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Feasibility of bioremediation of trichloroethylene contaminated sites by nitrifying bacteria through cometabolism with ammonia

Lei Yang [a,*], Yu-Feng Chang [b], Ming-Shean Chou [c]

[a] *Department of Marine Environment and Engineering, National Sun Yat-sen University, Kaohsiung 804, Taiwan*

[b] *Environmental Science, Taipei 114, Taiwan*

[c] *Institute of Environmental Engineering, National Sun Yat-sen University, Kaohsiung 804, Taiwan*

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Abstract

The autotrophic ammonia-oxidizing bacteria (*Nitrosomonas* sp.) are able to dechlorinate trichloroethylene (TCE) through cometabolism using ammonia (NH_3) as a growth substrate. Cometabolic kinetics models suggest that TCE is a potent competitive inhibitor of NH_3 oxidation because it competes with NH_3 for oxidation by the enzyme of ammonia monooxygenase (AMO). In this study, an enriched culture of nitrifying bacteria was used to investigate the efficiencies of cometabolism of TCE by AMO. In addition, the relationships among specific growth substrate (NH_3) utilization rate (qNH_3), specific nongrowth substrate (TCE) cometabolic rate (qTCE), NH_3 and TCE concentrations, and NH_3/TCE and TCE/NH_3 ratios were also analyzed. We found that the relationships between qNH_3 and NH_3 for the systems with and without TCE followed the Alvarez–Cohen competitive inhibition model and Monod model, respectively. Our results demonstrate that TCE could be cometabolized in a nitrification system when sufficient oxygen and NH_3 were supplied. In addition, the high levels of TCE (> 200 $\mu g/l$) were also found to show inhibitory effects towards NH_3 oxidation in enriched nitrifying culture. We also found that the NH_3/TCE ratio rather than TCE concentrations alone exhibited strong correlation with qNH_3, much the same as the Ely activity recovery model presented. Our results suggest that the relationship between qTCE and TCE concentrations followed the Oldenhuis enzyme inactivation model for systems without NH_3.

Keywords: *Nitrosomonas* sp.; Trichloroethylene; Cometabolism; Ammonia monooxygenase; Competitive inhibition; Enriched culture; Monod model; Alvarez–Cohen model; Oldenhuis model; Ely model

* Corresponding author. Fax: +886-7-5255068.

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1. Introduction

Trichloroethylene (TCE) are used widely in industry and laundry as one of the solvents for degreasing and dry cleaning. TCE migrates quickly in groundwater and exhibits slow breakdown rates in the subsurface. Because of these factors, TCE is the most widely distributed organic groundwater and soil pollutant in the USA [1,2]. According to a report from USEPA, this chlorinated hydrocarbon is also the most prevalent hazardous organic compound at 246 out of 1035 Superfund Sites [3]. Due to its suspected carcinogenicity and toxicity [4], an extensive study of its fate in the environment is necessary to reduce the risk to human health and to be able to apply remediation techniques successfully.

A variety of biological treatment technologies for the destruction of chlorinated hydrocarbons, such as TCE, are now under development. Microorganisms that can grow on TCE as a sole carbon or energy source have not yet been isolated. However, several physiologically diverse types of bacteria can cometabolically dechlorinate and then partially or fully degrade TCE. For instance, Vogel and McCarty [5] found that TCE could be cometabolically transformed when used as a nongrowth-supporting electron acceptor by methanogens under anaerobic conditions, while Wilson and Wilson [6] found that methanotrophic bacteria could cometabolize TCE aerobically by means of the enzyme methane monooxygenase (MMO) when methane was supplied as a primary substrate. Recently, several studies [4,7–12] have indicated that chlorinated aliphatic compounds, including TCE, can be cometabolized by nitrifying bacteria (*Nitrosomonas europaea*). It was found that the enzyme ammonia monooxygenase (AMO) was involved in these reactions. Usually, aerobic cometabolic processes are preferred over anaerobic ones because TCE can be mineralized to CO_2, H_2O and Cl^- without the accumulation of stable and carcinogenic intermediates such as vinyl chloride [5]. However, due to the low solubility of methane gas, it is difficult to handle this primary substrate for methanotrophs in cometabolism of TCE. Thus, using the more soluble of ammonia/ammonium instead of methane as a primary substrate for nitrifying bacteria may increase the operation efficiencies in in situ bioremediation of TCE.

Hyman et al. [12] reported that there were three factors which might influence the maximal sustainable rate of TCE degradation in cometabolism with ammonia (NH_3) oxidation by *N. europaea*. These are the competition between growth substrate (NH_3) and nongrowth substrate (TCE) for oxidation by AMO, the toxicity associated with TCE oxidation which would result in a loss of AMO activity, and the ability of cells to recover from the damage caused by such toxicity, respectively [12]. Ensley [13] thought that the simultaneous presence of the two substrates for the same enzyme would result in a competitive inhibition of the oxidation of the primary growth-supporting substrate (NH_3). Ely et al. [11] have developed a kinetics model for TCE cometabolism by *N. europaea*. The model solution showed TCE to be a competitive inhibitor of NH_3 oxidation, with TCE affinity for AMO being about four times greater than that of NH_3 for the enzyme. The extent of competitive inhibition of NH_3 oxidation by TCE determined the relative amounts of both NH_3 and TCE oxidation [12]. As to the factor of toxicity, it has been suggested that this toxicity is caused by a reaction of AMO with short-lived reactive intermediates generated during TCE oxidation [14]. It was also

observed that such an inactivating effects on AMO activity resulting from the toxicity associated with TCE oxidation could be recovered in a process requiring de novo protein synthesis [15]. However, no mixed or enriched culture of nitrifying bacteria has been studied for TCE cometabolism. The purpose of this study was to use an enriched nitrifying culture, instead of a pure culture, to investigate the cometabolic efficiencies of TCE by AMO under different environmental conditions. In addition, the present study has investigated effects of competitive inhibition on both NH_3 and TCE oxidation through the analysis of kinetics by using parameters of both specific growth and nongrowth substrates utilization rates.

