J. Microbiol. Biotechnol. (2006), **16**(3), 469–474

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Study on Operational Factors in a Nitrite-Accumulating Submerged Membrane Bioreactor

YOO, IK-KEUN*, KYOUNG JO LIM, WON SIK LEE, DONG JIN KIM[1], AND GI CHEOL CHA[2]

School of Chemical Engineering & Bioengineering, University of Ulsan, Ulsan 680-749, Korea
[1]Department of Environmental Systems Engineering, Hallym University, Kangwon 200-702, Korea
[2]Division of Environmental Engineering, Yonsei University, Kangwon 220-710, Korea

Received: May 11, 2005
Accepted: September 22, 2005

Abstract Partial nitrification blocking of the oxidation of nitrite (NO_2^-) to nitrate (NO_3^-) has cost-efficient advantages such as lower oxygen and organics demand for nitrification and denitrification, respectively. A nitrifying membrane bioreactor of submerged type was operated for the treatment of synthetic ammonium wastewater with the purpose of nitrite build-up without affecting the efficiency of ammonium oxidation. A high ammonium concentration (1,000 mg/l) was completely converted to nitrate at up to 2 kg N/m^3 day under sufficient aeration. The control of pH under sufficient aeration was not a reliable strategy to maintain stable nitrite build-up. When the dissolved oxygen concentration was kept at 0.2–0.4 mg/l by adjusting the aeration rate, about 70% of nitrite content was obtained with ammonium oxidation efficiency higher than 93%. The increase of suction pressure due to membrane fouling was not significant under lowered aerating environment over a 6-month period of operation. The composition of nitrifier community, including relative abundance of nitrite oxidizers in a nitrite-accumulating condition, was quantified by fluorescence *in situ* hybridization analysis.

Key words: Aeration rate, FISH, nitrifier, nitrite, suction pressure, submerged membrane bioreactor

Most of the conventional biological technologies still pose major problems for nitrogen removal. Firstly, nitrification requires a long retention time, owing to the low growth rate of nitrifiers. Secondly, organic carbon from an external source must be supplied during denitrification of nitrate [9, 21]. To address the first problem, we suggest a submerged membrane bioreactor (MBR) in which the membrane enables complete retention of slowly growing microbes within the reactor. A nitrite-denitrification pathway has been proposed to overcome the second problem, since it requires 40% less organic substrate than with the nitrate-denitrification pathway [1, 5–7].

Nitrite build-up can occur owing to selective inhibition of free ammonia (FA) to nitrite oxidizers [1, 3, 5, 18]. However, it seems difficult to stably maintain an inhibitory concentration of FA in real plants, since the FA concentration depends on several factors such as pH, temperature, and proportional ammonium nitrogen (NH_4^+-N) concentration [27]. Moreover, acclimated nitrite oxidizers are capable of tolerating FA levels as high as 40 mg NH_3-N/l [26]. Although the SHARON process is reliable for nitrite build-up [10, 19], it is only suitable for wastewaters that are originally of high temperature. In a packed-bed biofilm reactor of plug flow type [28], inhibition by FA can be implemented, since the concentration profile of ammonium varies along the axial direction of the reactor. In the case of a well-mixed reactor such as MBR, it is difficult to maintain an inhibitory concentration of FA on nitrite oxidizers, because of the low concentration of targeted ammonium nitrogen in the effluent. Therefore, FA inhibition, decoupled with disturbance in ammonium oxidation efficiency, is not recommended as an efficient strategy to promote nitrite build-up in the MBR. A low concentration of dissolved oxygen (DO) also limits nitrite oxidation, since the oxygen saturation coefficients for ammonium oxidation and nitrite oxidation are known to be 0.3 and 1.1 mg DO/l, respectively [27]. The bulk of DO concentration can easily be controlled to some extent by changing the aeration intensity and can quickly be determined from a DO sensor. Therefore, manipulation of the aeration level rather than application of FA inhibition can be suggested to be a more practical alternative in mixed systems with sludge retention. The maintenance of DO at a low level is also advantageous in the following denitrification stage, since denitrification occurs under oxygen-limited condition [14, 25]. In several studies,

---

**Corresponding author*
Phone: 82-52-259-2251; Fax: 82-52-2591689;
E-mail: ikyoo@uou2.ulsan.ac.kr

nitrite buid-up has been linked to oxygen-limited conditions [6, 7, 22, 23].

