Partial Nitrification in an Airlift Activated Sludge Reactor with Experimental and Theoretical Assessments of the pH Gradient inside the Sponge Support Medium

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ABSTRACT: An airlift-type activated sludge reactor using a sponge support medium was fed with wastewater containing 500 mg-N/L of NH_4^+-N. In order to establish partial nitrification, the reactor was kept at 35°C. Moreover, the reactor was started up with the $NaHCO_3$ solution as an alkali agent to supply inorganic carbon for enhancement of partial nitrification. The partial nitrification was established during the period when $NaHCO_3$ was used as an alkali agent. However, soon after changing the alkali agent to a mixture of Na_2HPO_4 and NaOH, the production of NO_2^--N started to decrease. The change in pH inside the sponge medium revealed by the model simulation and microelectrode study showed relatively high free ammonium concentration inside the medium. Thus, partial nitrification was proved to be affected by inorganic carbon in the alkali agent. Furthermore, it was suggested that the establishment of partial nitrification in this study was due to the combination of high operating temperatures, addition of inorganic carbon, and the inhibition of NOB by the free ammonium concentration.

Key words: Nitrite accumulation, Sponge cube, Microelectrode, pH gradient, Model simulation

INTRODUCTION

Anaerobic ammonium oxidation (ANAMMOX) is a novel autotrophic denitrification process, first reported by Delft University in 1995, which employs NH_4^+-N as an electron donor and NO_2^--N as an electron acceptor according to the reaction $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ (Mulder *et al*., 1995; Kuenen and Jetten 2001). To complete this reaction, 50% of the NH_4^+-N in wastewater must be oxidized to NO_2^--N, also referred to as partial nitrification. Theoretically, the amount of oxygen required for partial nitrification is only 32.5% of that required for complete conversion of NH_4^+-N to NO_3^--N, i.e., nitrification. In addition, since ANAMMOX does not require any organic sources for denitrification, the process is considered to be a promising and economi-

cally viable biological wastewater treatment method for nitrogen removal (Fux *et al*., 2002). However, there are several problems associated with the practical implementation of the ANAMMOX process, one of which is the establishment of an appropriate technology for partial nitrification. To date, attempts at partial nitrification have focused on (a) inhibition of nitrite oxidizing bacteria (NOB) by free ammonium or free nitrous acid (Guidad *et al*., 2005; Chung *et al*., 2005; Ruiz *et al*., 2003; Yun *et al*., 2003), (b) operation under low dissolved oxygen (DO) concentrations (Bernet *et al*., 2002; Jianlong *et al*., 2004; Tokutomi *et al*., 2004; Wyffels *et al*., 2004), (c) operation at high temperatures (Hellinga *et al*., 1998), (d) addition of inorganic carbon (IC) (Tokutomi *et al*., 2006; Jun *et al*., 2000), (e) operation with high salinity (Yu *et al*., 2004), and (d)

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operation of the process under a combination of these conditions (Bae *et al*., 2002). Operation at temperatures as high as 30 to 35°C may be the effective means of establishing a reliable partial nitrification process because the growth rate of ammonium oxidizing bacteria (AOB) under such conditions is markedly higher than that of NOB (Hellinga *et al*., 1998). By exploiting this relationship, the SHARON (Single tank reactor for High activity Ammonium Removal Over Nitrite) process has become well known as one the most effective partial nitrification processes (Hellinga *et al*., 1998). The SHARON process works without biomass retention, meaning that the hydraulic retention time (HRT) is equal to the sludge retention time. With high temperatures and no sludge retention, the dilution rate is set in such a way that the AOB can grow fast enough to stay in the reactor, while NOB is washed out (Hellinga *et al*., 1998). In the previous study, pilot scale experiments were performed to evaluate the potential of partial nitrification process with an airlift reactor at 30°C under oxygen limitation conditions below 1.5 mg-O_2/L (Tokutomi *et al*., 2006). Although partial nitrification continued over 100 days, but nitrate formation increased after 150 days of operation. To overcome this phenomenon, IC was fed to the reactor by changing pH adjustment reagent from NaOH to Na_2CO_3 and nitrite accumulation was recovered successfully without changing DO concentration. This indicated that a high concentration of IC is also one of the control parameters for accumulating nitrite in biofilm nitrification system.

In this study, a laboratory scale airlift-activated sludge reactor using a sponge support medium was applied to the partial nitrification of ammonia-rich artificial wastewater. The airlift reactor was started up by maintaining the temperature of the reactor at 35°C. During the first 84 days from the start up, solution of $NaHCO_3$ was used as an alkali agent to adjust pH at 7.5 inside the reactor. This also aimed to investigate the effect of IC for the enhancement of partial nitrification even under high DO concentration (> 3.0 mg-O_2/L). Thus, by employing the high temperature operation and IC addition for restraint of growth of NOB, we expected that the attachment and growth of AOB, both inside and on the surface of the sponge media, would promote the retention of a large amount of AOB biomass inside the reactor. If this is achieved, a more effective partial nitrification process will be established. Furthermore, the pH gradients inside the sponge medium were investigated experimentally using a pH microelectrode and employing simulations to discuss the factors affecting partial nitrification.

