J Ind Microbiol Biotechnol (2008) 35:1331–1338
DOI 10.1007/s10295-008-0415-9

ORIGINAL PAPER

Simultaneous effect of temperature, cyanide and ammonia-oxidizing bacteria concentrations on ammonia oxidation

Hyojin Do · Juntaek Lim · Seung Gu Shin · Yi-Ju Wu · Johng-Hwa Ahn · Seokhwan Hwang

Received: 24 March 2008 / Accepted: 29 July 2008 / Published online: 20 August 2008

Abstract For biological nitrification, a set of experiments were carried out to approximate the response of lag period along with ammonia oxidation rate with respect to different concentrations of cyanide (CN^-) and ammonia-oxidizing bacteria (AOB), and temperature variation in laboratory-scale batch reactors. The effects of simultaneous changes in these three factors on ammonia oxidation were quantitatively estimated and modeled using response surface analysis. The lag period and the ammonia oxidation rate responded differently to changes in the three factors. The lag period and the ammonia oxidation rate were significantly affected by the CN^- and AOB concentrations, while temperature changes only affected the ammonia oxidation rate. The increase of AOB concentration and temperature alleviated the inhibition effect of cyanide on ammonia oxidation. The statistical method used in this study can be extended to estimate the quantitative effects of other environmental factors that can change simultaneously.

H. Do · J. Lim · S. G. Shin · S. Hwang (✉)
School of Environmental Science and Engineering,
Pohang University of Science and Technology (POSTECH),
San 31, Hyoja-dong, Nam-gu, Pohang,
Gyungbuk 790-784, South Korea
e-mail: shwang@postech.ac.kr

Y.-J. Wu
Department of Environmental Engineering,
National Cheng Kung University, No. 1 University Road,
Tainan City 701, Taiwan

J.-H. Ahn
Division of Environmental and Geosystem Engineering,
College of Engineering, Kangwon National University,
Hyoja-2-dong, Chuncheon, Kangwon 200-701, South Korea

Keywords Ammonia oxidation · Response surface analysis · Cyanide · Temperature · Ammonia-oxidizing bacteria

Introduction

Ammonia-oxidizing bacteria (AOB), chemoautotrophs, oxidize ammonia to nitrite, which is the first and rate-limiting step of nitrification. AOB are highly sensitive to many environmental factors such as pH, temperature, and dissolved oxygen (DO) concentration [28, 29, 31]. Growth of AOB is also affected by industrial contaminants, including cyanide [3, 11, 16, 17].

Cyanide is toxic to many living organisms, because it inhibits activities of enzymes such as the heme–copper oxidase superfamily of cytochrome oxidases [4, 5]. Cyanide is often found in various industrial wastewater such as coke oven gas wastewater, blast-furnace blowdown water, photoprocessing wastewater, electroplating wastewater, and chemical fertilizer wastewater, which also contains high levels of ammonium [12, 19, 21, 30, 33]. In these industrial wastewaters, ammonia oxidation can be highly susceptible to disruption by cyanide.

Temperature and AOB concentration as well as cyanide directly influence the ammonia oxidation. The activity of living organisms involves enzymatic reactions in cells; these reactions are affected by temperature. According to the van't Hoff–Arrhenius equation, the growth rate of microorganisms doubles with each 10°C increment in temperature [7]. The concentration of AOB is another important factor that affects ammonia oxidation, because ammonia oxidation is the process of substrate utilization by AOB.

Researchers have investigated the effects of various environmental factors on ammonia oxidation. Several pure culture studies have demonstrated the effects of temperature on AOB [14, 15, 28] and have reported 30–35°C as the optimum temperature. The toxic effects of cyanide on AOB activity have also been investigated [17, 19], and it was shown that this inhibition is irreversible [10].

It must be noticed that most studies have investigated the effect of one environmental factor at a time. The effect of temperature on nitrification, for example, has recently been studied at a fixed concentration of cyanide [19]. In a full-scale wastewater treatment plant (WWTP), however, the environmental factors affecting ammonia oxidation are likely to change simultaneously and independently. For example, influent cyanide concentration to the industrial wastewater treatment plant usually fluctuates, because it is released in different amounts at variable flow rates with respect to the operational modes of the unit processes. Temperature of the WWTP is also affected by ambient temperature, because almost all full-scale WWTPs are located outside. Microbial populations in such treatment system also change according to the influent quality along with the growth conditions such as temperature. Therefore, it is essential to understand the simultaneous effects of changes in these factors on ammonia oxidation to predict and control the ammonia oxidation in full-scale WWTP.

