacteria hybrid with a group of probes labeled with FITC, it shows green, the populations of ammonia oxidizing bacteria accounted for the majority, shown as Fig. 3B; DAPI stains the total bacteria, it shows blue, shown as Fig. 3A.

**Fig. 3** Sludge microflora changes in partial nitrification reactor.

A. The total bacteria after cultivation, blue color represents total bacteria (labelled with DAPI); B. Ammonia oxidizing bacteria after cultivation, green color indicates the ammonia oxidizing bacteria (labelled with FITC).

4. Conclusion

The temperature was controlled by heating rod at 35±1℃, the pH value was automatically regulated at 7.5 by adding 2 mol/L NaOH, influent ammonia concentration varied from 200 mg N/L to 700 mg N/L, the dissolved oxygen concentration was below 2.5 mg/L, a stable nitrite accumulation were achieved in a swim-bed reactor. FISH showed that the bacteria in the reactor were mainly ammonia oxidizing bacteria, the nitrite oxidizing bacteria were inhibited reproductively and died out.

Acknowledgments

This work was financially supported by Yingkou Institute of Technology Project (YBL201815) fund.

References

[1] APHA, 1995. Standard Methods for the Examination of Water and Wastewater, American Public Health Association, New York.

[2] Blackburne, R., Yuan, Z. and Keller, J., 2008. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation. 19 (2), 303-312.

[3] Egli, K., Langer, C., Siegrist, H.R., Zehnder, A.J.B., Wagner, M. and van der Meer, J.R., 2003. Community analysis of ammonia and nitrite oxidizers during start-up of nitritation reactors. Applied and environmental microbiology. 69 (6), 3213-3222.

[4] Joo, S.H., Kim, D.J., Yoo, I.K. 2000. Partial nitrification in an upflow biological aerated filter by O2 limitation. Biotechnology letters. 22 (11), 937-940.

[5] Kuai, L. and Verstraete, W. 1998. Ammonium removal by the oxygen-limited autotrophic nitrification-denitrification system. Applied and Environmental Microbiology. 64 (11), 4500-4506.

[6] Mulder, A., Graa, A.A., Robertson, L.A., 1995. Anaerobic ammonium oxidation discovered in a
denitrifying fluidized bed reactor. FEMS Microbiology Ecology. 16 (3), 177-184.

[7] Pynaert, K., Smets, B.F., Wyffels, S., 2003. Characterization of an autotrophic nitrogen-removing biofilm from a highly loaded lab-scale rotating biological contactor. Applied and environmental microbiology. 69 (6), 3626-3635.

[8] Schramm, A., De Beer, D., Gieseke, A., 2000. Microenvironments and distribution of nitrifying bacteria in a membrane-bound biofilm. Environmental microbiology. 2 (6), 680-686.

[9] Third, K.A., Sliekers, A.O., Kuenen, J.G., 2001. The CANON system (completely autotrophic nitrogen-removal over nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. Systematic and applied microbiology. 24 (4), 588-596.

[10] Vadivelu, V.M., Keller, J. and Yuan, Z., 2007. Effect of free ammonia on the respiration and growth processes of an enriched Nitrobacter culture.