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Kinetic analysis of the inhibitory effect of trichloroethylene (TCE) on nitrification in cometabolic degradation

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Abstract

In this study, the inhibitory effect of TCE on nitrification process was investigated with an enriched nitrifier culture. TCE was found to be a competitive inhibitor of ammonia oxidation and the inhibition constant (K_I) was determined as 666–802 μg/l. The TCE affinity for the AMO enzyme was significantly higher than ammonium. The effect of TCE on ammonium utilization was evaluated with linearized plots of Monod equation (e.g., Lineweaver–Burk, Hanes–Woolf and Eadie–Hofstee plots) and non-linear least square regression (NLSR). No significant differences were found among these data evaluation methods in terms of kinetic parameters obtained.

Abbreviations: I: – inhibitory (non-growth) substrate concentration, μg non-growth substrate/l; K_I: – inhibition constant of inhibitory (non-growth) substrate, μg non-growth substrate/l; K_{iu}: – dissociation constant of enzyme-substrate-inhibitory substance (ESI) complex, μg non-growth substrate/l; K_S^{app}: – apparent half-saturation constant for growth substrate, mg substrate/l; K_S: – half-saturation constant for growth substrate, mg growth substrate/l; q: – specific substrate utilization rate, mg substrate/g VSS h; $q_{NH_4\text{-}N}$: – specific ammonium utilization rate, mg NH_4-N/g VSS h; q_{max}: – maximum specific substrate utilization rate, mg substrate/g VSS h; $q_{max,\ NH_4\text{-}N}$: – maximum specific ammonium utilization rate, mg NH_4-N/g VSS h; $q^{app}_{max,\ NH_4\text{-}N}$: – apparent maximum specific ammonium utilization rate, mg NH_4-N/g VSS h

Introduction

Trichloroethylene (TCE) is widely used in various industrial processes (e.g., degreasing of fabricated metal parts, industrial dry-cleaning, textile manufacturing). It is among the most prevalent hazardous organic compounds present in groundwater and soil. Its widespread occurrence in soil and subsurface media is an important environmental issue due to carcinogenic and other serious health effects on humans (ATSDR 1997) and it is therefore strictly regulated.

Currently existing conventional treatment technologies, such as air stripping and activated carbon adsorption, transfer TCE from one medium to another without any significant reduction in volume or toxicity (EPA 1992). Hence, in recent years, biological treatment methods, which provide destruction of TCE, have gained importance. Although no microorganism has been isolated which can grow using TCE as a sole carbon or energy source (Hyman et al. 1995), several anaerobic and aerobic bacteria are known to degrade TCE by cometabolic transformation. Here, a non-growth-supporting substrate is transformed through the catalysis of non-specific enzymes synthesized by bacteria in the presence of a growth substrate. The majority of aerobic bacteria that

have so far been demonstrated to be capable of cometabolic TCE transformation through the catalysis of monooxygenase enzymes are methane oxidizers (Alvarez-Cohen & McCarty 1991; Anderson & McCarty 1997; Chang & Alvarez-Cohen 1995a; Chang & Alvarez-Cohen 1995b; Chu & Alvarez-Cohen 1999; Chu & Alvarez-Cohen 2000; Duba et al. 1996; Eguchi et al. 2001; Ely et al. 1997; Kang et al. 2001; Smith et al. 1997; Speitel & Segar 1995), propane oxidizers (Chang & Alvarez-Cohen 1995b), toluene oxidizers (Chang & Alvarez-Cohen 1995b; Fries et al. 1997; Guo et al. 2001; Sun et al. 1997) and phenol oxidizers (Chang & Alvarez-Cohen 1995b; Nakano et al. 1999; Speitel & Segar 1995). Alternatively, ammonia oxidizers (nitrifiers) are also capable of cometabolic TCE transformation through the catalysis of ammonia monooxygenase (AMO) enzyme. The use of nitrifying bacteria is an attractive solution because these bacteria are ubiquitously present in almost all environments, particularly in natural soils and wastewater treatment plants.

However, up to date, very few studies (Arciero et al. 1989; Ely et al. 1995; Ely et al. 1997; Hyman et al. 1995; Racshe et al. 1991; Yang et al. 1999) have been reported with nitrifiers and most of them were performed with pure *Nitrosomonas europaea* species. Hence, they do not represent a realistic case since in full-scale biological treatment and bioremediation systems pure nitrifying cultures are hardly employed and maintained. The study of Arciero et al. (1989) was the first demonstration that the species *Nitrosomonas europaea* catalyzed TCE degradation. The findings of this study suggested that TCE specifically interacted with the AMO enzyme. In later studies (Ely et al. 1995; Ely et al. 1997; Hyman et al. 1995; Rasche et al. 1991; Yang et al. 1999), TCE dependent inhibition of ammonium oxidation was observed. However, only a few of these studies (e.g., Ely et al. 1995b; Hyman et al. 1995) focused on the kinetics of this inhibitory effect. In the studies of Ely et al. (1995b) and Hyman et al. (1995), TCE was reported to be a potent competitive inhibitor of ammonium oxidation by *Nitrosomonas europaea*.