2. Materials and methods

2.1. Seeding and culturing an enriched nitrification system

An enriched stock nitrifying culture was used throughout. This stock culture was supplied with a solution containing ammonia sulfate [$(NH_4)_2SO_4$, 3.3 g/l], sodium bicarbonate ($NaHCO_3$, 7.5 g/l) and other trace nutrients ($MgSO_4$, 40 mg/l; $FeCl_3$, 0.4 mg/l; KH_2PO_4, 83.3 mg/l; $CuSO_4$, 0.2 mg/l) in a chemostat culturing system under a flow rate of 6.55 l/day. Due to large amounts of H^+ produced during nitrification, which would decrease the pH values, the pH inside the chemostat culture was controlled at 8.0 by a pH controller. The original seeding of nitrifying bacteria was sampled from the Kaohsiung City Human Body Waste Treatment Plant in Taiwan. The concentrations of biomass (expressed as mixed liquor volatile suspended solids, MLVSS) and nitrification oxygen uptake rate (NOUR, mg O_2/l h) were measured weekly to check the growth rates and activities of the nitrifying bacteria. When the value of NOUR was held constant in the range of 80–100 mg O_2/l h, it meant that the culture had reached a steady state. The culture was then ready to be used as stock nitrifying bacteria to run for the tests of this study.

2.2. Experimental procedures

When the enriched culture of nitrifying bacteria was at a steady state and the NOUR was up to the values that we expected, the batch tests of cometabolism of TCE by AMO were begun. The mixed liquor of nitrifying bacteria taken form the stock chemostat system was first rinsed by deionized (DI) water at least three times to remove the interfering materials. The rinsed nitrifying sludge was then mixed with the nutritious water aerated with air for dilution. The nutritious water was made from DI water, plus $MgSO_4$ (0.09 g/l), $CaSO_4$ (0.034 g/l), $FeSO_4$ (0.272 mg/l), KH_2PO_4 (0.1 g/l), $NaHCO_3$ (0.4 g/l) and $CuSO_4$ (0.16 mg/l). Three different experiments divided into 18 test runs were run in this study based on three different ways of supplying oxygen. For test runs 1–4, the rinsed and diluted nitrifying sludge sample was put into a 300-ml BOD bottle, and then different amounts of NH_3 and TCE were added to fill up the bottle and cover by water seal. The BOD bottle was then immediately put into a constant temperature tank maintained at 20°C to let the cometabolism occur in a quiet condition

(i.e., without shaking). The samples would be taken from the bottle daily for analyzing the concentrations of TCE and NH_3 changed with time. In test runs 5–10, the rinsed and diluted nitrifying bacteria sample was put into a 150-ml brown bottle to decrease the interference of light towards nitrification [17], and then it was filled up with different amounts of NH_3 and TCE. The solution inside the bottle had been pre-aerated by pure oxygen to increase its DO. The bottle was then covered (with a Teflon stopper) and placed in a shaking table with a shaking speed of 150 rpm to keep the sample completely mixed. The other experimental procedures were the same as the first test run set. In test runs 11–18, the experimental procedures were the same as the second test run set except for the final volume filled inside the bottle. The final volume was controlled at 125 ml, and thus 25 ml head space was left inside the bottle to make sure that enough oxygen was transferred into the liquid phase. A blank test was run as a control in both of the second and third test run sets by adding allythiourea (ATU), which could show 75% inhibitory effects to nitrification with a dose as low as 0.075 mg/l [18]. The amounts of nitrifying biomass (expressed as MLVSS) and initially applied concentrations of NH_3 and TCE controlled in each test run are summarized in Table 1.

2.3. Analytical methods

NOUR was measured using the Gilson Model 5/6H Oxygraph respirometer. The biomass concentrations of nitrifying bacteria expressed as MLVSS were measured by the method shown in the Standard Methods (209D) [16]. The concentrations of NO_2^- and NO_3^- were determined by using ''TOA ICA-5000 System'' Ion Chromatography (IC), while NH_3 was measured using the Nesslerization method with a spectrophotom-

Table 1
Controlling conditions for each test

Test run no.	Biomass (mg VSS/l)	NH_3–N (mg N/l)	TCE (μg/l)
1	4.1	4.25	0
2	4.1	4.50	0
3	4.1	0	94.6
4	6.3	0	102
5	28.7	16.8	0
6	28.7	0	141
7	28.7	1.5	151
8	28.7	16.8	142
9	28.7	38.0	140
10	0	15.0	138
11	36.8	17.4	0
12	36.8	19.7	125
13	36.8	19.2	191
14	36.8	18.8	365
15	65.4	12.8	0
16	65.4	12.2	138
17	105.5	12.7	130
18	141.2	12.4	131

eter (HACH, DR-2000) [16]. The concentrations of TCE were analyzed using the equipment of GC-FID (Shimadzu, GC-14A) with the samples pretreated through a purge-and-trap system (Tekmar, Purge and Trap Concentrator-2000). The operation conditions controlled for GC-FID were as follows: Column type: 60/80 Carbopack B/1% SP™-1000 (8′ × 1/8″ OD Stainless steel column); H_2 pressure: 0.6 kg/cm^2; air pressure: 0.5 kg/cm^2; N_2 pressure: 5 kg/cm^2; temp. increasing program: 40°C–220°C (8°C/min); inj. temp.: 150°C; det. temp.: 250°C.