Response surface analysis (RSA) is a collection of mathematical and statistical techniques useful for designing experiments, building models, and evaluating relative significance of several independent variables (i.e., environmental factors) [2, 26]. In most RSA problems, the relationship between the response and the independent variables is unknown. Thus, the first step in RSA is to approximate the function using a low-order polynomial in some region of the independent variables. If the response is not linear, then a higher order polynomial must be used to approximate the response. Almost all RSA studies utilize one or more of these approximating polynomials [22, 23]. Thus, this study was conducted to approximate continuous response surfaces of the change in AOB activity with respect to the simultaneous effects of cyanide concentration, AOB concentration, and temperature using RSA. To quantify the effects on ammonia oxidation, changes in the lag period and the rate of ammonia oxidation were investigated, because the lag period is the time in which the cells adapt to new environments or stressors and the ammonia oxidation rate represents the activity of the AOB in exponential growth period. Statistical model equations of the simultaneous effects of changes in these three factors are expressed in terms of the lag period and the ammonia oxidation rate.

Materials and methods

Enrichment culture and seed preparation

A batch reactor with a 10 L working volume was set up to have a sufficient amount of seed to inoculate the subsequent batch cultures for RSA. The reactor was inoculated with a steady-state effluent of a lab-scale completely stirred tank reactor (CSTR) treating ammonia containing wastewater (i.e., 300 mg NH_4^+–N/L) of a steel-processing industry. The CSTR was operated at 10 days hydraulic retention time and at 28 ± 20°C. pH and dissolved oxygen concentration was controlled to be above 7.5 and 2.0 mg DO/L, respectively. The enrichment batch system was inoculated with 10% (v/v) of the CSTR effluent, and 90% (v/v) of the identical wastewater was adjusted to contain 500 mg/L NH_4^+–N. The other operational conditions were same as those of the inoculum system. Ten liters of the enrichment culture was centrifuged at 14,000*g* for 5 min. The supernatant was discarded and the pellet was washed with 20 mM $NaHCO_3$. After another centrifugation and washing step, the concentrated seed was prepared and aerated until it was used as the inoculum for the subsequent batch trials.

DNA extraction

DNA was extracted from the sample for the quantitative real-time PCR (QPCR) assay. An aliquot (1 mL) of sample was centrifuged at 16,000*g* for 10 min, and 900 μL of the supernatant was decanted. The pellet was washed twice with following steps: (1) the pellet was added to 100 μL of deionized distilled water (DDW) and resuspended; (2) the pellet was centrifuged; (3) an aliquot (100 μL) of supernatant was decanted. Finally, the pellet was prepared in 100 μL of total volume and applied to an automated nucleic acid extractor (Magtration System 6GC, PSS Co., Japan). The purified DNA was eluted with 100 μL of DDW and the concentration of the AOB was measured with the QPCR assay.

Quantitative real-time PCR

In previous studies, the mixed liquor volatile suspended solids (MLVSS) have often been used to represent the concentration of microorganisms [18, 32]. However, even the same concentrations of MLVSS may display different ammonia oxidation rates, because they contain different portion of AOB. The sludge samples from different WWTPs showed different AOB concentrations, in the range of 10^4–10^8 copies/mL [8]. The AOB concentration per MLVSS in each sludge sample also differed [6]. The β-proteobacterial AOB, a subgroup of AOB, are likely to

be found in most engineered biological nutrient removal processes [24]. In this study, the β-proteobacterial AOB concentration, one of the independent variables, was measured using a QPCR (LightCycler 1.2, Roche Diagnostics) with the TaqMan system, which employs two PCR primers and a TaqMan probe. The TaqMan probe contains a fluorescent reporter dye FAM and a quencher dye TAMRA, which were attached to the 5′ and the 3′ ends, respectively. The primers and probe bind to the target DNA during the annealing step of PCR and the TaqMan probe is cleaved by the exonuclease activity of *Taq* polymerase during primer extension.

TaqMan probe and primer sets, designed to specifically detect subgroups of AOB (i.e., clusters of *Nitrosomonas europaea*, *N. nitrosa*, *N. cryotolerans*, and *Nitrosospira multiformis*) [24], were used to quantify the AOB in the seed. Plasmids, constructed using type strains belonging to the clusters and normalized to be 10^6 copies of corresponding 16S rRNA genes/µl, were used as target AOB templates to construct standard curves. Different initial 16S rRNA gene copy numbers covering six orders of magnitude for each target were amplified using the QPCR with the corresponding sets. Logarithmic values of the different 16S rRNA gene amounts were plotted against the threshold cycle (C_T) numbers from each QPCR assay. All sets were capable of detecting the corresponding targets as low as two 16S rRNA gene copies per reaction mixture.

A two-step amplification of the target DNA, combining the annealing and extension steps, was performed. An initial 10 min incubation at 94°C for *Taq* DNA polymerase activation was followed by 45 cycles of denaturation at 94°C for 10 s, and simultaneous annealing and extension at 60°C for 30 s. The transition rate was 20°C/s for all segments of the two-step cycling.