MATERIALS & METHODS

The airlift reactor used in this study is shown in Fig. 1. The working volume of the reactor was 3 L. The HRT was fixed at 5.3 hours (nitrogen loading rate: 2.0 - 2.5 g-N/L/d). The DO concentration was maintained at above 3.0 mg-O_2/L (3.73 mg-O_2/L on average during entire experimental period) to investigate the effect of IC for partial nitrification under high DO conditions. The temperature of the reactor was kept at 35°C using a water jacket and the pH of the mixed liquor was adjusted to 7.5 by an automatic pH controller (TP-252, Automatic System Research Co. Ltd., Japan). The following alkali agents were used to adjust the pH at 7.5; 50 g/L of $NaHCO_3$ for Phase 1 (day 0 to 84), 24 g/L of Na_2HPO_4 and 17 g/L of NaOH for Phase 2 (day 85 to 107), 20 g/L of NaOH for Phase 3 (day 108 to 120), 24 g/L of Na_2HPO_4 and 17 g/L of NaOH for Phase 4 (day 121 to 150), and 47 g/L of Na_2HPO_4 and 17 g/L of NaOH for Phase 5 (day 151 to 177). A partition was installed in the center of the reactor and aeration was supplied to the reactor bottom to generate a vertical rotary flow. Polyurethane sponge cubes (3 mm x 3 mm x 3 mm) were used as a support medium. The reactor was filled with 1 L of sponge cubes and seeded with activated sewage sludge before start-up. The NH_4^+-N of the artificial wastewater was adjusted to 500 mg-N/L using $(NH_4)_2SO_4$. Other ingredients of the wastewater, which was a modification of the ANAMMOX culture, are shown in Table 1 (Tokutomi *et al*., 2004). The NH_4^+-N, NO_2^--N, and NO_3^--N in the wastewater and the effluent were analyzed using a HACH water quality analyzer (DR-2000, HACH, USA). All other analytical procedures were carried out according to Standard Methods for the Examination of Water and Wastewater (APHA 2005). Determination of the pH profile within a sponge cube was conducted using a pH microelectrode as described previously (Yamaguchi *et al*., 2001). A 100 µL glass pipette (Drummond Scientific Company, USA) was stretched using a micropipette-tensioning device (Shimadzu MPT-1, Japan). The 10 µm tip was filled with a silane solution (a mixture of carbon tetrachloride and 20% (v/v) of trimethylchlorosilane), before drying at 130°C. An ion-exchange membrane solution composed of 10% (w/v) tridodecylamine and 7% (w/v) tetraphenylborate sodium salt was dissolved in *o*-nitrophenyl octyl ether before being exposed to 100% CO_2 gas for 16 h. The glass pipette was filled with the electrolytic solution and an Ag/AgCl wire (0.3 mm diam.) was inserted into the electrolytic solution. The device used for the determination of the pH gradient in the sponge cube is shown in Fig. 2. The mixed liquor from the reactor was forwarded directly to the device and the overflow was returned to the reactor. The reference sensor of the pH microelectrode was submerged into the overflow liquor and the sponge medium was affixed to the top of a plastic bottle.
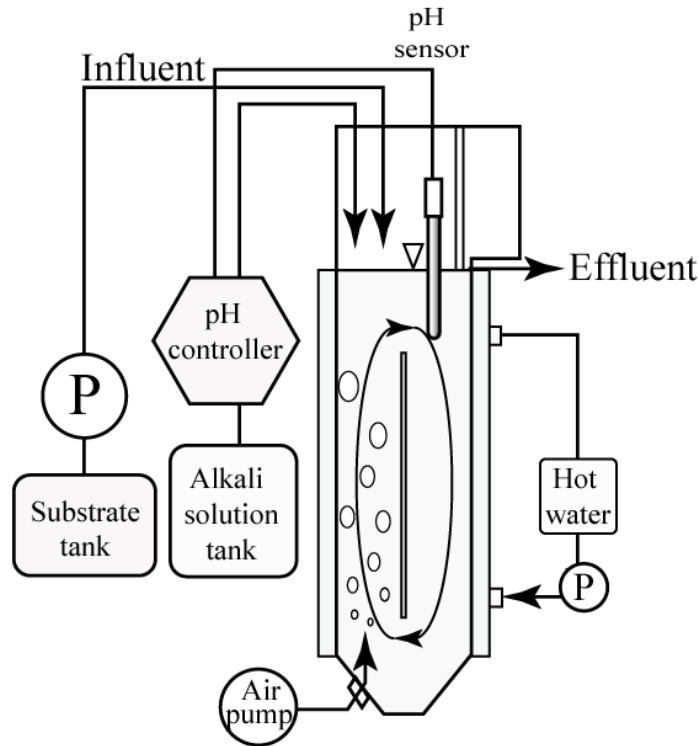
Fig. 1. Experimental set-up of the airlift activated sludge reactor