3. Results and discussion

3.1. Cometabolic efficiencies of TCE by enriched nitrifying culture

The experiments in test run nos. 1, 2, 3, 4, 5, 6, 11 and 15 were designed as controls for the nitrifying system with growth (NH_3) and nongrowth substrates (TCE) only. The experimental results are shown in Figs. 1 and 2. According to Fig. 1, we found that NH_3 was not completely oxidized in test run nos. 1, 2 and 5, while complete nitrification was observed in test run nos. 11 and 15 for the systems with NH_3 supplied only. This was because the method used to supply oxygen to the system could not provide sufficient oxygen to the nitrifying bacteria for nitrification in test run nos. 1, 2 and 5 (no head space left). However, in test run nos. 11 and 15, due to sufficient oxygen supplied to the systems through the head space inside the bottles plus shaking, it took only 1 day to completely oxidize NH_3. This result demonstrated that the activities of the enriched nitrifying bacteria used in this study were high. As seen from Fig. 2, we observed that TCE also could not be co-oxidized completely under these three initially applied concentrations of TCE for the systems without NH_3 added. The reasons might be the insufficient oxygen supplied, or the inhibitory effects of TCE to AMO, which needed further study. The experiments in the test run nos. 7, 8 and 9 were designed to compare the efficiencies of cometabolism under the conditions of same biomass concentration (28.7 mg VSS/l) and initial TCE level (150 μg/l) but different initial NH_3 concentra-

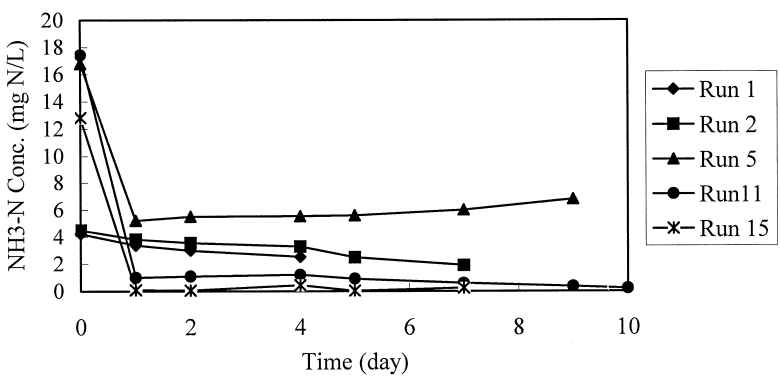

Fig. 1. The variations of NH_3 with time in the systems without TCE.

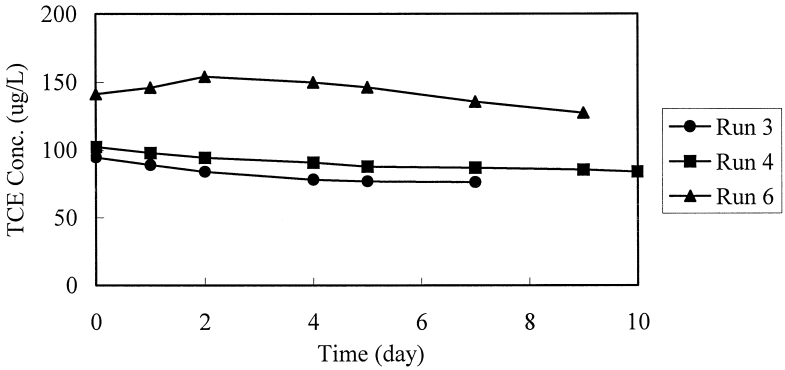

Fig. 2. The variations of TCE with time in the systems without NH_3.

tions. Test run no. 10 was employed as a control test with no nitrifying bacteria added into the test bottle. The experimental results are shown in Figs. 3 and 4. According to Fig. 3, the parts of control tests shows that less amounts of TCE were lost in all the three systems [run nos. 6, 6(A) and 10]. This means that most of the TCE removed in the test run nos. 7, 8 and 9 was due to the effects of cometabolism with NH_3. However, according to Figs. 3 and 4, we also found that both of the TCE and NH_3 oxidations were not complete even after 9-days running in the test applied with higher NH_3 concentrations (38.0 mg N/l). The reasons might be due to TCE-mediated inactivation to AMO, or to deficiency of DO in the test bottle. Thus, more tests were needed with improved efficiencies of oxygen supplied to the systems.

In the experiments during test run nos. 11–14, 25 ml head space was left inside the bottle to make sure that sufficient oxygen was supplied to the culture during the test run. The experimental results are shown in Figs. 5 and 6. According to Fig. 5, the control tests, which were achieved by adding ATU into the systems, revealed that less amounts of the TCE were removed from the systems due to the effects of evaporation and

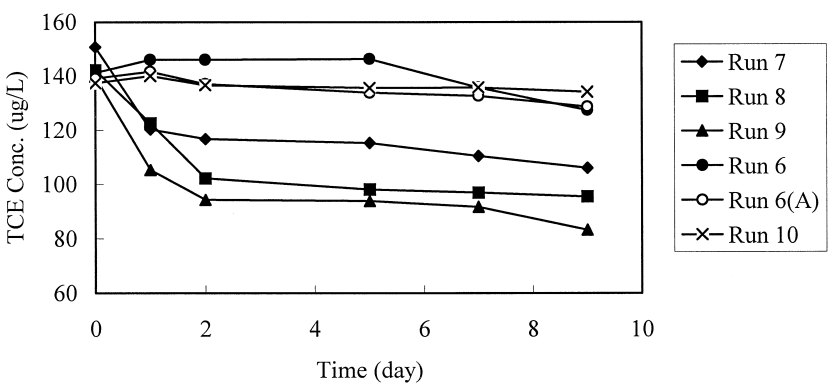

Fig. 3. The variations of TCE with time in the systems with both NH_3 and TCE existed and biomass of 28.7 mg VSS/l (A: adding ATU).