Additional kinetic studies are necessary to provide a complete understanding of the inhibitory characteristics of TCE on ammonium oxidation by a mixed nitrifying culture. In this study, the kinetics of the inhibitory effect of TCE on nitrification was studied using an enriched nitrifier culture. The inhibition type and inhibition coefficient were determined for a broad range of NH_4-N and TCE using both linearized plots of Monod equation (e.g., Lineweaver–Burk, Hanes–Woolf, Eadie–Hofstee) and non-linear least square regression (NLSR) analysis. In literature, such a comparison of different data analyses methods has not been made in the determination of inhibition type and inhibition constants. Therefore, the findings of this research not only provide information about the kinetics of TCE inhibition on ammonium oxidation but also on comparison of different evaluation methods.

Materials and methods

Enrichment of a nitrifier culture

A mixed liquor was taken from the Istanbul Pasakoy Advanced Biological Sewage Treatment Plant and enriched in terms of nitrifiers in a batch reactor for about 4 months. The culture was daily fed with a stock synthetic feed [37.75 g/l $(NH_4)_2SO_4$, 95 g/l $NaHCO_3$] and a stock mineral [2 g/l $MgSO_4{\cdot}7H_2O$, 0.103 g/l $CaCO_3$, 0.4 g/l $FeSO_4{\cdot}7H_2O$, 0.2 g/l $MnSO_4{\cdot}H_2O$, 0.325 g/l K_2HPO_4] solution based on fill-and-draw principle. All chemicals used in the preparation of stock synthetic feed and mineral solutions were supplied from Merck KGaA., Germany. The initial ammonium (NH_4-N) concentration at the start was always kept around 200 mg/l. The pH was in the range of 7–8. The dissolved oxygen concentration was maintained around 4 mg/l by supplying air at a rate of 5.5 l/min, which also provided complete mixing. The temperature was held in the range of 20–25 °C. The sludge age was kept around 14 days. The enrichment of culture for nitrifiers was monitored through specific ammonium utilization rate ($q_{NH_4\text{-}N}$), which reached a steady value of 20–25 mg NH_4-N/g VSS h at the end of enrichment period as reported previously (Alpaslan Kocamemi & Çeçen 2005). Also after the enrichment period, this stock culture was fed in a same manner as described above.

Sludge samples taken from the stock enriched nitrifier culture immediately after enrichment and seven months later were analyzed by molecular biology techniques. FISH analysis was performed using the NSO 190 oligonucleotide probe to

monitor the *Nitrosomonas* species in this culture (Mertoglu et al. 2005; Mobarry et al. 1996). All microorganisms were visualized by DAPI staining (blue). Denaturing gradient gel electropheresis (DGGE) analyses (Nicolaisen & Ramsing 2002) were performed to screen whether any changes occurred in the diversity of amoA gene sequences 7 months after the enrichment period. The aim was to ensure that all experiments were performed with the same microbial population.

Experimental procedure

Our previous results have shown that both the specific oxygen uptake rate (SOUR) and the specific ammonium utilization rate ($q_{NH_4\text{-}N}$) decreased by 50% in a TCE concentration range of 1000–2000 μg/l (Alpaslan Kocamemi & Çeçen 2005). Therefore, present experiments were conducted at rather low TCE levels ranging from 40 to 845 μg/l. All experiments were performed in 200 ml capped glass bottles and at a constant wastewater temperature of 25 °C. Complete mixing was achieved by magnetic stirring. All experiments were started at the same initial pH value, which was around 8. Throughout the 2 h experimental period, nitrification resulted in a slight pH decrease and the experiments were ended at a pH value around 7. Therefore, all experiments were performed in the same pH range. Since TCE is a highly volatile substance, aeration was not done by diffusers, but the enriched nitrifier culture was initially supersaturated to a DO concentration of 35–40 mg/l with pure O_2 gas. Since the duration of our experiments was too short (maximum 2 h) and the initial NH_4-N/VSS ratio in these experiments was too small, the change in cell concentrations was considered insignificant and the VSS measurement was performed at the end of each experiment.