Table 1. Substrate composition

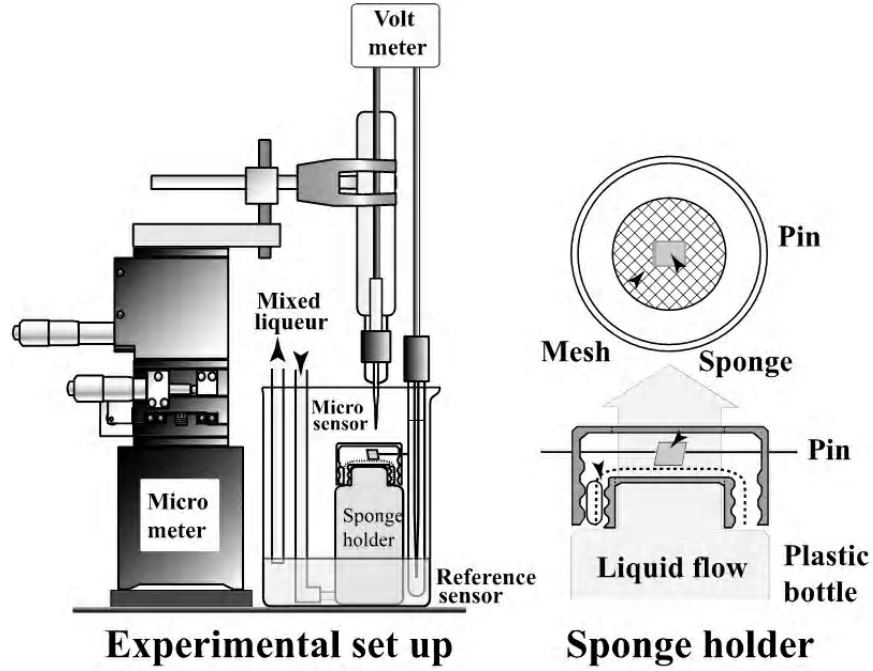| Substrate composition		
$(NH_4)_2SO_4$	2.38	g/L
$MgSO_4 \cdot 7H_2O$	0.2	g/L
$CaCl_2 \cdot 2H_2O$	0.02	g/L
KH_2PO_4	0.114	g/L
Basal element solution A	0.1	mL/L
Basal element solution B	0.1	mL/L
Basal solution A		
$EDTA \cdot 2Na$	50	g/L
$FeSO_4 \cdot 7H_2O$	50	g/L
Basal solution B		
$EDTA \cdot 2Na$	25	g/L
$ZnSO_4 \cdot 7H_2O$	4.3	g/L
$CoCl_2 \cdot 6H_2O$	2.4	g/L
$MnCl_2 \cdot 4H_2O$	9.9	g/L
$CuSO_4 \cdot 5H_2O$	2.5	g/L
$NaMoO_4 \cdot 2H_2O$	2.2	g/L
$NiCl_2 \cdot 6H_2O$	1.9	g/L
$NaSeO_4 \cdot 10H_2O$	2.1	g/L
H_3BO_4	0.14	g/L

Fig. 2. Description of the experimental device used for pH microelectrode study

MODEL SIMULATION

Assuming that penetration of the substrate into the sponge carrier progresses according to Fick's law, the flux per unit area will occur in proportion to the concentration gradient, which can be represented as follows:

$$J = -D_e \frac{dC}{dx} \qquad (1)$$

J: flux [mmol/cm^2/s]
D_e: diffusion coefficient [cm^2/s]
C: substrate concentration [mmol/cm^3]
x: distance [cm]

A definition of the model coordinates is shown in Fig. 3, where the lengths of a, b, and c are expressed in cm (a d" b and a d" c). The dV_i is a very small volume between two adjacent cubes, i.e., a cube having distance x_i from the center with a surface area of A_i [cm^2] and another with distance x_{i+1} ($x_{i+1} = x_i + dx$) with a surface area of A_{i+1}. The material balance in dV_i is expressed in equation 2 below:

$$\varepsilon \cdot dx \cdot A_i \cdot \frac{dC}{dt} = A_i \cdot J_i - A_{i+1} \cdot J_{i+1} \qquad (2)$$

ε: void volume fraction [-]
t: time [s]