However, maintenance of a low DO concentration is disadvantageous in terms of the overall nitrification rate [12]. A possible solution to maintain a high overall nitrification rate under lowered DO concentrations is a system in which a high concentration of nitrifying biomass can stably be maintained. In the literature, immobilization of biomass in the form of a biofilm or retention of suspended biomass by membrane separation has been found to operate a reactor at a high volumetric loading rate [7, 8, 16]. Among two types of MBRs such as cross-flow MBRs and submerged MBRs, submerged MBRs have been popular, since the use of submerged modules reduces the power consumption of MBRs. Whereas the flow velocity of mixed liquor in cross-flow MBRs is a critical parameter for controlling membrane fouling, a relatively higher aeration intensity that may increase DO concentration is required to reduce membrane fouling in submerged MBRs [24]. Therefore, it is uncertain whether a submerged MBR is a reliable system capable of achieving a relatively high nitrite build-up while maintaining high ammonium oxidation efficiency and low membrane fouling.

The objective of this study was to investigate the possibility of using a submerged MBR for partial nitrification of ammonium without affecting the overall nitrification rate. The MBR used in this study was operated in a nitrifier-dominant environment by feeding it ammonium wastewater. The recent development of molecular biology in wastewater treatment has resulted in the application of fluorescent-labeled 16S rRNA targeted probes for single-cell identification of bacteria samples from activated sludge [2, 11, 15]. Application of fluorescence *in situ* hybridization (FISH) was also carried out to study the community composition of nitrifying sludge in the MBR. Nitrogen concentrations in the effluent were monitored by varying operational parameters such as pH, ammonium load, and aeration rate over a one-year period.

A rectangular type reactor with an 8-l working volume, in which one element of hollow fiber membrane (0.2 m^2, Mitsubishi Rayon Co., Japan) was submerged, was constructed. The temperature was kept at 25°C, and pH was controlled by using 1 M sodium bicarbonate. The pH was maintained at 7.5 except for the period of experiments on pH effect. The pressure gauge was installed in order to monitor the variation of the suction pressure between the membrane and suction pump. Air was supplied through a coarse bubble diffuser located underneath the membrane module. The diffuser was a 1.5-cm inner diameter tube with 1-mm openings, so that coarse bubbles could be produced. The reactor was seeded with nitrifying activated sludge from an industrial wastewater treatment plant. The composition of synthetic wastewater was as follows: $(NH_4)_2SO_4$, 100–1,000 mg N/l; $NaHCO_3$ (as $CaCO_3$), 3–7.1 mg/mg N; $MgSO_4\cdot 7H_2O$, 50 mg/l; $CaCl_2\cdot 2H_2O$, 50 mg/l; $KH_2PO_4\cdot H_2O$, 50 mg/l; $FeSO_4\cdot 7H_2O$, 2 mg/l; $MnSO_4\cdot H_2O$, 1 mg/l; yeast extract, 50 mg/l. No organic substrate was fed to the reactor, except that present in yeast extract. The permeate was extracted by a suction pump operated periodically in a 10-min cycle (8 min on and 2 min off). Since the permeate membrane flux was kept at 3.4 l/m^2h, the hydraulic retention time was maintained at 12 h. Sludge was withdrawn daily to keep the sludge retention time (SRT) at 60 days.