Experiments in the absence of TCE

The first set experiments (blank experiments) was performed to determine $q_{max,\ NH_4\text{-}N}$ and K_S of the enriched nitrifier culture in the absence of TCE under saturated oxygen conditions. In each experiment, the stock enriched nitrifier culture was rinsed and diluted with deionized water to a MLVSS concentration of 320–510 mg/l. It was then fed with feed and mineral solutions (see Section 2.1) to maintain an initial NH_4-N concentration of 25–400 mg/l. NH_4-N measurements were carried out at certain time intervals. The specific ammonium utilization rate ($q_{NH_4\text{-}N}$) was calculated from the slope of NH_4-N/VSS versus time plottings through linear regression analysis. $q_{NH_4\text{-}N}$ values were further processed with respect to the initial NH_4-N concentrations to determine the apparent $q_{max,\ NH_4\text{-}N}$ and K_S values.

Experiments in the presence of TCE

In each of four experimental sets, the initial NH_4-N ranged from 25 to 400 mg/l at a fixed TCE concentration. The initial TCE was 40, 110, 325, 845 μg/l in the second, third, fourth and fifth sets, respectively. In each experiment, the stock enriched nitrifier culture was rinsed and diluted with deionized water to a MLVSS concentration of 210–530 mg/l. NH_4-N measurements were performed as described in Section 2.2.1.

Analytical methods

NH_4-N concentrations were analyzed using the Nessler Method and the Hach DR/2000 spectrophotometer. The DO concentrations and pH were measured by the WTW OxiLevel-2 DO meter and WTW Inolab-1 pH meter, respectively. VSS analyses were performed using the Method 2540E in Standard Methods (APHA, AWWA, WEF 1998). Liquid samples for TCE analysis were extracted into *n*-pentane by the EPA Method 502.1. The extracts were analyzed by the Hewlett Packard 5890 Gas Chromatograph equipped with a J&W prosteel megabore column (0.53 mm ID, 30 m length) and an electron capture detector (ECD). The chromatograph was operated isothermally at 100 °C oven, 250 °C injection port and 250 °C detector temperatures for 5 min with a nitrogen carrier gas flow of 10 ml/min. Nitrogen gas (extra pure: $>99.99\%$) was obtained from BOS Inc., Turkey. The GC calibration standards were prepared with a TCE solution (200 μg/ml) supplied from Crescent Chemical Company, Inc., New York.

Results and discussions

FISH and DGGE analyses of stock enriched nitrifier culture

FISH analysis of the stock culture (Figure 1) clearly indicated that the dominant species in all

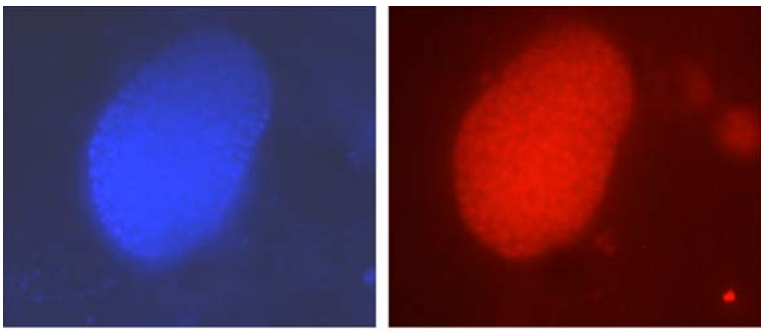

Figure 1. Photomicrographs of FISH with oligonucleotide probe NSO190 (red) for *Nitrosomonas* species. All microorganisms were visualized by DAPI staining (blue). Photomicrograph couple represents the same field of the microscopic view.

microorganisms were the members of the genus *Nitrosomonas*. This finding ensured the enrichment for nitrifiers. As seen from the figure, *Nitrosomonas* species spherically formed dense microcolonies. All experiments were carried out with this culture.

The DGGE analyses of sludge samples following the enrichment period and 7 months later are shown in Figure 2. No changes had occurred in the diversity of amoA gene sequences during this period. This finding ensured that all experiments were performed with the same microbial population. Therefore, the kinetic results are not expected to change due to a probable shift in microbial population.

Evaluation of ammonium utilization in the absence of TCE

The specific ammonium utilization rates ($q_{NH_4\text{-}N}$) were first investigated at the initial NH_4-N concentrations of 25, 50, 100, 200 and 400 mg/l to determine the $q_{max,\,NH_4\text{-}N}$ and K_S values. Figure 3(a) demonstrates an example for the analysis of $q_{NH_4\text{-}N}$ values. $q_{NH_4\text{-}N}$ values determined through these experiments are shown in Figure 4.

$$q_{NH_4\text{-}N} = q_{max,NH_4\text{-}N}\frac{S}{K_S + S} \qquad (1)$$

where S is the bulk substrate concentration (mg/l)