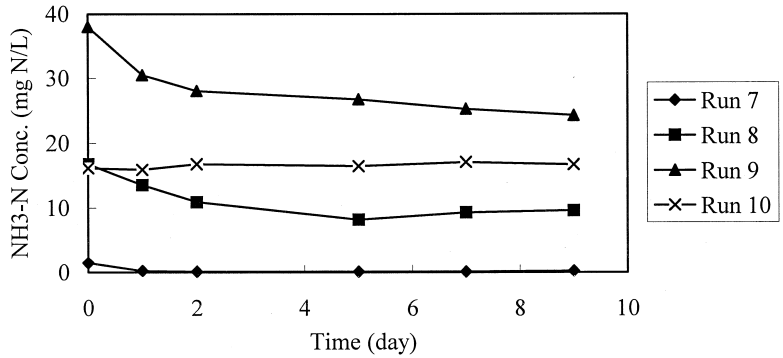

Fig. 4. The variations of NH_3 with time in the systems with both NH_3 and TCE existed and biomass of 28.7 mg VSS/l.

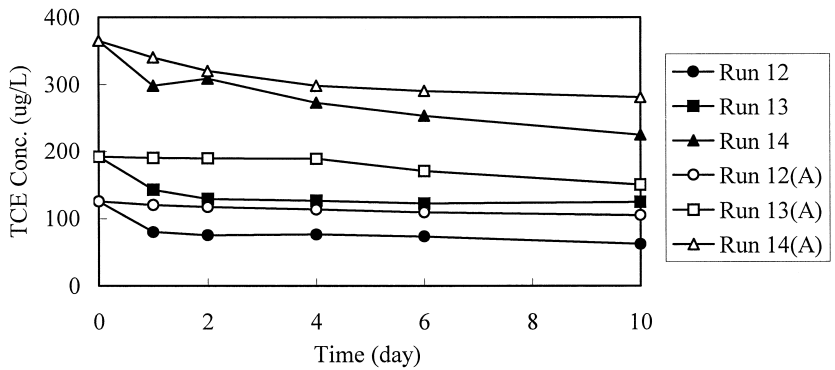

Fig. 5. The variations of TCE with time in the systems with both NH_3 and TCE existed and biomass of 36.8 mg VSS/l (A: adding ATU).

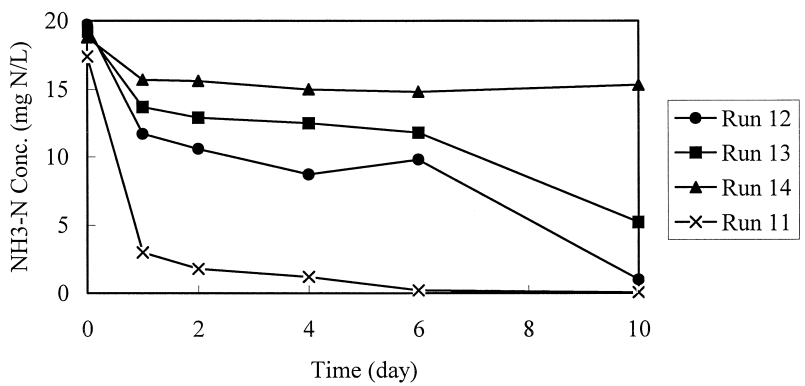

Fig. 6. The variations of NH_3 with time in the systems with both NH_3 and TCE existed and biomass of 36.8 mg VSS/l.

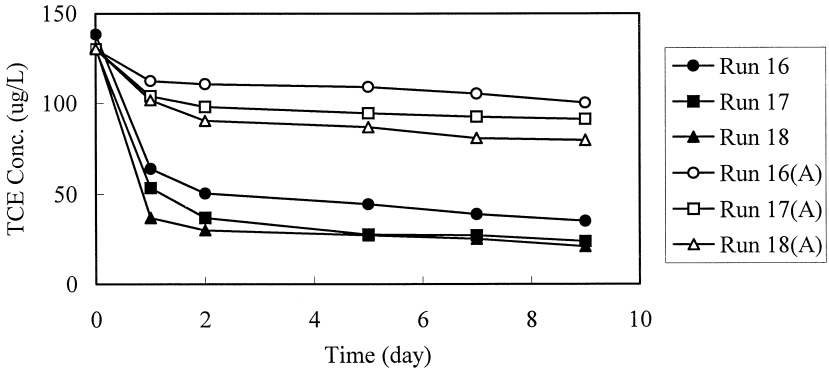

Fig. 7. The variations of TCE with time in the systems with both NH_3 and TCE existed at same levels (A: adding ATU).

adsorption. As seen from the same figure, the initial removal rates of TCE were similar for all three systems with initial TCE concentrations of 125, 191 and 365 μg/l, respectively, while the amounts of NH_3 oxidation were increased with decreased TCE levels initially applied to the system, as shown in Fig. 6. This figure also demonstrated that the ''head space'' culturing systems could supply sufficient oxygen resulting in a complete nitrification occurred in the system with nitrifying bacteria and NH_3 only. Hence, the reason of the incomplete TCE cometabolism and NH_3 oxidation found in the test runs 7, 8 and 9 was not the inhibitory effect of TCE to AMO, but due to less oxygen supplied to the system. It was thus concluded that the minimum level of TCE showing inhibition to nitrification was at least over 200 μg/l.