Applying Fick's law to equation 2, we get the following equation:

$$\varepsilon \frac{\partial C}{\partial t} = D_e \left(\frac{1}{A_i} \frac{\partial A_i}{\partial x} \frac{\partial C}{\partial x} + \frac{\partial^2 C}{\partial x^2} \right) \qquad (3)$$

The surface area A_i of the cube and "A_i/"x are expressed by equations 4 and 5, respectively.

$$A_i = 8\left(3x^2 + 2y_0 x + 2z_0 x + y_0 z_0\right) \qquad (4)$$

$$\frac{\partial A_i}{\partial x} = 16\left(y_0 + z_0 + 3x\right) \qquad (5)$$

y, z: distance from the origin on the y and z-axes, $y_0 = (b-a)/2$, $z_0 = (b-a)/2$
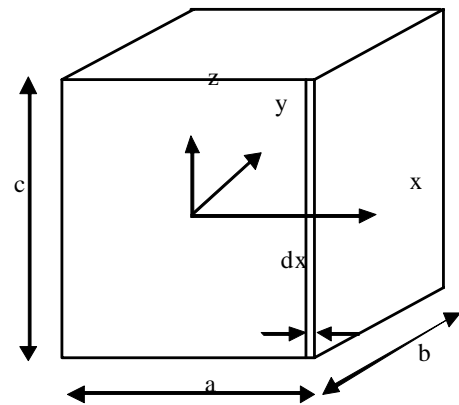

Fig. 3. Definition of the model coordinates

Combining equations 3, 4, 5, and a term for the biological conversion rate, the following substrate diffusion/consumption model in the sponge carrier can be obtained:

$$\varepsilon \frac{\partial C}{\partial t} = D_e \left\{ \frac{2(y_0 + z_0 + 3x)}{3x^2 + 2y_0 x + 2z_0 x + y_0 z_0} \cdot \frac{\partial C}{\partial x} + \frac{\partial^2 C}{\partial x^2} \right\} + r \tag{6}$$

r: biological conversion rate [mmol/g-VSS/s]
VSS: volatile suspended solids
Boundary conditions are given as follows:

$$x = 0 : \frac{dC}{dx} = 0 \tag{7}$$

$$x = a/2 + \delta : C = C_b \tag{8}$$

A: length of the narrow side of the sponge carrier [cm]
δ: thickness of the boundary film [cm]
C_b: substrate concentration in the liquor [mmol/cm^3]
Ammonium oxidation per sponge volume over time (r_a) can be calculated using the Michaelis-Menten equation below:

$$r_a = -v_{a,max} \frac{C_a}{k_a + C_a} \frac{C_{DO}}{k_o + C_{DO}} X \tag{9}$$

$v_{a,max}$: maximum specific ammonium oxidation rate [mmol-NH$_4^+$-N/g-VSS/s]
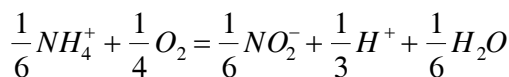C_a: concentration of ammonium nitrogen [mmol-NH$_4^+$-N/cm^3]
C_{DO}: dissolved oxygen [mmol-O$_2$/cm^3]
K_a: Michaelis constant for ammonium oxidation [mmol-NH$_4^+$-N/cm^3]
K_O: Michaelis constant for oxygen consumption [mmol-O$_2$/cm^3]
X: biomass concentration in the sponge cube [mg-VSS/cm^3]
The equation for the conversion of ammonium to nitrite is

$$\frac{1}{6} NH_4^+ + \frac{1}{4} O_2 = \frac{1}{6} NO_2^- + \frac{1}{3} H^+ + \frac{1}{6} H_2O$$

which means that the nitrite production rate is

$$r_N = -r_a \tag{10}$$

Finally, the concentration profile of NH$_4^+$-N and NO$_2^-$-N in a sponge cube can be expressed using the following equations:

$$\varepsilon \frac{\partial C_a}{\partial t} = D_e \left\{ \frac{2(y_0 + z_0 + 3x)}{3x^2 + 2y_0 x + 2z_0 x + y_0 z_0} \frac{\partial C_a}{\partial x} + \frac{\partial^2 C_a}{\partial x^2} \right\} + r_a \tag{11}$$

$$\varepsilon \frac{\partial C_N}{\partial t} = D_e \left\{ \frac{2(y_0 + z_0 + 3x)}{3x^2 + 2y_0 x + 2z_0 x + y_0 z_0} \frac{\partial C_N}{\partial x} + \frac{\partial^2 C_N}{\partial x^2} \right\} - r_a \tag{12}$$