$q_{max,NH_4\text{-}N}$ and K_S values in Equation 1 can be estimated either by transforming the non-linear Monod equation into linearized forms by Lineweaver–Burk, Hanes–Woolf and Eadie–Hofstee plots (Cornish-Bowden 1995) or by applying NLSR analysis. In the analysis of data shown in Figure 4, all these methods were applied in order to compare them. In the case of Lineweaver–Burk, Hanes–Woolf and Eadie–Hofstee methods, data were plotted as $1/q_{NH_4\text{-}N}$ versus $1/NH_4$-N, NH_4-N/$q_{NH_4\text{-}N}$ versus NH_4-N and $q_{NH_4\text{-}N}$ versus $q_{NH_4\text{-}N}$/NH_4-N, respectively. Then, $q_{max,\,NH_4\text{-}N}$ and K_S values were found through linear regression analysis. The analysis of the same data by non-linear least square regression was performed with the

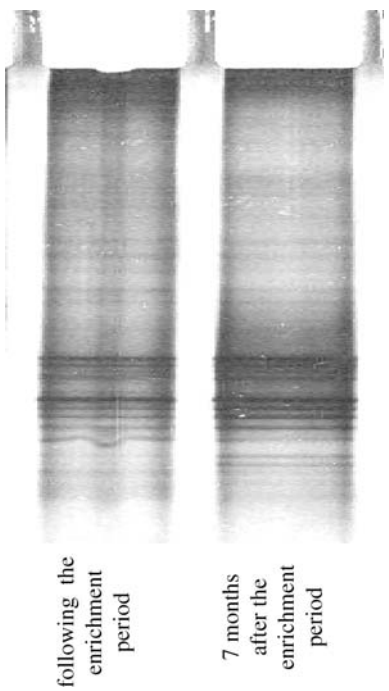

Figure 2. DGGE results of amoA genes of nitrifiers.

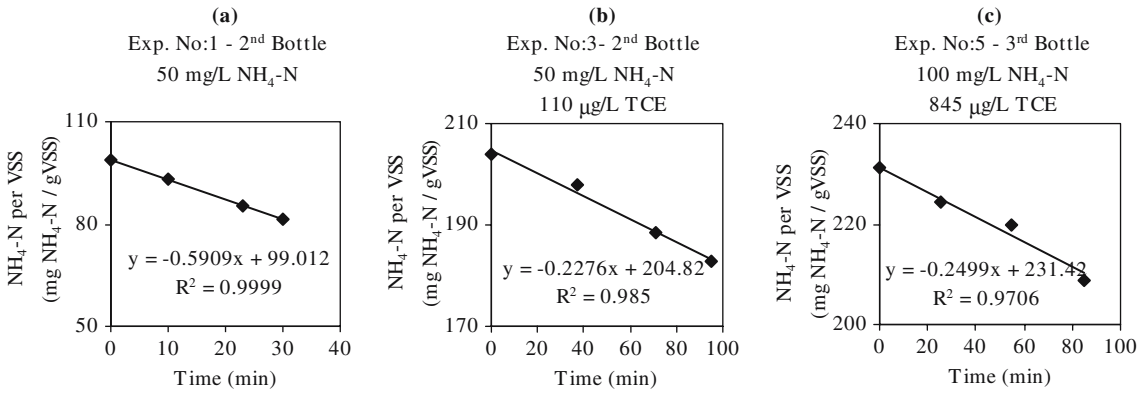

Figure 3. Specific ammonium utilization in batch experiments (a) at 50 mg/l initial NH_4-N concentration in the absence of TCE, (b) at 50 mg/l initial NH_4-N and 110 μg/l initial TCE concentrations, (c) at 100 mg/l initial NH_4-N and 845 μg/l initial TCE concentrations.

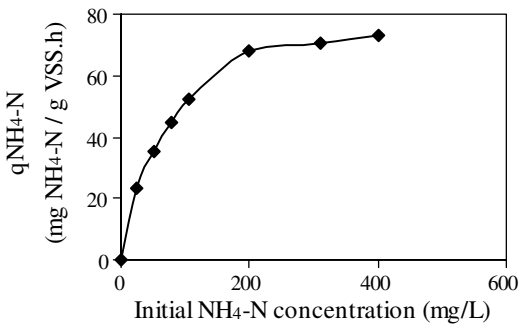

Figure 4. Specific ammonium utilization rates ($q_{NH_4\text{-}N}$) of the enriched nitrifier culture in the absence of TCE.

Ez-Fit5 package program of Perella Scientific Inc., US. This program curve-fits experimental substrate (NH_4-N) and rate ($q_{NH_4\text{-}N}$) data to the Michaelis–Menten model and tests statistically whether experimental data is randomly distributed around the fitted curve at a 95% confidence level. $q_{max,\ NH_4\text{-}N}$ and K_S values determined from these analyses are shown in Table 1. As seen from this table, by linearization and NLSR analysis $q_{max,\ NH_4\text{-}N}$ and K_S were found in the ranges of 86.2–87.8 mg NH_4-N/g VSS h and 66.36–70.09 mg/l, respectively.