Experimental design and batch tests

The RSA experiment was designed with a central composite cube design [27] and batch ammonia oxidation reactions were conducted at each condition (Table 1). Each batch trial was performed with the ISO 9509 method [13]. Concentrated (tenfold) synthetic wastewater was prepared with 2.65 g/L $(NH_4)_2SO_4$ for a final concentration of 56 mg/L NH_4^+–N. The concentrated wastewater also contained 16.8 g/L $NaHCO_3$ as buffering agent, which helped maintain the pH at 7.5 during ammonia oxidation. A concentrated KCN solution (100 mg/L), the concentrated seed, and the synthetic wastewater were added to 250-mL flasks, with DDW to a total volume of 125 mL. The flasks were kept in a shaking incubator, stirred at 130 rpm [18], at the desired temperature of 23, 28, or 33°C. The cultures were periodically sampled until the ammonium was completely depleted.

Analytical methods

The nitrite concentration of each experimental trial was monitored with the Griess method [20]. The concentrations of ammonium ions were analyzed with an ion chromatography (Metrohm 790 Personal IC) and were plotted to

Table 1 Experimental design and observed lag periods and ammonia oxidation rates

Trial	Independent variables			Dependent variables	
	CN^- concentration (mg/L)	AOB concentration (10^9 copies/L)	Temperature (°C)	Rate (NH_4^+–N mg/L/h)	Lag (h)
Linear design					
1	0.1	30	33	10.86	1.4
2	0.1	30	23	5.64	3.7
3	0.1	3	33	3.16	18.4
4	0.1	3	23	1.72	40.7
5[a]	5	16.5	28	3.90	161.0
6	10	30	33	2.60	159.9
7	10	30	23	2.40	216.0
8	10	3	33	2.69	482.3
9	10	3	23	0.72	678.8
Quadratic design					
10	0.1	16.5	28	6.01	4.0
11	5	30	28	5.89	86.7
12	5	16.5	33	2.26	168.3
13	5	16.5	23	1.65	201.2
14	5	3	28	2.27	439.8
15	10	16.5	28	1.61	240.2

[a] Center point. The experiment was replicated three times and the data represent average values

calculate the lag periods and ammonia oxidation rates using the modified Gompertz model (Eq. 1) [34].

$$y = A\exp\left\{-\exp\left[\frac{\mu_{\max}\cdot\exp(1)}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

where

A Asymptote, the maximum value of growth reached;
μ_m Maximum specific growth rate, tangent to the growth curve at the inflection point;
λ Lag time, t-axis intercept of the tangent at the inflection point on the growth curve.

Results

Range of variables and RSA

Cyanide concentration, AOB concentration, and temperature in the biological nitrification facility of a steel-producing industry were monitored to determine the experimental ranges of the variables approximately for a year (Fig. 1). The cyanide concentration varied from 0 to 7.2 mg/L and the AOB concentration ranged from 3.85×10^9 and 2.36×10^{10} copies/L in the nitrification facility. The lowest and highest temperatures of the facility were 23 and 33°C for the period. Thus, ranges of the independent variables were determined based on these monitored data for the industrial full-scale WWTP, and are summarized in Table 2. The culture condition at the center point was 5 mg CN^-/L, 1.65×10^{10} copies of AOB/L, and 28°C. Repeat observations at the center were used to estimate the experimental error in the RSA.

A total of 17 trials, including a center point, were run to estimate the response surface for the lag period and the ammonia oxidation rate. A first-order model was initially tested, but it was not statistically significant based on the regression coefficient, the lack of fit of the first-order model, the P values of the parameter estimations, and the residual plots (data not shown). Additional six trials (10–15 in Table 1) were added to the previous 11 trials to form a central composite cubic design. Various models, from interaction to partial cubic, were tested sequentially with data from the whole 17 trials (Table 1) to estimate the lag periods and ammonia oxidation rates.

Effects on the lag period

The effects of the variables on the lag period were adequately estimated with the partial cubic model (Eq. 2) based on statistical analysis ($P = 0.0009$ at a 0.1% α-level, $R^2 = 0.993$) and a residual plot (Fig. 2a). The residual