Ammonium concentration was measured by a Nesslerization method by reading absorbance at 425 nm [4]. Nitrite and nitrate were determined by ion chromatography (Basic IC, Metrohm, Switzerland). Nitrite was also measured by a US EPA-approved method utilizing the Hach Laboratory method 8507 (DR/4000, Hach). DO, pH, and membrane flux were monitored daily. A DO meter (YSI 55) was used for measuring DO concentration in the MBR, and FISH analysis of fixed sludge was performed as detailed in our previous study [13], which followed the protocol of Manz *et al.* [17]. The following rRNA-targeted oligonucleotide probes were used: probe EUB338 for quantifying members of the domain Bacteria; probe NSM156 specific for *Nitrosomonas* spp.; probe NSV443 specific for *Nitrosospira* spp.; probe NTSPA662 specific for *Nitrospira* spp.; probe NIT3 specific for *Nitrobacter* spp. The relative population of specifically hybridized cells was quantified using Visual Digital Image Analyzer (Zeiss, Germany).

Following the seeding of the MBR, the aeration rate was kept at 10 l/min, resulting in an initial DO concentration higher than 3 mg/l. The enrichment and acclimation of nitrifying sludge was performed by gradually increasing the ammonium concentration for an initial 3-month period. Since the aeration rate was not changed, DO decreased steadily to less than 1 mg/l with an increase of sludge concentration and ammonium load. This nitrifier-enriched MBR showed full conversion of ammonium of up to an ammonium concentration of 1,000 mg N/l and a load of 2 kg N/m^3 day. After 3 months, the pH was changed from 6.5 to 8.5 in steps, with a sufficient pause of more than two weeks at each pH value. It has been reported that the optimum pH for nitrification ranges from 7.5 to 8.0, and that pH affects free ammonia and nitrous acid (HNO_2) concentrations, which will influence the activity of nitrifiers, especially nitrite oxidizers [3, 20]. In our experiment, the efficiency of ammonium oxidation significantly decreased at pH 6.5 (range fluctuating between 70% and 90%). Nitrite peak from 100 to 400 mg N/l was transiently and unsteadily observed. At pH 8.5, the ammonium oxidation efficiency was higher than that at pH 6.5, but the NH_3-N concentration increased because of a pH-dependent equilibrium shift from NH_4^+ to NH_3. The increased NH_3-N also caused transient nitrite build-up, but nitrite oxidation to nitrate occurred within a few days, possibly due to biomass adaptation to free

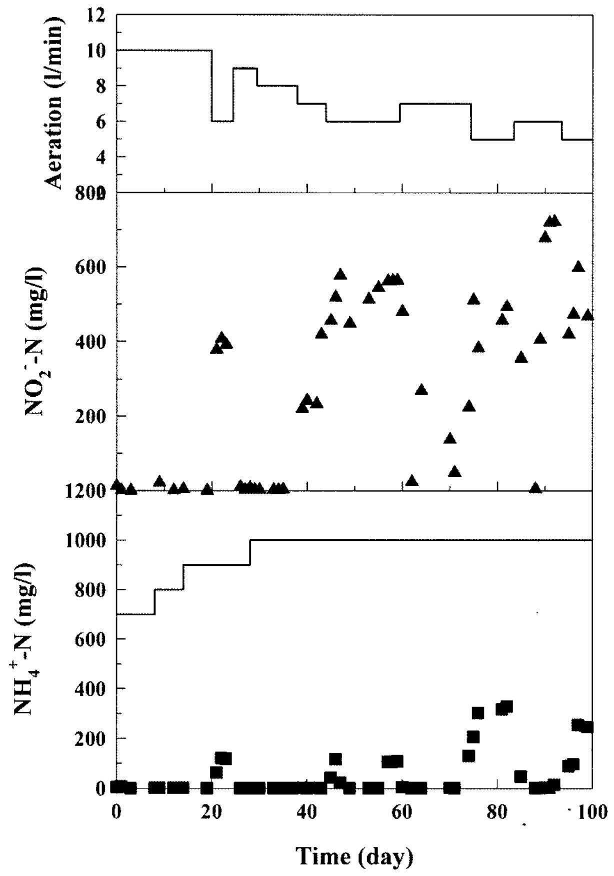

Fig. 1. Time courses of aeration rate, nitrogen concentrations, and nitrogen loss in a submerged membrane bioreactor; effluent NO_2^--N (▲); effluent NH_4^+-N (■); influent NH_4^+-N (—).