The reported K_S values (66.36–70.09 mg/l) reflect the 'apparent' values and are significantly higher than those reported for nitrifiers in literature. This discrepancy can be explained as follows: All experiments were started at an initial DO concentration of 35–40 mg/l and ended when DO decreased to about 4 mg/l. During this period, a very small decrease was observed in the initial NH_4-N concentration. Therefore, data analyses were performed with respect to the initial NH_4-N concentrations rather than the bulk NH_4-N concentrations at any time t. Actually, in the application of the Monod equation (Equation 1), the bulk substrate concentration must be taken. The use of the initial substrate concentration, as in the case of the present study, results in overestimation of the K_S value. On the other hand, in experiments with diffused aeration, continuous oxygen supply throughout experiments allowed that all of the initial substrate was utilized by bacteria. In that case, data analysis could be performed in terms of bulk substrate concentrations and exact K_S value could be determined. Preliminary studies with the same culture under diffused air conditions resulted in a K_S range of 6.4–7.8 mg/l (the data are not shown) when data were analyzed with respect to bulk NH_4-N concentrations (Alpaslan Kocamemi 2005). This value is also higher than those reported in literature. The reason is most probably related to mass transfer limitation under our experimental conditions.

Effect of TCE on ammonium utilization

The effects of TCE on maximum specific ammonium utilization rate ($q_{max,NH_4\text{-}N}$) and half-saturation constant (K_S) were investigated in batch experiments. The initial NH_4-N and TCE concentrations were kept in the ranges of 25–400 mg/l and 40–845 μg/l, respectively. Figures 3(b) and 3(c) demonstrate examples for the analysis of

Table 1. Estimation of the apparent maximum specific ammonium utilization rates ($q^{app}_{max, NH_4\text{-}N}$) and apparent half-saturation constants K_S^{app} by several linearization methods and non-linear regression analysis

Method	Without TCE			40 μg/l TCE			110 μg/l TCE			325 μg/l TCE			845 μg/l TCE		
	R^2	$q^{app}_{max, NH_4\text{-}N}$ (mg NH_4-N/g VSS h)	K_S^{app} (mg/l)	R^2	$q^{app}_{max, NH_4\text{-}N}$ (mg NH_4-N/g VSS h)	K_S^{app} (mg/l)	R^2	$q^{app}_{max, NH_4\text{-}N}$ (mg NH_4-N/g VSS h)	K_S^{app} (mg/l)	R^2	$q^{app}_{max, NH_4\text{-}N}$ (mg NH_4-N/g VSS h)	K_S^{app} (mg/l)	R^2	$q^{app}_{max, NH_4\text{-}N}$ (mg NH_4-N/g VSS h)	K_S^{app} (mg/l)
Lineweaver–Burk ($1/q$ versus $1/S$)	0.99	86.2	67.5	0.99	87.7	250.0	0.99	87.7	312.5	0.99	90.9	353.2	0.99	85.4	501.3
Hanes–Woolf (S/q versus S)	0.99	86.2	66.3	0.97	85.4	235.5	0.99	80.6	263.2	0.99	90.9	352.9	0.98	90.0	532.1
Eadie–Hofstee (q versus q/S)	0.98	87.5	69.7	0.92	87.8	246.1	0.99	81.4	267.5	0.98	90.7	351.5	0.98	89.2	526.2
NLSR analysis	0.97	87.8 ± 3.4	70.0 ± 8.1	0.95	82.7 ± 8.8	215.5 ± 47.3	0.99	78.8 ± 1.1	251.8 ± 7.1	0.99	90.4 ± 3.4	348.5 ± 23.3	0.99	88.9 ± 2.1	523.0 ± 19.8

$q_{NH_4\text{-}N}$ values. $q_{NH_4\text{-}N}$ values determined through these experiments are shown in Figure 5.