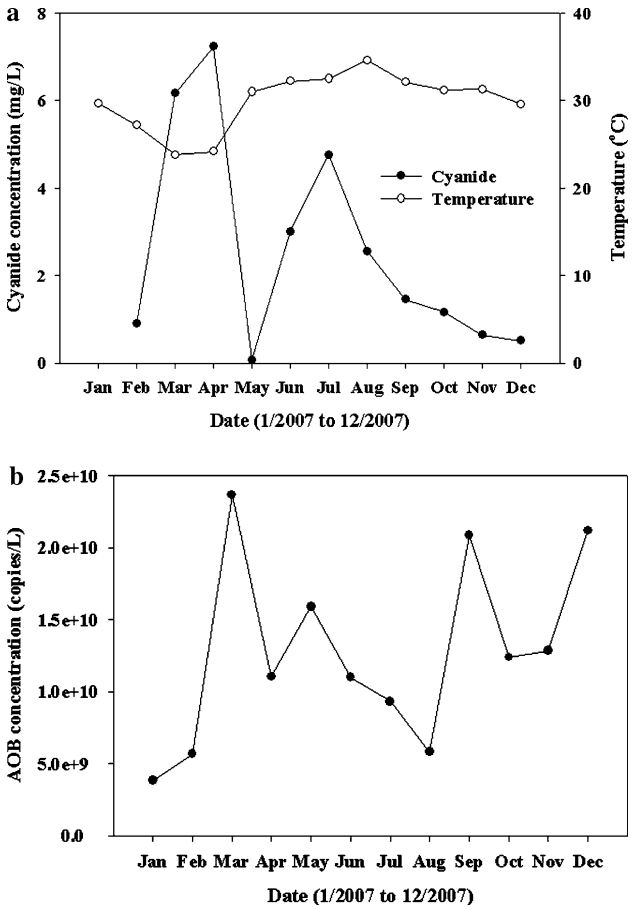

Fig. 1 Variation of cyanide concentration and temperature (**a**), and AOB concentration (**b**) in the biological nitrification facility treating wastewater of the steel-processing industry

Table 2 Experimental ranges of the three variables based on monitoring data for a steel-producing industrial wastewater treatment plant

Independent variables	Low	Center	High
CN^- concentration (mg/L)	0.1	5	10
AOB concentration (10^9 copies/L)	3	16.5	30
Temperature (°C)	23	28	33

plots for the models and data set showed no patterns or trends. A check of the constant variance assumption also could be addressed, because a random plot of residuals meant homogeneous error variances across the observed values.

$$\begin{aligned} Y_a = {} & 341 + 118X_1 - 1.19\times10^{-8}X_2 - 16.4X_3 - 5.99 \\ & \times 10^{-9}X_1X_2 + 0.325X_1X_3 \\ & + 2.97\times10^{-10}X_2X_3 - 1.95X_1^2 + 9.05\times10^{-20}X_2^2 \\ & + 0.182X_3^2 + 7.30\times10^{-20}X_1X_2^2 \\ & + 2.19\times10^{-10}X_1^2X_2 - 0.146X_1^2X_3 \end{aligned} \quad (2)$$

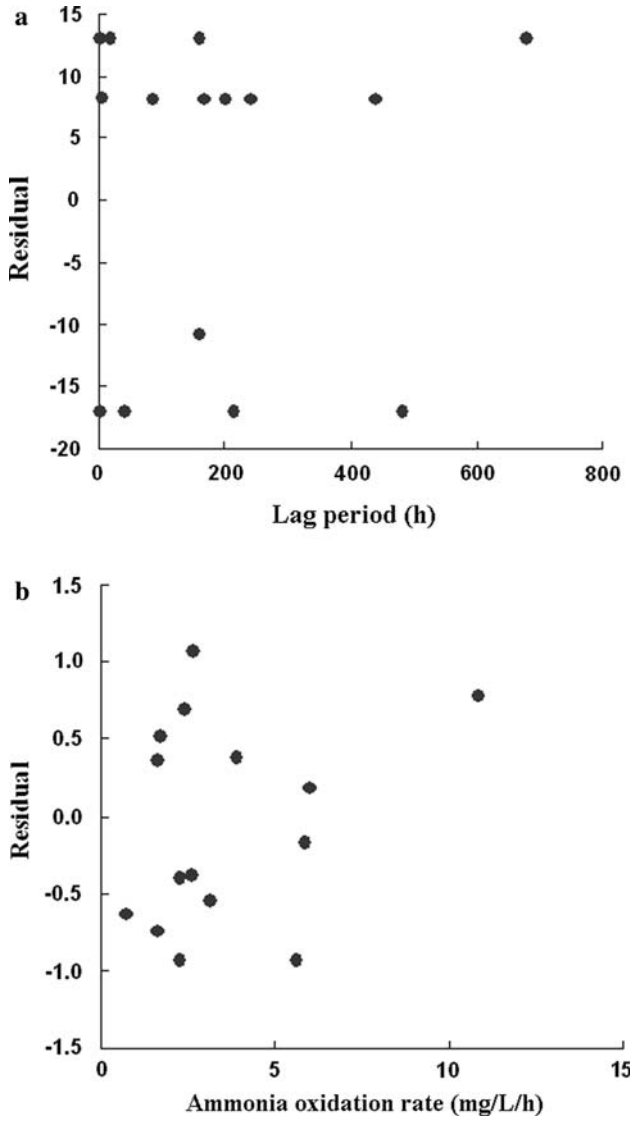

Fig. 2 Residual plots of the partial cubic model for the lag period (**a**), and the quadratic model for the ammonia oxidation rate (**b**). Each residual was calculated using Eqs. (2) and (3), respectively