ammonia. Moreover, with an influent concentration of 1,000 mg N/l at pH 8.5, even a 7% change of ammonium oxidation efficiency led to a fluctuation of NH_3-N concentration in the range of 0.1–10.5 mg N/l. This unsteady variation of ammonium conversion efficiency and the biomass acclimation to free ammonia made it difficult to maintain stable nitrite build-up by controlling the NH_3-N concentration (data are not plotted here because of deviations). On a long-term basis, therefore, pH control was not recommended to promote nitrite accumulation in the MBR.

As an alternative variable to pH, the aeration rate was investigated during the latter operational period of the same MBR. Figure 1 shows the time profiles of nitrogen concentrations in the effluent according to aeration rate investigated (5–10 l/min). At a constant pH of 7.5 and an aeration rate of 10 l/min, the feeding concentration of ammonium was decreased to 700 mg/l at day 0 in order to fully activate the nitrite converion in the MBR. From day 0 to day 19, a complete conversion of ammonium to nitrate was confirmed, implying an abundance of nitrite oxidizers. Nitrate concentrations were found in the range of 590–660 mg/l. At days 21–25, the nitrite peak of about 400 mg/l was observed immediately with a decrease of aeration rate to 6 l/min. After ammonium concentration was increased again to 1,000 mg/l from day 29, the aeration rate was controlled at each fixed value in the range of 5–8 l/min. The DO concentration ranged from 0.2 to 0.4 mg/l at 6 l/min of aeration, whereas it measured from 0.4 to 0.8 mg/l at 5 l/min. The higher concentration of DO at a lower aeration rate resulted from a decreased nitrification rate. An effluent ammonium concentration of up to 300 mg/l was observed at the aeration of 5 l/min. When the aeration rate was kept at 6 l/min, more than 700 mg/l of maximum concentration of nitrite was observed with mostly ammonium concentration of less than 50 mg/l. A small variation in the aeration rate seems to cause a relatively large change in the oxidation of nitrite as well as ammonium. The effect of DO concentration on the nitrification characteristics was studied in a biofilm airlift suspension (BAS) reactor [7], and both nitrite and nitrate concentrations were found to decrease at below 1 mg/l DO concentrations, owing to decreased nitrification efficiency: They obtained a maximum nitrite ratio of 0.5 at 196 mgNH_4-N/l under the bulk DO concentrations of 1–2 mg/l. The higher efficiency of ammonium conversion in our reactor might have resulted from the different population of active ammonium oxidizers and/or the oxygen transfer efficiency. Whereas the nitrifiers in the MBR were in the suspension state of a floc-like form, the cells in the BAS reactor were usually attached to the carrier surface as a bioflim. A DO concentration higher than 2 mg/l is usually required for efficient nitrification. In contrast, DO concentration of less than 0.5 mg/l in our MBR was sufficient for almost complete ammonium conversion. This was due to a high concentration of nitrifiers kept in the MBR, suggesting a potential use of the MBR as a highly efficient nitrification system.