As seen from Figure 5, at various TCE concentrations, $q_{NH_4\text{-}N}$ pattern still followed the Monod model. Increasing the TCE concentration resulted in a relative decrease in $q_{NH_4\text{-}N}$ with respect to the base $q_{NH_4\text{-}N}$ in the absence of TCE, indicating the inhibitory effect of TCE on nitrification. The analyses of data by NLSR and linearization methods are illustrated in Figures 5 and 6, respectively. These analyses yielded the apparent $q_{max, NH_4\text{-}N}$ ($q^{app}_{max, NH_4\text{-}N}$) and K_S (K_S^{app}) values summarized in Table 1. These values were placed into Equation 1. Then, the differences between model and experimental results were statistically evaluated. For this purpose, the residuals between experimental data and model results were examined. A few examples are illustrated in Figure 7. Residuals appeared to be randomly distributed and hence provided evidence that all data analysis methods had no serious deficiencies. As a next step, the analysis of variance (ANOVA) was performed at a 95% confidence level [significance level $(\alpha) = 0.05$] using the MINITAB package program (Minitab Inc., US). The obtained probability values (p-values) were greater than $\alpha = 0.05$ indicating no significant differences among linearized methods and NLSR. Regarding this finding, it may be concluded that problems with linearization, which are commonly reported in literature (Cornish-Bowden 1995; Knightes & Peters 2000), can be compounded when the rate data are obtained for a wide substrate range or at substrate concentrations greater than K_S as in the case of present study.

Inhibitory characteristics of TCE on ammonium utilization

Various types of inhibition (e.g, competitive, uncompetitive, mixed) have a different influence on linearized plottings of Monod equation. Therefore, the inhibitory characteristics of a compound can roughly be predicted by comparing linearized plottings with the schematic figures given in literature (Bailey & Ollis 1986). In the present study, the inhibitory characteristics of TCE was initially evaluated by comparing Figure 6 with the schematic figures shown in literature (Bailey & Ollis 1986). This comparison clearly indicated a typical competitive inhibition characteristic. Inhibition types may in principle be sub-classified as

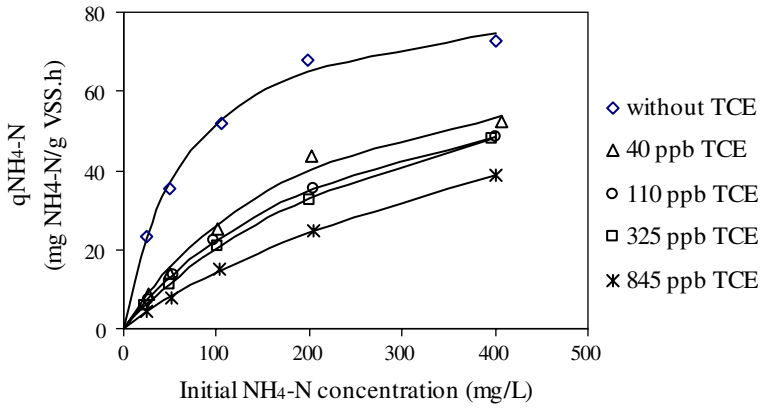

Figure 5. Specific ammonium utilization rates ($q_{NH_4\text{-}N}$) in the presence of TCE (experimental data and model fitting).

competitive, uncompetitive and mixed according to the q_{max} and K_S parameters (Cornish-Bowden 1995). The net effect of a competitive inhibitor, which can only bind to the free enzyme (E), is to increase K_S by a factor $1 + I/K_I$ while leaving q_{max} unchanged. An uncompetitive inhibitor can only bind to the enzyme-substrate (ES) complex and decrease both q_{max} and K_S by a factor $1/(1 + I/K_{iu})$. On the other hand, in the case of mixed inhibition, the inhibitory compound can bind both to the E and ES complex with dissociation constants of K_I and K_{iu}, respectively. The net effect of a mixed inhibitor on substrate utilization is to increase or decrease K_S by a factor $(1 + I/K_I)/(1 + I/K_{iu})$ while decreasing q_{max} by a factor of $1/(1 + I/K_{iu})$ (Cornish-Bowden 1995).

In order to diagnose the type of TCE inhibition on nitrification, the apparent $q_{max,\ NH_4\text{-}N}$ ($q^{app}_{max,\ NH_4\text{-}N}$) and K_S (K^{app}_S) values in Table 1 were compared with the base $q_{max,\ NH_4\text{-}N}$ and K_S determined in the absence of TCE. As the initial TCE concentration increased, K^{app}_S also increased, whereas $q^{app}_{max,\ NH_4\text{-}N}$ remained almost constant. These results exactly coincide with the characteristics of a competitive inhibitor which is usually a substrate analog competing for the active site of the enzyme (Shuler & Kargi 2001). Therefore, TCE was found to be a competitive inhibitor of ammonia oxidation. This finding is in consistency with the results of Hyman et al. (1995) and Ely et al. (1995), who concluded that TCE was a competitive inhibitor. They used a Dixon plot for oxygen uptake rate measurements and a model solution, respectively. However, it should be noted that their results were obtained for pure *Nitrosomonas europaea* cultures as in many other studies.