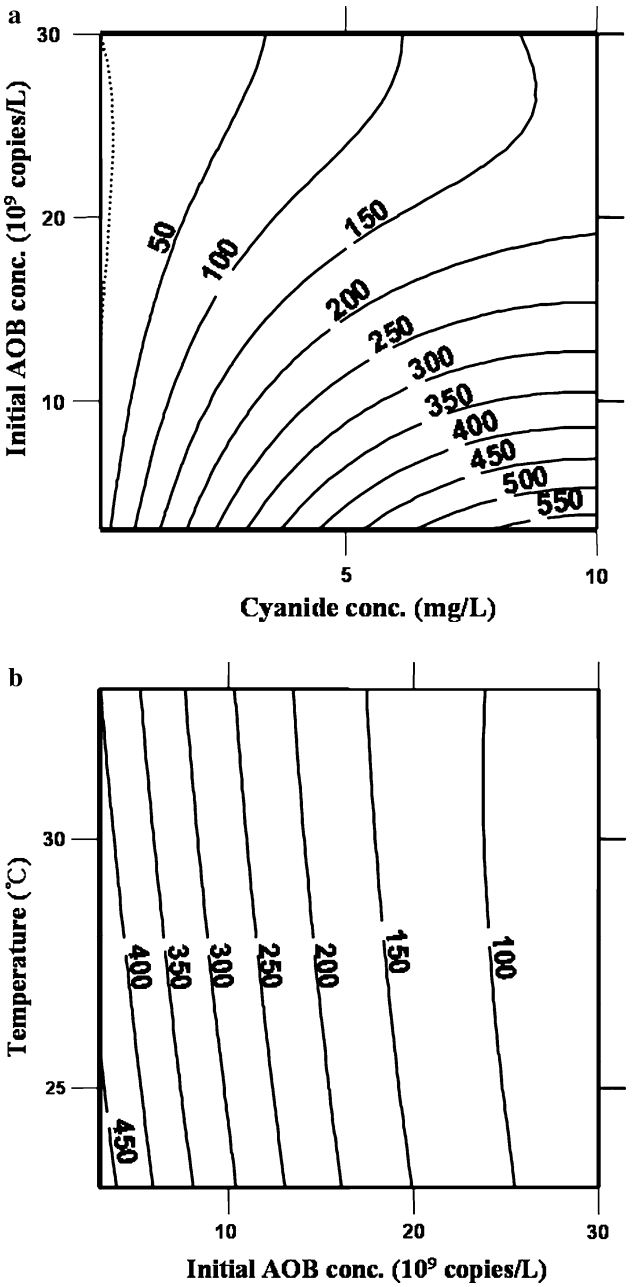

Fig. 3 Two-dimensional contour plots of the partial cubic model for the lag period of ammonia oxidation with respect to cyanide concentration and initial AOB concentration within the design boundary at 28°C (**a**), and with respect to the initial AOB concentration and temperature at a cyanide concentration of 5 mg/L (**b**)

where

Y_a Lag period;

X_k Independent variable k (k = cyanide concentration, initial AOB concentration, or temperature).

With this model equation, the change in the lag period can be quantified when the variables change simultaneously. If cyanide concentration, initial AOB concentration, and temperature were 5 mg/L, 16.5 $\times$ 10^9 copies/L, and 23°C, respectively, then the lag period would be 193 h. Using the equation, the lag period was expected to decrease to 174 h if cyanide concentration and temperature increased to 10 mg/L and 33°C, respectively. It could also be predicted that the lag period would not decrease even if the cyanide concentration decreased from 10 to 2 mg/L when the initial AOB concentration also decreased to 3 $\times$ 10^9 copies/L at 33°C.

Figure 3a, b shows the effects of the cyanide and initial AOB concentrations and the effects of the initial AOB concentration and temperature on the lag period, respectively. Temperature had no significant effect on the lag period (Table 3).

Table 3 Results of the response surface analysis and the effects of each term

Term	Regression analysis					
	Lag			Rate		
	Coefficient	Effect[a]	*P* value	Coefficient	Effect[a]	*P* value
Intercept	3.41×10^{2}	173		-4.34×10^{1}	3.50	
X_1	1.18×10^{2}	236	0.0056	3.49×10^{-1}	−3.47	0.0016
X_2	-1.19×10^{-8}	−355	0.0012	-3.76×10^{-11}	3.36	0.0020
X_3	-1.64×10	−33.5	0.4854	3.11	1.89	0.0309
X_1X_2	-5.99×10^{-9}	−183	0.0011	1.88×10^{-11}	−2.51	0.0149
X_1X_3	3.25×10^{-1}	−57.0	0.0591	-2.26×10^{-2}	−1.12	0.1956
X_2X_3	2.97×10^{-10}	40.1	0.1396	3.72×10^{-12}	0.502	0.5411
X_1^2	−1.95	−59.3	0.0346	2.41×10^{-2}	0.590	0.4114
X_2^2	9.05×10^{-20}	83.7	0.0113	4.62×10^{-21}	0.843	0.2525
X_3^2	1.82×10^{-1}	4.55	0.8210	-5.13×10^{-2}	−1.28	0.0996
$X_1X_2^2$	7.30×10^{-20}	132	0.0539			
$X_1^2X_2$	2.19×10^{-10}	145	0.0408			
$X_1^2X_3$	-1.46×10^{-1}	−35.8	0.5031			