Figure 2 shows variation of sludge concentration in a reactor and suction pressure between the membrane module and a suction pump. MLSS concentration was stabilized at

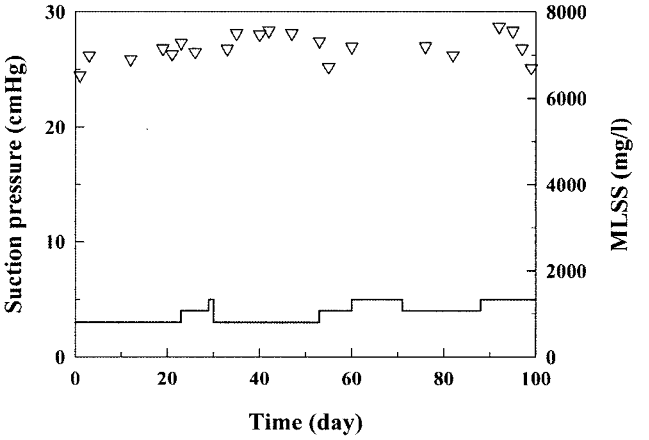

Fig. 2. Variation of suction pressure and sludge concentration; suction pressure (—); MLSS (▽).

around 6,500–7,500 mg/l at a constant SRT of 60 days. Since the MBR was fed with a synthetic medium without a major organic source, the environment within the MBR was appropriate for enriching nitrifiers mostly. Based on the SRT of 60 days and the nitrogen load of 2 kg N/m^3 day, the specific nitrifying sludge production was found to be 0.05 g MLSS/g N. The low yield is comparable to 0.03 g VSS/g N in a cross-flow MBR for the nitrification of sludge reject water [16]. Higher aeration is usually favorable for a submerged MBR, since turbulence induced by uplifting air and liquid flow is conducive to cleaning the membrane surface [24]. However, the aeration rate in our MBR was intentionally kept at a relatively lower value for the accumulation of nitrite under a low DO concentration. Thus, the operating condition in our MBR may not be proper for lessening the progress of membrane clogging. For the purpose of controlling the DO concentration by adjusting aeration intensity, a cross-flow MBR may be better than a submerged MBR, since the purpose of aeration in a cross-flow MBR can be decoupled with the purpose of cleaning membrane surface. However, rapid decrease of specific sludge activity was observed in a cross-flow MBR, due to a shear stress resulting from a high recirculation rate of biomass through a pump [8]. The cross-flow MBR also requires a higher power consumption, and therefore, the submerged type was proposed as a nitrite-accumulating nitrification system in this study. During more than 6 months of whole operation, an aeration of 5–10 l/min was sufficient to prevent membrane fouling, and hence, no membrane cleaning was conducted. A suction pressure of less than 6 cmHg was maintained. The nitrite-accumulating MBR was successfully operated even under the lowered aeration rate of 5–6 l/min without an abrupt increase of suction pressure. More extensive study on the long-term effect of aeration rate on the membrane fouling should be conducted in future.

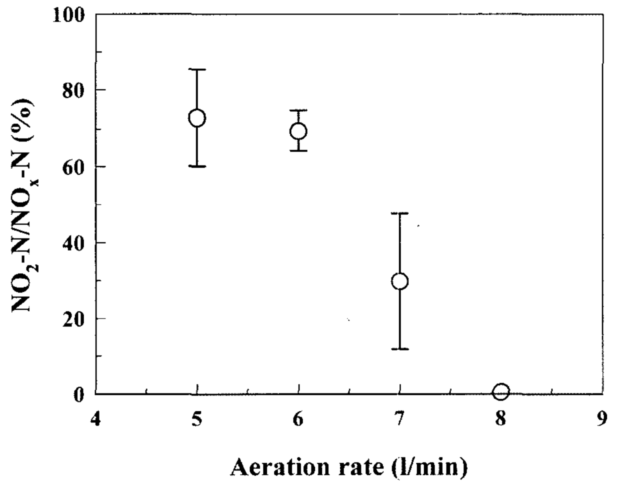

Fig. 3. Effect of aeration rate supplied to a MBR on nitrite ratio (NO_2-N/NO_x-N) in the effluent. Error bar indicates the standard deviation.