Determination of the inhibition coefficient (K_I) of TCE

The net effect of a competitive inhibitor on substrate utilization is to increase K_S by a factor $(1 + I/K_I)$ while leaving that of q_{max} unchanged as shown in the Equation 2 (Cornish-Bowden 1995).

$$q = q_{max}\frac{S}{K_S(1 + \frac{I}{K_I}) + S} \quad \text{where} \quad K_S(1 + \frac{I}{K_I}) = K^{app}_S, q_{max} = q^{app}_{max} \tag{2}$$

The simplest approach to find the K_I value in Equation 2 is to plot K^{app}_S values against the inhibitor concentrations [I]. However, since the K^{app}_S can never be estimated as accurately as K^{app}_S/q^{app}_{max} a better approach to find the K_I value is to plot K^{app}_S/q^{app}_{max} against the inhibitor concentration [I] (Cornish-Bowden 1995). The intercept on the x-axis will give the $-K_I$ value. Plottings of the K^{app}_S/q^{app}_{max} values in Table 1 against the initial TCE concentrations were linearized and R^2 values were found as 0.98–0.99. The calculated K_I values are summarized in Table 2 together with the K_I values reported for TCE in previous studies with pure *Nitrosomonas europaea* cultures. The K_I value for TCE found in the present study is low compared to those with pure cultures. This discrepancy may be attributed to different experimental conditions, such as temperature, nitrifier

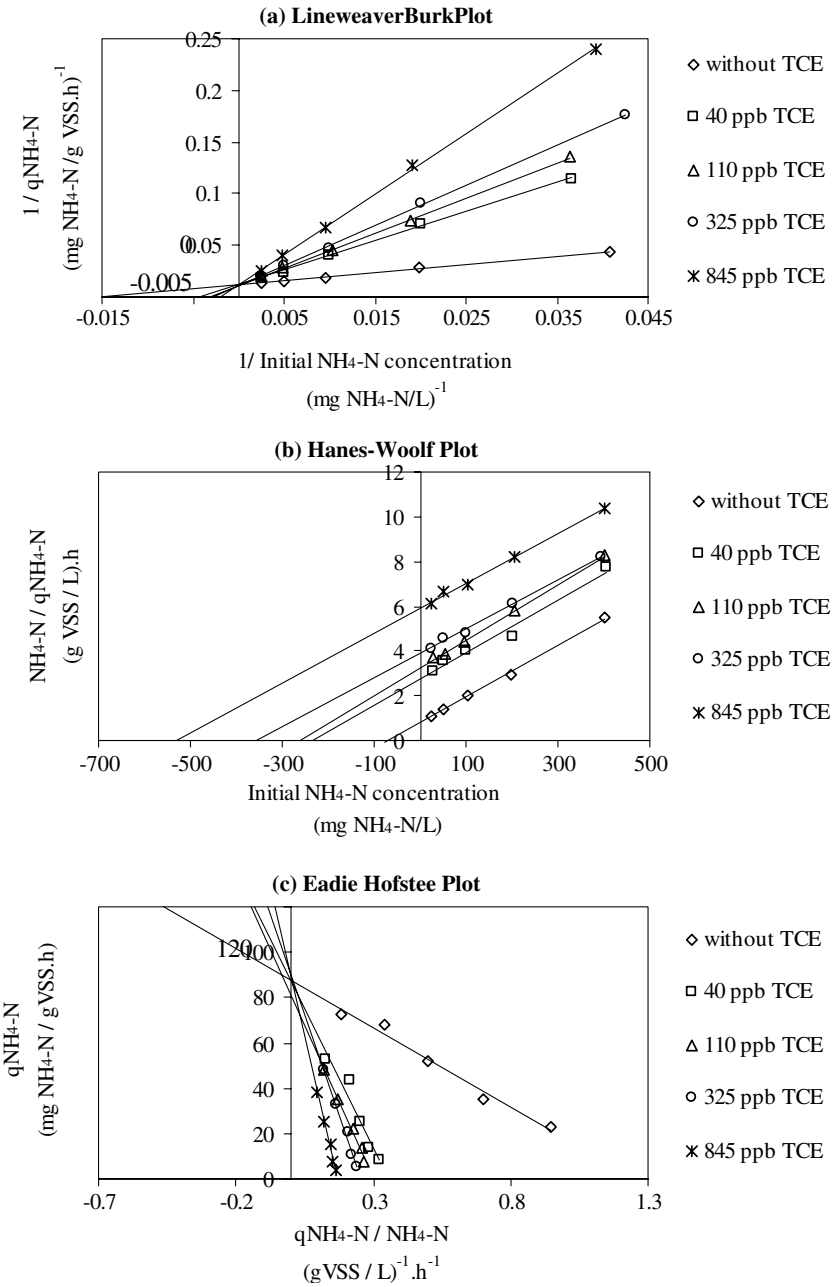

Figure 6. Effects of TCE on ammonium utilization rate as reflected by (a) Lineweaver–Burk plot, (b) Hanes–Woolf plot and (c) Eadie–Hofstee plot.

concentrations, and the use of an enriched culture instead of a pure culture.