Conversely, although the pH in the sponge cube is considered to vary with depth, it can be predicted theoretically by employing thermodynamics. In this study, the major electrolyte compounds in the mixed liquor are (NH$_4$)$_2$SO$_4$, NaHCO$_3$, MgSO$_4$, and KH$_2$PO$_4$. The charge balance of these electrolytes in the mixed liquor and the sponge cube are expressed in equation 13 below:

$$\tag{13}$$

$[Na^+]+[K^+]+[NH_4^+]+[Mg_2^+]+[H^+]=2[SO_4^{2-}]+[NO_2^-]$

$+[HCO_3^-]+2[CO_3^-]+[H_2PO_4^-]+2[HPO_4^{2-}]+3[PO_4^{3-}]+[OH^-]$

Concentrations of Na, K, Mg, SO$_4^{2-}$, H$_2$PO$_4^-$, HPO$_4^{2-}$, and PO$_3^{3-}$ can be determined based on the composition of the artificial wastewater (Table 1), and concentrations of HCO$_3^-$ and CO$_3^{2-}$ can be inferred based on the pH of the mixed liquor being adjusted to 7.5. Concentrations of individual anions can also be expressed as functions of the dissociation constant and H$^+$, which means that the pH gradient inside the sponge cube can be calculated empirically. For the determination of V_{max} (Table 2), sludge was squeezed from the 176 sponge cubes and washed with phosphate buffer before being centrifuged at 3000 rpm. After decanting, substrate with 30 mg L^{-1} of NH$_4^+$-N in the form of (NH$_4$)$_2$SO$_4$ was added to the sludge. The sludge mixture was then agitated using a Vortex mixer, and then incubated at 35°C. Michaelis constants for ammonium oxidation and DO consumption were used as described previously (Henze *et al*., 1995), and the diffusion coefficients for substrates and the ingredients in water were obtained from the Manual for Chemical Engineers (The society of Chemical Engineers 1978) (Table 2). Because the concentration of biomass in the sponge cubes was as high as 10000 mg-VSS/L, it was assumed that the diffusion coefficients in the sponge cubes would decrease to 80% of the tabulated values (Warnner and Gujer 1986).

Table 2. Constants used for model development

Substrate	*De* (cm²/d)	V_{max} (mmol-NH_4^+-N/g-VSS/s)	*Ks* (mmol/L)
NH_4^+	1.2	3.72×10^{-4}	0.107
O_2	2.0	6.38×10^{-4}	0.016
NO_2^-	1.2	-	-
CO_2	1.7	-	-

RESULTS & DISCUSSION

Data collected over 177 days of continuous reactor operation is shown in Fig. 4. The average nitrogen loading rate was 2.28 g-N/L/d for the duration of the experimental period. The average DO in the reactor was maintained at 3.73 mg-O_2/L. During the first 10 days, NH_4^+-N decreased with an increase in NO_2^--N. Then, partial nitrification was subsequently established and progressed during the remainder of Phase 1 (see arrow *a* in Fig. 4). The concentration of NO_3^--N increased to around 100 mg-N/L until day 15, after which it drastically decreased to approximately 10 mg-N/L by day 22 (average of 12.1 mg-N/L throughout Phase 1). Thus, despite the high DO concentration, successful establishment of partial nitrification was confirmed in Phase 1 and it is considered that this may have been due to not only the high operating temperatures (30-

35°C) but also the effect of IC addition. There have been prior some reports in the literature that have demonstrated that IC addition is effective for partial nitrification (Tokutomi *et al.*, 2006; Jun *et al.*, 2000); because the alkali agent used in Phase 1 was $NaHCO_3$, it was expected that a large proportion of the IC was dissolved in the mixed liquor during Phase 1.