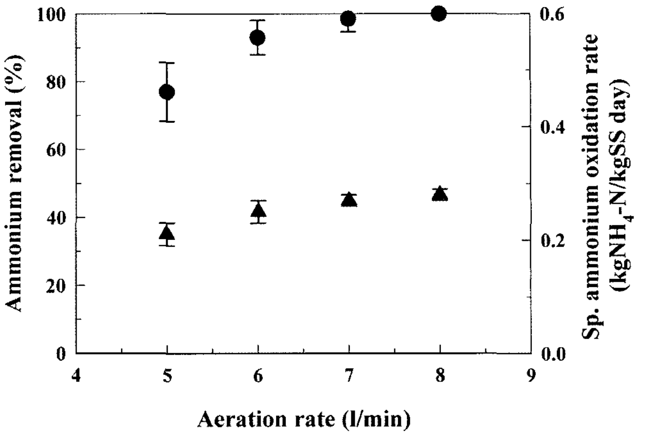

Fig. 4. Effect of aeration rate supplied to a MBR on ammonium removal efficiency (●) and specific ammonium oxidation rate (▲).

Error bar indicates the standard deviation.

Figures 3 and 4 summarize the effect of aeration rate on nitrite ratio, ammonium removal efficiency, and specific ammonium oxidation rate from day 29 to day 99, as shown in Fig. 1. On average, the nitrite ratio increased to 29.8, 69.4, and 72.8% as the aeration rate decreased to 7, 6, and 5 l/min, respectively. The nitrification efficiency significantly decreased from 93.0 to 76.9%, when the aeration was reduced from 6 to 5 l/min. The specific ammonium oxidation rate decreased from 0.28 to 0.21 kg NH_4-N/kgMLSS day as the aeration rate was reduced from 8 to 5 l/min, respectively. The specific ammonium oxidation rate in the present study was relatively higher than the 0.16 kg N/kg SS day that was reported for a previous MBR of Ghyoot' *et al.* [8], whose MBR was fed with organics as well as ammonium. Considering both the high ammonium oxidation and nitrite build-up, the optimum aeration rate was determined to be between 6 to 7 l/min. The DO concentration corresponding to this aeration rate was 0.2–0.4 mg/l. Although the optimum aeration rate and the corresponding DO concentration for nitrite accumulation were dependent on various factors such as MLSS concentration, reactor type, ammonium load, and temperature, the present study suggests that the MBR can be used as a reliable nitrite accumulating system with a proper control of aeration rate.

Since the nitrite peak of around 400 mg/l was observed at days 21–25, the MBR sludge was taken at day 25 and fixed immediately for FISH analysis to verify the existence of nitrite oxidizers even under the conditions that the oxidation of nitrite was inhibited by low DO concentration. If the nitrite peak was due to wash-out of nitrite oxidizers at days 21–25, then the nitrite oxidizers would not be detected by the FISH analysis. The probes corresponding to *Nitrosomonas* spp. (NSM156) and *Nitrosospira* spp.