[a] Effect is cR, if R is the range of the response term in the data and c is the estimated coefficient

Effects on the ammonia oxidation rate

The quadratic model (Eq. 3) was the only adequate model for the ammonia oxidation rate based on statistical analysis ($P = 0.006$ at a 1% α-level, $R^2 = 0.912$) and a residual plot (Fig. 2b).

$$Y_b = -43.4 + 3.49 \times 10^{-1}X_1 - 3.76 \times 10^{-11}X_2 + 3.11X_3 - 1.88 \times 10^{-11}X_1X_2 - 2.26 \times 10^{-2}X_1X_3 + 3.72 \times 10^{-12}X_2X_3 + 2.41 \times 10^{-2}X_1^2 + 4.62 \times 10^{-21}X_2^2 - 5.13 \times 10^{-2}X_3^2 \quad (3)$$

where

Y_b Ammonia oxidation rate;
X_k Independent variable k (k = cyanide concentration, initial AOB concentration, or temperature).

The change in the ammonia oxidation rate could be predicted with this model equation. When cyanide concentration, initial AOB concentration, and temperature were 10 mg/L, 3.0×10^9 copies/L, and 33°C, respectively, the estimated ammonia oxidation rate for NH_4^+–N was 1.86 mg/L per hour. Even if the cyanide concentration and temperature decreased from 10 to 0.1 mg/L, and from 33 to 23°C, respectively, the ammonia oxidation rate would decrease to 1.04 mg NH_4^+–N/L/h when the initial AOB concentration was kept at 3.0×10^9 copies/L. Figure 4a, b shows the effects of the cyanide concentration and initial AOB concentration on the ammonia oxidation rate and the effects of the initial AOB concentration and temperature on the ammonia oxidation rate, respectively. When the cyanide concentration is lower than 5 mg/L and the initial AOB concentration is higher than 16.5×10^9 copies/L, the ammonia oxidation rate is predominantly influenced by the linear combination of the cyanide concentration and the initial AOB concentration (Fig. 4a). The ammonia oxidation rate is significantly affected by the cyanide concentration, the initial AOB concentration, and temperature (Table 3).

Discussion

Cyanide is extremely toxic to AOB and to biological ammonia oxidation. A cyanide concentration of 0.34 mg/L inhibits nitrification during the activated sludge process [32]. A recent study with a pure culture of *Nitrosococcus mobilis* showed that even a cyanide concentration of 0.026 mg/L can have an adverse effect on ammonia oxidation [29]. Despite the severe toxicity of cyanide, microorganisms can adapt to high concentrations of cyanide after constant and long exposure to it [33]. This was confirmed in this study by the observation that ammonia oxidation was still occurring after almost 28 days (678 h) of the lag period, when the cyanide concentration was as high as 10 mg/L (Table 1).

The length of lag period was significantly affected by the cyanide concentration and the initial AOB concentration (Table 3). The cyanide concentration had a negative effect on duration of the lag period, because the AOB required prolonged period to adapt to high cyanide toxicity. Conversely, the initial AOB concentration had a positive effect to shorten the lag period. When the initial AOB concentration was 3.0×10^9 copies/L, the lag period was extended to 578 h as the cyanide concentration increased from 0.1 to 10 mg/L (Fig. 3a). However, the lag period was maintained for less than 191 h if the initial