K_S and K_I are indicators of the affinity of the substrate and the inhibitory compound for the enzyme, respectively. As seen from Table 1, the K_S^{app} values in the absence of TCE are in the range of 66–70 mg/l NH_4-N which corresponds to 4300–5000 μM. The comparison of this value with the K_I value (5.0–6.1 μM) in Table 2 clearly indicates that the affinity of the TCE for AMO enzyme was significantly higher than that of ammonium. As discussed before, the actual K_S value determined under diffused air conditions was in the range of 6.4–7.8 mg/l NH_4-N which

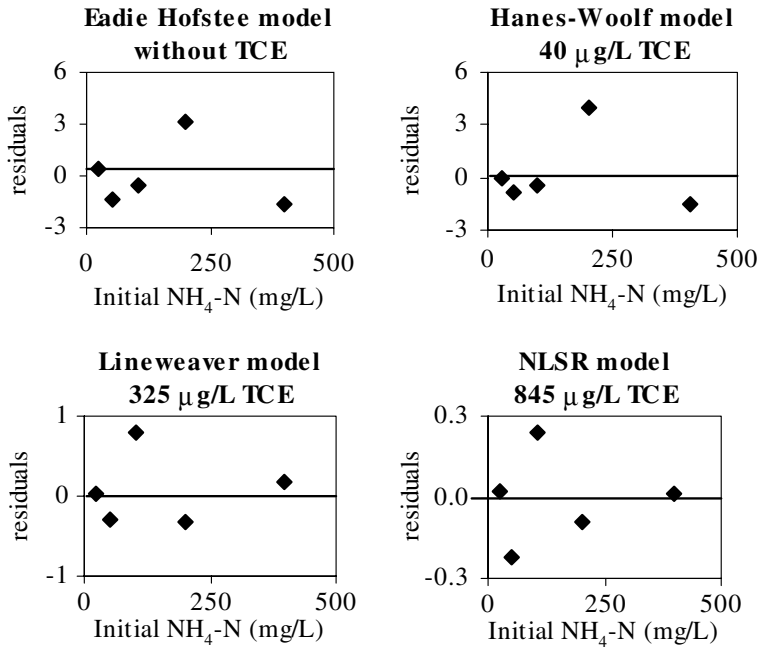

Figure 7. Residuals analyses in data linearization methods and non-linear least square regression (NLSR).

Table 2. Inhibition constant (K_I) of TCE estimated in the present study and reported in previous studies

Source of data	Estimated K_I μg/l [μM]
This study (Lineweaver–Burk Plot results)	802 [6.1]
This study (Hanes–Woolf Plot results)	715 [5.4]
This study (Eadie–Hofstee Plot results)	733 [5.5]
This study (NLSR results)	666 [5.0]
Hyman et al., 1995	3942 [30]
Ely et al., 1997	1563 [11.9]

corresponded to 458–558 μM. Comparison of the K_I value with the this actual K_S value also indicates the higher affinity of TCE for the AMO enzyme.

Conclusions

The use of nitrifiers for cometabolic removal of pollutants has been studied to a much smaller extent than other organisms. This kinetic analysis provides information about the effectiveness and practical applicability of cometabolic TCE degradation by nitrifiers in treatment and bioremediation systems. In cometabolic degradation processes, the effects of cometabolic substrate on growth substrate should be evaluated and vice versa. In this study, the kinetics of cometabolic degradation of TCE by nitrifiers was studied in terms of the inhibitory effect of TCE on nitrification.

The results demonstrated that TCE inhibited ammonium utilization and TCE was identified as a competitive inhibitor for nitrification. The affinity of the TCE for the AMO enzyme was found to be significantly higher than that of ammonium. No significant differences were found among the linearized plots of Monod equation (e.g., Lineweaver–Burk, Hanes–Woolf and Eadie–Hofstee plots) and NLSR in the calculation of kinetic parameters. This finding indicated that the usual problems reported for data linearization could be compounded when the rate data are obtained for a wide substrate range or at substrate concentrations greater than K_S.

Although in practice, bioremediation and treatment are usually performed with mixed cultures, most of the former studies were done with pure nitrifier cultures, especially *Nitrosomonas europaea*. Therefore, the data reported to date for nitrifiers are insufficient to analyze the TCE degradation capability of a mixed nitrifying

culture. The findings of this study partially filled a gap in the literature about the cometabolic degradation of TCE by nitrifiers. However, a complete kinetic analysis of the process requires also the effect of the ammonium level on TCE degradation, which will be discussed in another article.

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