In the test run nos. 15, 17 and 18, the experiments were operated under same NH_3 and TCE levels (12 mg N/l and 140 μg/l, respectively) but different biomass concentrations (65.4, 105.5 and 141.2 mg VSS/l, respectively). The purpose of this set of test runs was to study the effects of nitrifying biomass concentrations on the cometabolic efficiencies of TCE by AMO. The experimental results are shown in Figs. 7 and 8. According to Fig. 7, the amounts of TCE removed by cometabolism were greater

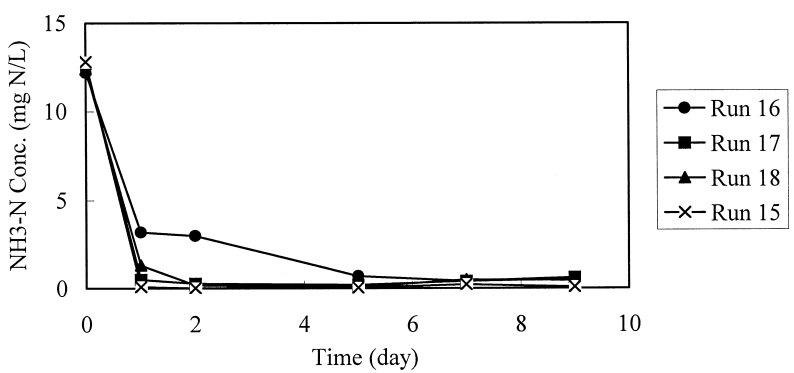

Fig. 8. The variations of NH_3 with time in the systems with both NH_3 and TCE existed at same levels.

than the other two sets of test runs after deducting the amounts of loss through evaporation and adsorption as learned from the control tests (i.e. adding ATU to the systems) shown in the same figure. Also, we found that the higher the concentrations of biomass were applied to the systems, the larger the amounts of TCE that were cometabolized. The reasons might be that the amounts of cometabolic enzyme of AMO generated by *Nitrosomonas* sp. were increased with the nitrifying biomass concentrations. On the other hand, according to Fig. 8, we found that TCE did not show inhibitory effects because of NH_3 oxidation in this set of test runs, in which we found that over 80% of NH_3 were removed in only 1-day running for all these three tests. According to the experimental results achieved in test run nos. 8, 12, 16, 17 and 18, which were run under similar TCE and NH_3 levels but different biomass concentrations, we concluded that the removal efficiencies of TCE through cometabolism by AMO could be increased by increasing the amounts of nitrifying bacteria in the treatment systems. As seen from Fig. 6, we also observed that the less nitrifying biomass (36.8 mg VSS/l) needed the longer lag time (about 10 days) for the nitrifying bacteria to oxidize NH_3 even with sufficient oxygen supplied in systems, in which the TCE levels were controlled at 125 and 200 μg/l, respectively. However, the lag time was decreased down to 1 day when the concentrations of nitrifying biomass were increased over 65.4 mg VSS/l as shown in Fig. 8. Therefore, the amounts of nitrifying biomass applied were also an important factor in the NH_3–TCE cometabolic systems. Hence, the specific NH_3 metabolic (dNH_3/dt/biomass) and TCE cometabolic rates (dTCE/dt/biomass) instead of NH_3 metabolic (dNH_3/dt) and TCE cometabolic rates ($dTCE/dt$) would be useful as parameters for kinetic analysis.

3.2. Kinetic analysis of cometabolism between NH_3 and TCE by AMO

Using the experimental results run in each test shown in Table 1, the specific growth substrate (NH_3) utilization rates (qNH_3) and nongrowth substrate (TCE) co-oxidation rates (qTCE) were calculated by dividing the initial NH_3 utilization and TCE co-oxidation rates by the concentrations of nitrifying biomass and are shown in Table 2.

Through regression analysis, the relationships between qNH_3 and initially applied NH_3 concentrations are expressed graphically in Fig. 9. As seen from this figure, for both conditions with and without TCE existing in the systems, qNH_3 presented a positive linear relationship against NH_3 concentrations with correlation coefficients (R^2) equal to 0.7102 and 0.8749, respectively. According to the Monod model, the equation is expressed as:

$$(dS/dt)/X = (kS)/(K_m + S) \quad (1)$$

where dS/dt is the utilization rate of the growth substrate (NH_3), X is the biomass concentration, S is the growth substrate concentration, k is the maximum specific utilization rate of the growth substrate, K_m is the half-saturation constant of the growth substrate, and $(dS/dt)/X$ is the specific utilization rate of the growth substrate (i.e. qNH_3). When S is much smaller than K_m, qNH_3 is proportional to S (i.e. NH_3 concentrations) linearly, which coincides with the regression result without TCE as