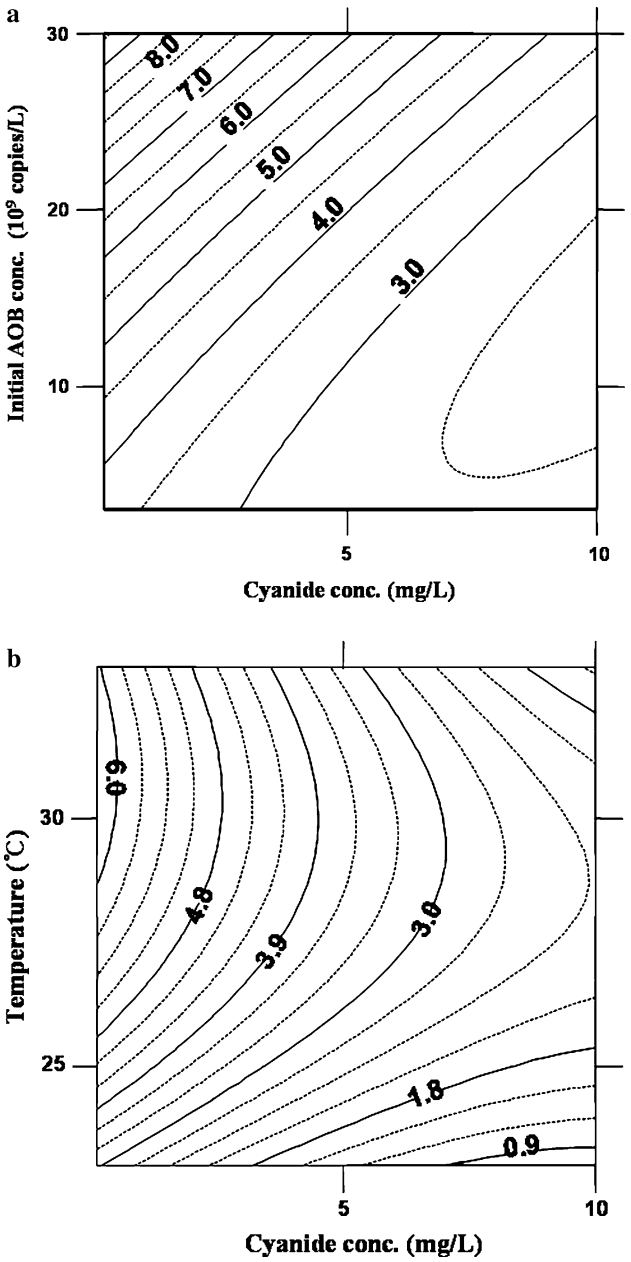

Fig. 4 Two-dimensional contour plots of the quadratic model for the ammonia oxidation rate with respect to the cyanide concentration and initial AOB concentration within the design boundary at 28°C (**a**), and with respect to the cyanide concentration and temperature within the design boundaries at an initial AOB concentration of 16.5×10^9 mg/L (**b**)

AOB concentration was higher than 2.0×10^{10} copies/L, as the cyanide concentration changed within the experimental range. The changes in the lag period with respect to the independent variables in this study may suggest that the functional redundancy of the system can be achieved by increasing the concentration of functional microorganisms.

The temperature for the highest ammonia oxidation rate decreased from 31 to 28.8°C as the cyanide concentration increased from 0.1 to 10 mg/L when the initial AOB concentration was 16.5×10^9 copies/L (Fig. 4b). In previous research, the optimum temperature for AOB was reported to be 35°C [1, 9, 14], which is a little higher than the results in this study. Temperature is one of the most important environmental factors for microbial growth [25] and is the result of two interactive processes under normal conditions: the anticipated increase in the reaction rate with an increase in temperature and protein denaturation above a critical temperature [7]. It is also suggested that the optimum temperature varies slightly with other environmental factors [25]. The toxicity of cyanide tended to reduce the optimum temperature for ammonia oxidation.

This study shows the quantitative effects of simultaneous changes in three important factors affecting ammonia oxidation. These results can provide a guide for the operation of full-scale WWTP, especially with quantitative information about the AOB concentration, which has a significant effect on reducing the toxic effects of cyanide.

A factorial design, used most often in biological research, yields discrete results according to the variable values that the investigator assigns. It is hard to visualize the effect of the variables that were not assigned (for example, the middle point between two variable values). In this paper, RSA was successfully applied to approximate the continuous response of the AOB activity with respect to the simultaneous changes in cyanide and AOB concentrations, and operational temperature. This concept allows researchers to study interactions of control variables or to determine the collective properties of the mixture of microbes selected by the conditions imposed upon them. The results can also be applied to relevant biological areas, when using mixed populations of microbes for better process control. With the proper selection of a variable interval, RSA continuously approximates the response surface, which allows the researcher to investigate the entire region of variables.

Acknowledgments This research was supported in part by the BK-21 program, Advanced Environmental Biotechnology Research Center (Grant No: R11-2003-006), and Ministry of Environment as "The Eco-technopia 21 project".

References

1. Bae W, Baek S, Chung J, Lee Y (2001) Optimal operational factors for nitrite accumulation in batch reactors. Biodegradation 12:359–366
2. Box GEP, Draper NR (1987) Empirical model-building and response surfaces. Wiley, New York
3. Brandt KK, Hesselsøe M, Roslev P, Henriksen K, Sørensen J (2001) Toxic effects of linear alkylbenzene sulfonate on