In order to investigate the effect of IC on partial nitrification of the airlift reactor, the alkali agent was changed from $NaHCO_3$ to a mixture of Na_2HPO_4 and NaOH for Phase 2 (day 85 to 107). Soon after changing the alkali agents to a mixture of Na_2HPO_4 and NaOH (Phase 2), a decrease of NO_2^--N production was observed (see arrow *b* in Fig. 4). This tendency of NO_2^--N decrease continued in the following Phases during which no IC was added. Accumulation of NO_3^--N was still negligible during Phase 2 (9.1 mg-N/L on average during Phase 2), meaning that proliferation of NOB had still been restricted during Phase 2 as well as during Phase 1. However, after day 108 when the alkali agent was changed to NaOH only (Phase 3), production of NO_3^--N increased markedly and the maintenance of partial nitrification deteriorated rapidly (see arrow *c* in Fig. 4). It should be noticed that inhibition of ammonium oxidation itself started in Phase 4, and finally it deteriorated at the end of the experiment on day 177 in
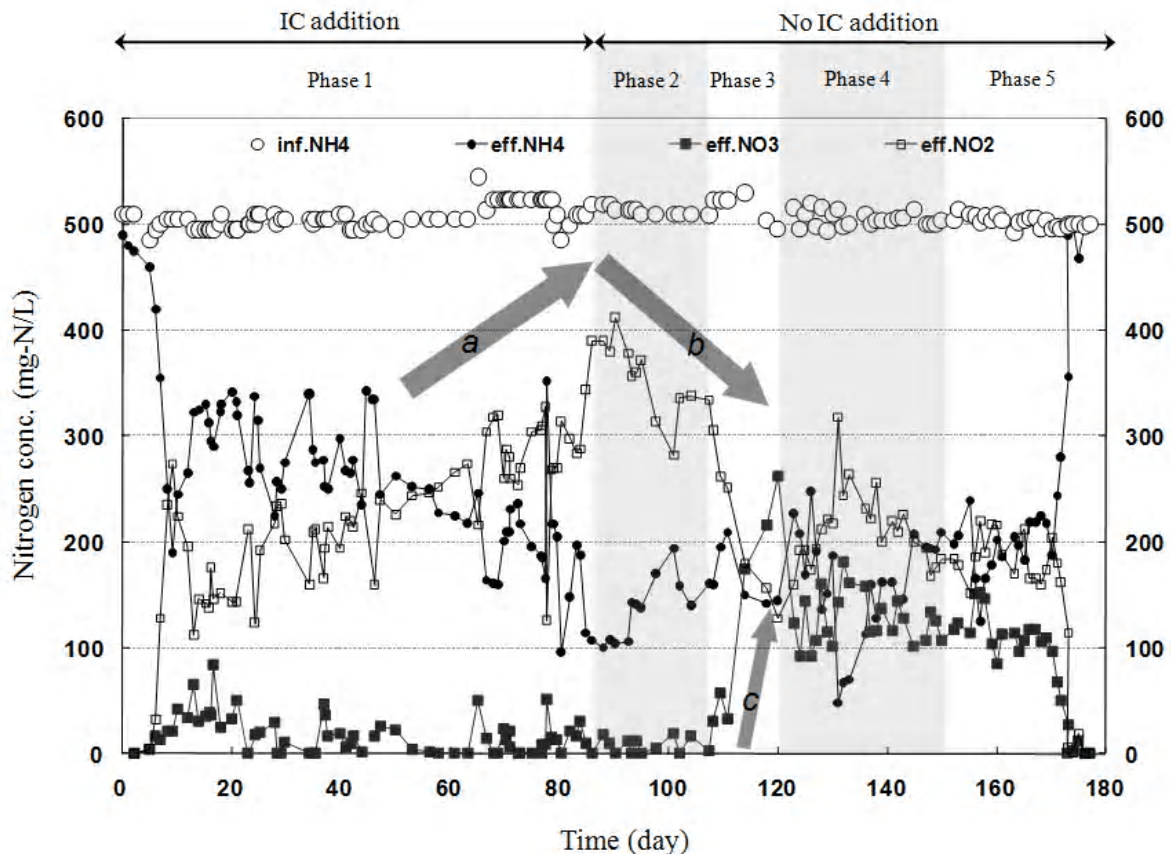

Fig. 4. Performance of the airlift reactor with respect to partial nitrification

Phase 5. Thus, addition of IC proved to be a prerequisite for not only partial nitrification but also ammonium oxidation (Denecke *et al*., 2003).

Accumulation of free nitrous acid (HNO_2) or free ammonia (NH_3OH), which results from a decrease or an increase in pH, has been reported to inhibit NOB, causing partial nitrification (Antonosen *et al*., 1976). As far as concentrations of total nitrous acid (the sum of the ionized and free nitrous acid concentrations) or total ammonia (the sum of ionized and free ammonium concentrations) are known, free nitrous acid or free ammonia concentrations can be calculated as functions of pH by using the equation of Anthonisen *et al* (1976). In this study, the pH of the mixed liquor was adjusted to 7.5 for all of the phases. However, the possibility exists that, particularly in Phase 1, NOB were inhibited by free nitrous acid or by free ammonia that had accumulated inside the sponge, which would have been caused by a change in pH deep inside the sponge medium. Therefore, we investigated the pH gradient inside the sponge cubes using a pH microelectrode (Fig. 5). The microelectrode study was conducted in the final days of Phases 1and 2, during which relatively stable partial nitrification was maintained, and Phase 3 when nitrate production was progressed (Fig. 4). In addition, we also simulated the results associated with a change in pH inside the sponge cubes and plotted these for comparison. The simulation data shows that the change in pH was almost negligible when either $NaHCO_3$ (Phase 1) or a mixture of Na_2HPO_4 and NaOH (Phase 2) were used as alkali agents (Fig. 5). The microelectrode study showed that pH started decreasing slightly at a point 1 mm into the sponge for each alkali agent. This decrease in pH continued inside the sponge cube but, even at a point deep within the sponge, i.e., 1.0-1.5 mm from the sponge surface, the decrease in the pH was still negligible (decreasing to only 7.3-7.4).