Table 2
Measurements of qNH_3 and qTCE at each condition

Test no.	dNH_3/dt (mg/l day)	$dTCE/dt$ (μg/l day)	VSS (mg)	qNH_3 (mg/mg VSS day)	qTCE (μg/mg VSS day)	qNH_3/qTCE
1	0.15	–	4.1	0.04 (no TCE)	–	–
2	0.68	–	4.1	0.16 (no TCE)	–	–
5	12.7	–	28.7	0.40 (no TCE)	–	–
11	17.4	–	36.8	0.47 (no TCE)	–	–
15	12.7	–	65.4	0.19 (no TCE)	–	–
3	–	4.08	4.1	–	0.99 (no NH_3)	–
4	–	4.00	6.3	–	0.63 (no NH_3)	–
6	–	3.44	28.7	–	0.12 (no NH_3)	–
7	1.24	28.5	28.7	0.04	0.99	0.04
8	3.20	17.7	28.7	0.11	0.62	0.18
9	7.50	32.5	28.7	0.26	1.13	0.23
12	8.00	42.4	36.8	0.22	1.15	0.19
13	5.50	48.0	36.8	0.15	1.30	0.12
14	3.10	50.0	36.8	0.08	1.36	0.06
16	9.00	57.0	65.4	0.14	0.87	0.16
17	12.2	51.0	105.4	0.12	0.48	0.24
18	11.1	66.0	141.2	0.08	0.46	0.17

shown in Fig. 9. However, for a competitive inhibition model in cometabolism, the Alvarez–Cohen models are expressed as [19]:

$$(dS/dt)/X = (kS)/\left[K_m(1 + IK_I) + S\right] \quad (2)$$

and

$$(dI/dt)/X = (k_I I)/\left[K_I(1 + S/K_m) + I\right] \quad (3)$$

where dI/dt is the utilization rate of the nongrowth substrate (TCE), I is the nongrowth substrate (i.e., competitive inhibitor) concentration, k_I is the maximum specific utilization rate of the nongrowth substrate, K_I is the half-saturation constant of the nongrowth substrate, and $(dI/dt)/X$ is the specific utilization rate of the nongrowth substrate (i.e. qTCE). Therefore, according to Eq. (2), qNH_3 is varied with both NH_3 and TCE concentrations for the systems where NH_3 and TCE coexisted. However, when I is much smaller than K_I, Eq. (2) is similar than Eq. (1). According to Ely et al. [4], the estimated K_I was about 10.7 μM TCE, or 1.4 mg/l TCE when using pure cultures of NH_3-oxidizing bacteria, *N. europaea*, to degrade TCE cometabolically in the presence of NH_3. The concentrations of TCE used in this study were in a range between 94 and 365 μg/l, which are much smaller than the K_I value mentioned previously. Hence, qNH_3 is still presented linearly against NH_3 for the systems with TCE as shown in Fig. 9.

According to Fig. 9, the K value (i.e. k/K_m, the slope of the line), which is the kinetic constant for the enriched nitrifying culture of this study, was equal to about 0.0231 for the systems without adding TCE. This number is approximately three times higher than the one ($K = 0.0079$) for the systems with TCE applied. However, Ely et al. [4] also found that TCE had about four times greater affinity than NH_3 for AMO. Thus,

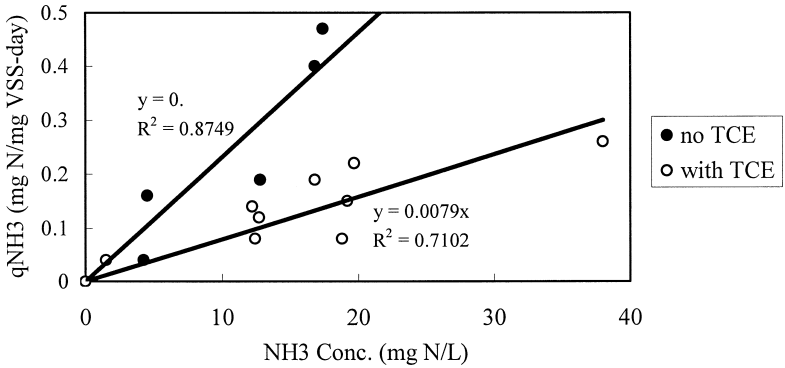

Fig. 9. Relationships between qNH_3 and NH_3 concentrations in the systems with and without TCE.

it is demonstrated that TCE is a competitive inhibitor for the enriched cultures of NH_3-oxidizing bacteria of this study against NH_3.

Through regression analysis, the relationship between qTCE and the initially applied TCE concentrations is presented graphically in Fig. 10. As seen from this figure, the relationships between qTCE and TCE concentrations for the conditions with and without NH_3 added into the enriched nitrifying systems are quite different. For the batch cultures without adding NH_3, a strong negative linear relationship between qTCE and TCE concentrations is observed with R^2 equal to 0.926. Oldenhuis et al. [20] have developed the following model for enzyme inactivation resulting from product toxicity:

$$V_{\max,t} = V_{\max,0} - p(S_a - S_t) \tag{4}$$

where $V_{max,0}$ and $V_{max,t}$ represent the activity of the cells at time points 0 and t, respectively, S_a is the total amount of substrate added, S_t is the amount of substrate left at time t, and p is the inactivation constant. Those researchers used pure methan-

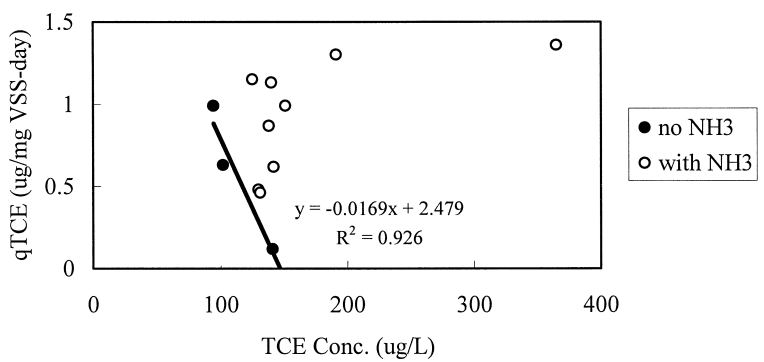

Fig. 10. Relationships between qTCE and TCE concentrations in the systems with and without NH_3.