(NSV443) among the ammonia oxidizers were applied for hybridization. Cells hybridizing with probe NSM156 accounted for 22–27%, whereas those with NSV443 accounted for 10–14% of the cells hybridizing with eubacterial probe EUB338. The probes specific for *Nitrospira* spp. (NTSPA662) and *Nitrobacter* spp. (NIT3) were used for the hybridization of nitrite oxidizers. Cells hybridizing with probe NIT3 accounted for 4–5%, whereas those with NTSPA662 accounted for 16–20% of the EUB338 hybridizing cells. Thus, it can be said that the nitrite peak was not due to the absence of nitrite oxidizers, but rather to the inhibition of nitrite oxidizers by some operating factors kept in the MBR. Liebig *et al.* [16] determined the microbial community composition in a MBR by FISH analysis, by operating the MBR under complete sludge retention over 300 days and also feeding sludge reject water containing 600 mg N/l to the reactor: In their FISH analysis on ammonia oxidizers, no hybridization signals for *Nitrosospira* clusters were observed whereas comparable amounts of *Nitrosomonas*-like ammonia oxidizers were detected. With regard to nitrite oxidizers, only *Nitrospira* spp. was identified to contribute to nitrite oxidation. Compared with the above observations [16], the abundance of *Nitrosospira* spp. and *Nitrobacter* spp. in our MBR seems to have resulted from a difference in operating conditions, such as the composition of wastewater, sludge retention time, and mostly DO concentration. No further study on the community composition was carried out here. In conclusion, a high concentration of nitrifier in a MBR enabled almost complete ammonia conversion (more than 99% at the load and concentration of up to 2 kg NH_4-N/m^3 day and 1 g NH_4-N/l, respectively) under less than 0.5 mg/l DO concentration. Although the variation of free ammonia concentration was associated with the nitrite ratio during the whole operation period, nitrite accumulation due to inhibition of free ammonia was not stable. The aeration rate, which determines bulk DO concentration, was a reliable variable to effectively control nitrite build-up. The decrease of aeration resulted in a concomitant increase of nitrite content in the effluent. However, aeration to some extent was essential to maintain the efficiency of ammonium oxidation.

Acknowledgment

This work was supported by grant No. (R05-2002-000-00170-0) from the Basic Research Program of the Korea Science & Engineering Foundation.

REFERENCES

1. Abelling, U. and C. F. Seyfried. 1992. Anaerobic-aerobic treatment of high strength ammonium wastewater-nitrogen removal using nitrite. *Wat. Sci. Technol.* **26:** 1007–1015.
2. Ahn, I. S., M. W. Kim, H. J. La, K. M. Choi, and J. C. Kwon. 2003. Bacterial community composition of activated sludge relative to type and efficiency of municipal wastewater treatment plants. *J. Microbiol. Biotechnol.* **13:** 15–21.
3. Anthonisen, A. C., R. C. Loehr, T. B. S. Prakasam, and E. G. Srinath. 1976. Inhibition of nitrification by ammonia and nitrous acid. *J. Wat. Pollut. Control Fed.* **48:** 835–852.
4. APHA. 1992. *Standard Methods for the Examination of Water and Wastewater.* 18th Ed. APHA. Washington DC, U.S.A.
5. Balmelle, B., M. Nguyen, B. Capdeville, J. C. Cornier, and A. Deguin. 1992. Study of factors controlling nitrite build-up in biological processes for water nitrification. *Wat. Sci. Technol.* **26:** 1017–1025.
6. Bernet, N., P. Dangcong, J.-P. Delgenes, and R. Moletta. 2001. Nitrification at low oxygen concentration in biofilm reactor. *J. Environ. Eng.-ASCE* **127:** 266–271.
7. Garrido, J. M., W. A. J. van Benthum, M. C. M. van Loosdrecht, and J. J. Heijnen. 1997. Influence of dissolved oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. *Biotechnol. Bioeng.* **53:** 168–178.
8. Ghyoot, W., S. Vandaele, and W. Verstraete. 1999. Nitrogen removal from sludge reject water with a membrane-assisted bioreactor. *Wat. Res.* **33:** 23–32.
9. Gil, K. I. and E. S. Choi. 2002. Estimation of nitrite concentration in the biological nitritation process using enzymatic inhibition kinetics. *J. Microbiol. Biotechnol.* **12:** 377–381.
10. Hellinga, C., A. A. J. C. Schellen, J. W. Mulder, M. C. M. van Loosdrecht, and J. J. Heijnen. 1998. The Sharon process: An innovative method for nitrogen removal from ammonium-rich wastewater. *Wat. Sci. Technol.* **37:** 135–142.
11. Jeon, C. O., S. H. Woo, and J. M. Park. 2003. Microbial communities of activated sludge performing enhanced biological phosphorus removal in a sequencing batch reactor supplied with glucose. *J. Microbiol. Biotechnol.* **13:** 385–393.
12. Joo, S. H., D. J. Kim, I.-K. Yoo, K. Park, and G. C. Cha. 2000. Partial nitrification in an upflow biological aerated filter by O_2 limitation. *Biotechnol. Lett.* **22:** 937–940.
13. Kim, D. J., J. S. Chang, D. I. Lee, D. W. Han, I.-K. Yoo, and G. C. Cha. 2003. Nitrification of high strength ammonia wastewater and nitrite accumulation characteristics. *Wat. Sci. Technol.* **47:** 45–51.
14. Kim, S. H., S. H. Song, and Y. J. Yoo. 2004. The pH as a control parameter for oxidation-reduction potential on the denitrification by *Ochrobactrum anthropi* SY509. *J. Microbiol. Biotechnol.* **14:** 639–642.
15. Kim, W. K., R. Cui, and D. Jahng. 2005. Enrichment of ammonia-oxidizing bacteria for efficient nitrification of wastewater. *J. Microbiol. Biotechnol.* **15:** 772–779.
16. Liebig, T., M. Wagner, L. Bjerrum, and M. Denecke. 2001. Nitrification performance and nitrifier community composition of a chemostat and a membrane-assisted bioreactor for the nitrification of sludge reject water. *Bioproc. Biosys. Eng.* **24:** 203–210.
17. Manz, W., R. Amann, W. Ludwig, M. Wagner, and K. H. Schleifer. 1992. Phylogenetic oligodeoxynucleotide probes