The inhibition of nitrifying organisms, i.e., both AOB and NOB, has been shown to be initiated at concentrations of free nitrous acid of 0.22-2.8 mg-N/L (Antonosen *et al*., 1976). However, the range of free nitrous acid concentrations in this experiment was estimated to be only 0.01-0.02 mg-N/L at pH 7.2-7.5 by assuming that the total nitrous acid concentration was 200 mg-N/L at 35°C, which was significantly lower than the reported inhibition level (Antonosen *et al*., 1976). On the other hand, the concentration of free ammonium capable of inhibiting NOB has been reported to be in the range 0.1-1.0 mg-N/L (Antonosen *et al*., 1976). The free ammonium in this experiment was calculated to be 3.4-6.8 mg-N/L at pH 7.2-7.5 by assuming that the total ammonia concentration was 200 mg-N/L at 35°C, which is slightly greater than the reported inhibition level (Antonosen *et al*., 1976). We also investigated the pH decline in Phase 3. The decrease in pH inside the sponge cube in Phase 3 was observed to be minimal in both the experimental measurements and simulations (data not shown), indicating that a change in pH was unlikely to be responsible for the observed deterioration in partial nitrification in Phase 3. Thus, the high-temperature operation conditions of this reactor, the addition of IC, and the inhibition of NOB by the free ammonium concentration appear to have influenced the partial nitrification of Phase 1 observed in this study.

CONCLUSION

In this study, an airlift-type activated sludge reactor using a sponge support medium was fed with artificial wastewater containing 500 mg-N/L of NH_4^+-N for a period of 120 days. The temperature of the reactor was maintained at 35°C to establish partial nitrification, and alkali agents such as $NaHCO_3$, Na_2HPO_4 + NaOH, and NaOH alone were used to adjust the pH of
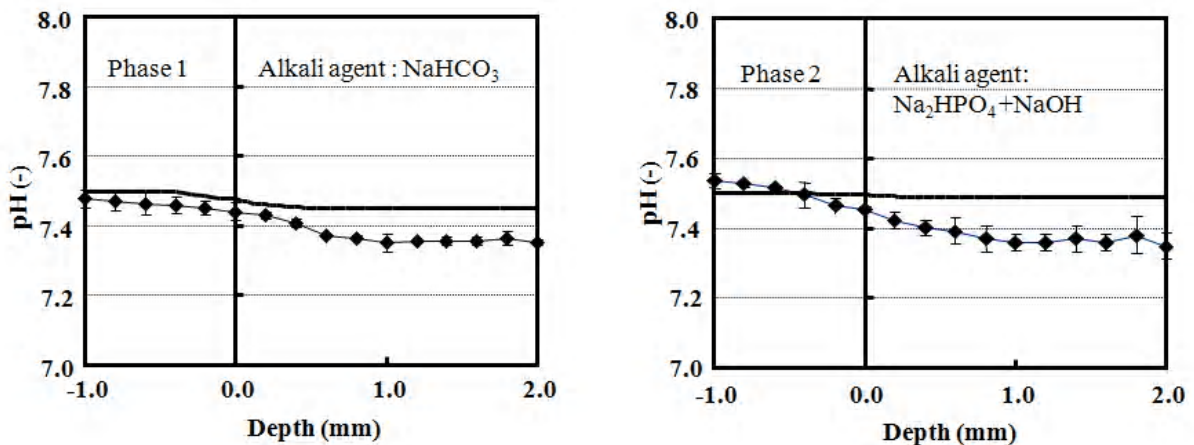

Fig. 5. pH gradient inside a sponge cube in Phases 1 and 2
Solid line: model simulation, symbols: pH microelectrode measurement

the mixed liquor to 7.5. The following conclusions can thus be drawn from this study.

1) Partial nitrification was established independently of high DO concentrations using $NaHCO_3$ as an alkali agent (average nitrogen loading rate: 2.3 g-N/L/d). However, soon after changing the alkali agent to solely NaOH, NO_2^--N accumulation started decreasing markedly and finally excessive accumulation of NO_3^--N was observed. Partial nitrification therefore appeared to be primarily affected by IC in the alkali agent used for pH adjustment even under high DO conditions.

2) According to the model simulation and microelectrode study, the change in pH was so negligible in all cases that inhibition of NOB by nitrous acid is considered to be insignificant. The free ammonium concentration, which was calculated from the pH inside the sponge cube, was higher than the reported inhibition level. It therefore appeared that the establishment of partial nitrification in this study may have been due to the combination of high operating temperatures, IC addition, and the inhibition of NOB by the free ammonium concentration.