otrophic cultures of *Methylosinus trichosporium* OB3b to run their enzyme inactivation model based on TCE degradation activity and restated the model as follows:

$$(\mathrm{d}I/\mathrm{d}t)/X = [(\mathrm{d}I/\mathrm{d}t)/X]_0 - p(I - I_0) \quad (5)$$

where I_0 denotes the initial applied concentration of the nongrowth substrate (TCE) and $[(\mathrm{d}I/\mathrm{d}t)/X]_0$ is the specific utilization rate of the nongrowth substrate (qTCE) under I_0. The other terms are defined as in Eqs. (3) and (4). As mentioned previously, the regression result between qTCE and TCE for systems without adding NH_3 shown in Fig. 10 is negatively linear, which agrees with the model presented in Eq. (5). According to Fig. 10, the inactivation constant p is determined to be 0.0169 (μg TCE/mg VSS day/μg TCE/l). The value of $[(\mathrm{d}I/\mathrm{d}t)/X]_0$ is thus calculated to be about 2.48 μg TCE/mg VSS day when I_0 is equal to 0. In addition, qTCE will be decreased to 0 when the concentration of TCE is increased to about 150 μg/l for the enriched nitrifying cultures without adding NH_3. This result following the Oldenhuis model needs to be demonstrated further by increasing the TCE levels in future studies. Actually, the value of R^2 (0.9963) by using multiple regression analysis is higher than that ($R^2 = 0.9260$) of using linear regression analysis with an empirical equation of $\mathrm{qTCE} = 2 \times 10^8(\mathrm{TCE})^{-4.23}$, which means that qTCE is near to zero when the concentration of TCE is unlimited large. This conclusion is different from that drawn by Oldenhuis et al. [20]. On the other hand, according to Eq. (2), when $S(NH_3)$ is greatly smaller than K_m, this equation can be simplified as follows:

$$(\mathrm{d}I/\mathrm{d}t)/X = (k_I I)/(K_I + I) \quad (6)$$

This equation now looks like the Monod model. According to Fig. 10, the relationship between qTCE and TCE for the systems in which both NH_3 and TCE co-existed also looks like following the Monod model presented in Eq. (6). Thus, this result can be explained by using the Alvarez–Cohen model.

These experimental results are also consistent with the findings in the study of Hyman et al. [12]. They found that the extent of TCE co-oxidation by the enzyme of AMO generated by *Nitrosomonas* sp. might be depressed by TCE itself due to the short-lived reactive intermediates produced during TCE oxidation, which showed toxicity to AMO. Therefore, the higher the TCE concentrations in the batch nitrifying culture, the more intermediates were generated during TCE cometabolism. These intermediates intensely reacted with AMO resulting in less active AMO available to continuously catalyze the TCE cometabolism which would make the qTCE values decreased. However, for the system to which both NH_3 and TCE co-existed, we found that NH_3, which served as a growth substrate for nitrifying bacteria, might be helpful to release the toxicity of the intermediate generated during TCE cometabolism to AMO to make qTCE increased with TCE concentrations at low TCE levels (< 200 μg/l). When the concentration of TCE was increased up to about 200 μg/l, the recovery ability of the enzyme of AMO seemed not to increase continuously even though NH_3 is cometabolized with TCE in the system that caused qTCE to be kept stable (as shown in Fig. 10).

The results studied by Ely et al. [21] showed that growth and nongrowth substrate utilization rates depend on each other and that they may be related by a constant called the first-order reaction rates ratio as follows:

$$\mathrm{d}S/\mathrm{d}t = \varepsilon(S/I)(\mathrm{d}I/\mathrm{d}t) \text{ or } \mathrm{d}S/\mathrm{d}I = \varepsilon(S/I) \quad (7)$$

where ε is the first-order reaction rates ratio [equal to $(k/K_m)/(k_I/K_I)$]. Thus, in order to investigate if NH_3 (S) and TCE (I) presented a co-effect on both qNH_3 and qTCE as mentioned in Eq. (7), the relationships among qNH_3, qTCE, qNH_3/qTCE (i.e. dS/dI), NH_3/TCE and TCE/NH_3 ratios were studied through regression analysis. According to the regression analytical results, we found that only the relationship between qNH_3 and NH_3/TCE is significantly linear learned by its R^2 values equal to 0.8374 as shown in Fig. 11. According to Eq. (7), this equation can be restated as follows:

$$(dS/dt)/X = [\varepsilon(dI/dt)/X](S/I) \tag{8}$$

i.e.,

$$qNH_3 = (\varepsilon\, qTCE)(NH_3/TCE) \tag{9}$$

If qTCE is not changed significantly, ε qTCE can be regarded as a constant, which means that the relationship between qNH_3 and NH_3/TCE is positively linear as shown in Fig. 11. In addition, we can restate Eq. (9) as follows:

$$qTCE = (qNH_3/\varepsilon)(TCE/NH_3) \tag{10}$$

As mentioned previously, if the variations of qNH_3 is not significantly changing, qNH_3/ε can also be regarded as a constant, which means that the relationship between qTCE and TCE/NH_3 is linear. However, the regression analytical result shows that this relationship is not linear with a low R^2 value (0.0207). The variabilities of qNH_3 and qTCE were also analyzed in this study. According to the statistic analytical results, the values of coefficient of variation for qNH_3 and qTCE are measured to be equal to 36% and 52%, respectively, which means that qNH_3 is relatively less variable than qTCE. That was the reason why we found that the specific NH_3 utilization rates of nitrifying bacteria (qNH_3) exhibited positive linear relationship with the factor of NH_3/TCE ratio (Fig. 11). It was inferred that the inactivation of NH_3 oxidizing activity by high levels of TCE might be recovered with increasing NH_3 concentration. The reasons could be that a relatively high NH_3 concentration to TCE concentration might increase the competitive ability of NH_3 against TCE for the enzyme of AMO [12]. In another words, when TCE