for the major subclasses of proteobacteria: Problems and solutions. *System. Appl. Microbiol.* **15:** 593–600.

18. Mauret, M., E. Paul, E. Puech-Costes, M. T. Maurette, and P. Baptiste. 1996. Application of experimental research methodology to the study of nitrification in mixed culture. *Wat. Sci. Technol.* **34:** 245–252.
19. Mulder, J. W., A. A. van de Graaf, L. A. Robertson, J. G. Kuenen. 2001. Full-scale application of the Sharon process for the treatment of rejection water of digested sludge dewatering. *Wat. Sci. Technol.* **43:** 127–134.
20. Painter, H. A. and J. E. Loveless. 1983. Effect of temperature and pH value on the growth rate constants of nitrifying bacteria in the activated sludge process. *Wat. Res.* **17:** 237–248.
21. Park, E. J., J. K. Seo, J. K. Kim, K. H. Suh, and S. K. Kim. 2000. Denitrification characteristics and microorganism composition of acclimated denitrifier consortium. *J. Microbiol. Biotechnol.* **10:** 410–414.
22. Pollice, A., V. Tandoi, and C. Lestingi. 2002. Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. *Wat. Res.* **36:** 2541–2546.
23. Ruiz, G., D. Jeison, and R. Chamy. 2003. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Wat. Res.* **37:** 1371–1377.
24. Shim, J. K., I.-K. Yoo, and Y. M. Lee. 2002. Design and operation considerations for wastewater treatment using a flat submerged membrane bioreactor. *Process Biochem.* **38:** 279–285.
25. Song, S. H., S. H. Yeom, S. S. Choi, and Y. J. Yoo. 2003. Effect of oxidation-reduction potential on denitrification by *Ochrobactrum anthropi* SY509. *J. Microbiol. Biotechnol.* **13:** 473–476.
26. Turk, O. and D. S. Mavinic 1989. Maintaining nitrite build-up in a system acclimated to free ammonia. *Wat. Res.* **23:** 1383–1388.
27. Wiesmann, U. 1994. Biological nitrogen removal from wastewater, pp. 113–154. *In* A. Fiechter (ed.), *Advances in Biochemical Engineering*. Springer-Verlag, Berlin.
28. Yun, H. J. and D. J. Kim. 2003. Nitrite accumulation characteristics of high strength ammonia wastewater in an autotrophic nitrifying biofilm reactor. *J. Chem. Technol. Biotechnol.* **78:** 377–383.