REFERENCES

Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S., and Srinath, E. G. (1976). Inhibition of nitrification by ammonia and nitrous acid. Journal of Water Pollution Control Federation, **48**, 835-852.

APHA, (2005). Standard Methods for the Examination of Water and Wastewater, 21st edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

Bae, W., Baek, S., Chung, J. and Lee, Y. (2002). Optimal operational factors for nitrite accumulation in batch reactors. Biodegradation, **12**, 359-366.

Bernet, N., Dangcong, P., Delgenes, J. P. and Moletta, R. (2002). Nitrification at low oxygen concentration in biofilm reactor. Journal of Environmental Engineering, **127**, 266-271.

Chung, J., Shim, H., Lee, Y. W. and Bae, W. (2005). Comparison of influence of free ammonia and dissolved oxygen on nitrite accumulation between suspended and attached cells. Environmental Technology, **26**, 21-33.

Denecke, M. and Liebig, T. (2003). Effect of carbon dioxide on nitrification rates. Bioprocess and Biosystems Engineering, **25**, 249-253.

Fux, C., Boehler, B., Huber, P., Brunner, I. and Siegrist, H. (2002). Biological treatment of ammonium-rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (ANAMMOX) in a pilot plant. Journal of Biotechnology, **99**, 295-306.

Giudad, G., Rubilar, O., Munoz, P., Ruiz, G., Chamy, R., Vergara, C. and Jeison, D. (2005). Partial nitrification of high-ammonia concentration wastewater as a part of a short-cut biological nitrogen removal. Process Biochemistry, **40**, 1715-1719.

Hellinga, C., Schellen, A. A. J. C., Mulde, J. W., van Loosdrecht, M. C. M. and Heijnen, J. J. (1998). The SHARON process: an innovative method for nitrogen removal from ammonium-rich waste water. Water Science and Technology, **37**, 135-142.

Henze, M., Gujer, W., Mino, T., Matuo, T., Wentzel, M. C. and Marais, G. V. R. (1995). *Activated* Sludge Model No.2, IAWQ Scientific and Technical Report, No.3, IWAQ, London.

Jianlong, W. and Ning, Y. (2004). Partial nitrification under limited dissolved oxygen conditions. Process Biochemistry, **39**, 1223-1229.

Jun, B. H., Tanji, Y. and Unno, H. (2000). Simulating accumulation of nitrifying bacteria in porous carrier by addition of inorganic carbon in a continuous flow fluidized bed wastewater treatment reactor. Journal of Bioscience and Bioengineering, **89**, 334-339.

Kuenen, J. G. and Jetten, M. S. M. (2001). Extraordinary anaerobic ammonium oxidizing bacteria. ASM news, **67**, 456-463.

Mulder, A., Graff, A. A., Robertson, L. A. and Kuenen, J. G. (1995). Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiology Ecology, **16**, 177-184.

Ruiz, G., Jeison, D. and Chamy, R. (2003). Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. Water Research, **37**, 1371-1377.

The Society of Chemical Engineers Japan (1978). Manual for Chemical Engineers, Maruzen, Tokyo, Japan.

Tokutomi, K. (2004). Operation of a nitrite-type airlift reactor at low DO concentration. Water Science and Technology, **49 (5-6)**, 81-88.

Tokutomi, T., Kiyokawa, T., Shibayama, C., Harada, H. and Ohashi, A. (2006). Effect of inorganic carbon on nitrite accumulation in an aerobic granular sludge. Water Science and Technology, **53**, 285-294.

Warner, O. and Gujer, W. (1986). A multispecies biofilm model. Biotechnology and Bioengineering, **28**, 314-328.

Yamaguchi, T., Yamazaki, S., Uemura, S., Tseng, I. C., Ohashi, A. and Harada, H. (2001). Microbial-ecological significance of sulfide precipitation within anaerobic granular sludge revealed by micro-electrodes study. Water Research, **35**, 3411-3417.

Yu, D., Peng, Y. and Zhang, K. (2004). Effects of seawater salinity on nitrite accumulation in short-range nitrification to nitrite as end product. Journal of Environmental Sciences, **16**, 247-251.

Yun, H. J. and Kim, D. J. (2003). Nitrite accumulation characteristics of high strength ammonia wastewater in an autotrophic nitrifying biofilm reactor. Journal of Chemical Technology and Biotechnology, **78**, 377-383.

Wyffels, S., van Hulle, S. W. H., Boeckx, P., Volcke, E .I. P., van Cleemput, O. Vanrolleghem, P. A. and Verstraete, W. (2004). Modeling and simulation of oxygen-limited partial nitritation in a membrane – assisted bioreactor (MBR). Biotechnology and Bioengineering, **86**, 531-542.