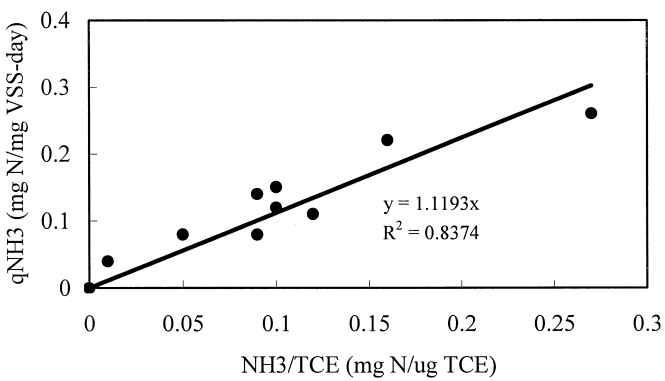

Fig. 11. Relationship between qNH_3 and NH_3/TCE in the systems with both NH_3 and TCE existed.

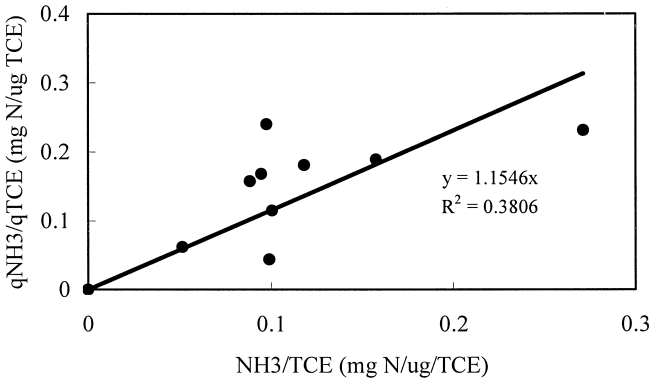

Fig. 12. Relationship between qNH_3/qTCE and NH_3/TCE in the systems with both NH_3 and TCE existed.

was relatively high to NH_3 concentration, TCE might exhibit strong competitive ability for AMO against NH_3 to make qNH_3 decreased gradually [11]. However, as seen from Fig. 12, the linear relationship between qNH_3/qTCE and NH_3/TCE does not follow the model presented in Eq. (7) as shown by its R^2 value of 0.3806. Nevertheless, according to this equation, the value of ε is measured to be about 1.15. This number is quite smaller than that ($\varepsilon = 20$) measured by Ely et al. [4], who used pure cultures of NH_3-oxidizing bacteria (*N. europaea*) in their study.

4. Conclusions

During this study TCE was found capable of being transformed biologically through cometabolism by the enzyme of AMO generated by *Nitrosomonas* sp. in the enriched batch culture. The environmental factor of oxygen was found to be important in the cometabolism of TCE by AMO, for which an oxygen deficiency would limit both NH_3 and TCE oxidation. However, when sufficient oxygen was provided, the systems applied with TCE concentrations of 125,200 and 350 μg/l still presented incomplete oxidation of TCE, while the NH_3 oxidation became significant only in the nitrifying systems maintained at low TCE levels of 125 and 200 μg/l with a lag period of 6 days. A high TCE level of 350 μg/l still showed inhibition to both TCE and NH_3 oxidation even when supplied with sufficient oxygen. The increase of concentrations of biomass of nitrifying bacteria could not only decrease the lag time down to 1 day for NH_3 oxidation by *Nitrosomonas* sp. in a nitrifying culture applied with TCE (150 μg/l), but also could increase the oxidation efficiency of TCE up to 80%. The results of kinetic analysis show that for both conditions with and without adding TCE, the specific NH_3 utilization rate (qNH_3) presents positive linear relationships against NH_3 concentration coinciding with the Monod model. For the systems without NH_3, we found that the relationship between specific TCE cometabolic rate (qTCE) and TCE concentration was negatively linear, which is coincident with the Oldenhuis enzyme inactivation model that p (inactivation constant) is measured to be 0.0169 (μg TCE/mg VSS day/μg TCE/l). In addition, we

found that the relationship between qTCE and TCE concentration for the systems with NH_3 also followed the model similar to the Monod model when S (NH_3) was much smaller than K_m in the Alvarez–Cohen competitive inhibition model. We also found that $qNH_3/qTCE$ would increase with NH_3/TCE linearly, which followed the Ely activity recovery model for which ε (first-order reaction rates ratio) was measured to be about 1.15. It was inferred that large amounts of NH_3 might enhance the recovery ability of AMO from the toxicity of the uncertain intermediate generated during TCE oxidation. Therefore, in order to increase the amounts of TCE removed through cometabolism by AMO, we suggest that some amounts of NH_3 should be maintained in the system to keep the enzyme of AMO active to cometabolize TCE.

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