metabolic activity, growth rate, and microcolony formation of *Nitrosomonas* and *Nitrosospira* strains. Appl Environ Microbiol 67:2489–2498
4. Cipollone R, Frangipani E, Federica T, Imperi F, Ascenzi P, Visca P (2007) Involvement of *Pseudomonas aeruginosa* rhodanese in protection from cyanide toxicity. Appl Environ Microbiol 73:390–398
5. Cooper M, Tavankar GR, Williams HD (2003) Regulation of expression of the cyanide-insensitive terminal oxidase in *Pseudomonas aeruginosa*. Microbiology 149:1275–1284
6. Dionisi HM, Layton AC, Harms G, Gregory IR, Robinson KG, Sayler GS (2002) Quantification of *Nitrosomonas oligotropha*-like ammonia-oxidizing bacteria and *Nitrospira* spp. from full-scale wastewater treatment plants by competitive PCR. Appl Environ Microbiol 68:245–253
7. EPA (1993) Nitrogen control. Office of Research and Development, Washington, DC
8. Geets J, de Cooman M, Wittebolle L, Heylen K, Vanparys B, De Vos P, Verstraete W, Boon N (2007) Real-time PCR assay for the simultaneous quantification of nitrifying and denitrifying bacteria in activated sludge. Appl Microbiol Biotechnol 75:211–221
9. Grunditz C, Dalhammar G (2001) Development of nitrification inhibition assays using pure cultures of *Nitrosomonas* and *Nitrobacter*. Water Res 35:433–440
10. Hooper AB, Terry KR (1973) Specific inhibitors of ammonia oxidation in *Nitrosomonas*. J Bacteriol 115:480–485
11. Hu Z, Chandran K, Grasso D, Smets BF (2003) Impact of metal sorption and internalization on nitrification inhibition. Environ Sci Technol 37:728–734
12. Huang P, Ma F, Qin SY (2006) Reclaimation of petroleum-based wastewater by novel ozone immobilized biological activated carbon process. J Harbin Inst Technol 13:753–757
13. ISO (1989) Water quality—method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste waters. International Organization for Standardization, Switzerland
14. Jones RD, Hood MA (1980) Effects of temperature, pH, salinity, and inorganic nitrogen on the rate of ammonium oxidation by nitrifiers isolated from wetland environments. Microb Ecol 6:339–347
15. Jones RD, Morita RY (1985) Low-temperatrue growth and whole-cell kinetics of a marine ammonium oxidizer. Mar Ecol 21:239–243
16. Juliastuti SR, Baeyens J, Creemers C, Bixio D, Lodewyckx E (2003) The inhibitory effects of heavy metals and organic compounds on the net maximum specific growth rate of the autotrophic biomass in activated sludge. J Hazard Mater 100:271–283
17. Kelly RT, Henriques IDS, Love NG (2004) Chemical inhibition of nitrification in activated sludge. Biotechnol Bioeng 85:683–694
18. Kim KT, Kim IS, Hwang SH, Kim SD (2006) Estimating the combined effects of copper and phenol to nitrifying bacteria in wastewater treatment plants. Water Res 40:561–568
19. Kim YM, Park D, Lee DS, Park JM (2007) Instability of biological nitrogen removal in a cokes wastewater treatment facility during summer. J Hazard Mater 141:27–32
20. Kleinbongard P, Rassaf T, Dejam A, Kerber S, Kelm M (2002) Griess method for nitrite measurement of aqueous and protein-containing samples. Methods Enzymol 359:158–168
21. Kumar MS, Vaidya AN, Shivaraman N, Bal AS (2003) Performance evaluation of a full-scale coke oven wastewater treatment plant in an integrated steel plant. Indian J Environ Health 45:29–38
22. Lee C, Kim J, Hwang S (2006) Optimization of adenosine 5′-triphosphate extraction for the measurement of acidogenic biomass utilizing whey wastewater. Biodegradation 17:347–355
23. Lee S, Bae H, Kim N, Hwang S (2008) Optimization of growth conditions of *Lentinus edodes* mycelium on corn processing waste using response surface analysis. J Biosci Bioeng 105:161–163
24. Lim J, Do H, Shin S, Hwang S (2008) Primer and probe sets for group-specific quantification of the genera *Nitrosomonas* and *Nitrosospira* using real-time PCR. Biotechnol Bioeng 99:1374–1383
25. Michael TM, Martinko JM, Parker J (2003) Brock biology of microorganisms. Pearson Education, Upper Saddle River
26. Montgomery DC (2001) Design and analysis of experiments. Wiley, New York
27. Myers RH, Montgomery DC (1995) Response surface methodology, process and product optimization using designed experiments. Wiley, New York
28. Neufeld R, Greenfield J, Rieder B (1986) Temperature, cyanide and phenolic nitrification inhibition. Water Res 20:633–642
29. Radniecki TS (2005) Physiological and transcriptional responses of *Nitrosococcus mobilis* to nitrification inhibitors. Yale University
30. Sirianuntapiboon S, Chuamkaew C (2007) Packed cage rotating biological contactor system for treatment of cyanide wastewater. Bioresour Technol 98:266–272
31. Stratton FE, McCarty PL (1967) Prediction of nitrification effects in the dissolved oxygen balance of streams. Environ Sci Technol 1:405–410
32. Tchobanoglous G, Burton FL, Stensel HD (2003) Wastewater engineering: treatment and reuse. McGraw-Hill, New York
33. Wild SR, Rudd T, Neller A (1994) Fate and effects of cyanide during wastewater treatment processes. Sci Total Environ 156:93–107
34. Zwietering MH, Jongenburger I, Rombouts FM, Vantriet K (1990) Modeling of the bacterial-growth curve. Appl Environ Microbiol 56:1875–1881