



Short Communication

Biological removal of the xenobiotic trichloroethylene (TCE) through cometabolism in nitrifying systems

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ABSTRACT

In the present study, cometabolic TCE degradation was evaluated using $\text{NH}_4\text{-N}$ as the growth-substrate. At initial TCE concentrations up to 845 $\mu\text{g/L}$, TCE degradation followed first-order kinetics. The increase in ammonium utilization rate favored the degradation of TCE. This ensured that biological transformation of TCE in nitrifying systems is accomplished through a cometabolic pathway by the catalysis of non-specific ammonia oxygenase enzyme of nitrifiers. The transformation yield (T_y) of TCE, the amount of TCE degraded per unit mass of $\text{NH}_4\text{-N}$, strongly depended on the initial $\text{NH}_4\text{-N}$ and TCE concentrations. In order to allow a rough estimation of TCE removal and nitrification at different influent TCE and $\text{NH}_4\text{-N}$ concentrations, a linear relationship was developed between $1/T_y$ and the initial $\text{NH}_4\text{-N/TCE}$ ratio. The estimated T_y values lead to the conclusion that nitrifying systems are promising candidates for biological removal of TCE through cometabolism.

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1. Introduction

Trichloroethylene (TCE) is a xenobiotic compound that is mainly used as degreaser and solvent in automotive, metal, electroplating, textile and paper industries. The large-scale use of TCE in various industries over the past years has led to widespread distribution of this toxic and carcinogenic compound in the environment, especially in groundwater media. TCE can also be monitored in surface media. But, the highly volatile characteristics usually results in quick evaporation in open surface water bodies.

Considerable effort has been focused on removal of TCE by biological processes. To date, no isolates or enrichment cultures utilizing TCE as growth-substrate under aerobic conditions have yet been obtained (Bradley, 2003; Field and Sierra-Alvarez, 2004). But, some aerobic bacterial cultures containing non-specific oxygenase enzymes (e.g., methane, toluene, phenol, propane and ammonia oxidizers) were identified capable to degrade TCE by cometabolism (Arciero et al., 1989; Chang and Alvarez-Cohen, 1995; Chu and Alvarez-Cohen, 2000; Han et al., 2007; Shukla et al., 2009). In this process, biotransformation of a non-growth compound (e.g., TCE) is accomplished concurrently with metabolism of a growth-supporting substrate through the catalysis of non-specific oxygenase enzymes. The organism obtains no benefit from cometabolism of the non-growth-supporting substrate.

Moreover, the presence of the non-growth-supporting substrate may inhibit the metabolism of the growth-supporting substrate, thereby decreasing or preventing bacterial growth (Ely et al., 1997). In addition, cometabolism of the non-growth-supporting substrate consumes reductant NAD(P)H , which can only be regenerated from growth-supporting substrate degradation. Therefore, in this process, a growth-supporting substrate must be available (at least periodically) to grow new cells, provide reductant and induce production of non-specific enzymes (Alvarez-Cohen and Speitel, 2001).

Among the organisms capable to degrade TCE cometabolically, this study focused on ammonia oxidizers (nitrifiers). In literature, the studies evaluating the cometabolism of TCE by nitrifying species (Arciero et al., 1989; Rasche et al., 1991; Ely et al., 1995, 1997; Hyman et al., 1995; Yang et al., 1999) are very limited compared to those performed with other organisms (e.g., methanotrophs). Nitrification studies up to date were mainly performed with pure *Nitrosomonas europaea* cultures. In our previous studies, using a mixed culture enriched in terms of nitrifying bacteria, we first evaluated the inhibitory effect of TCE on nitrification during cometabolic degradation for a broad TCE range and at a fixed growth-supporting substrate ($\text{NH}_4\text{-N}$) concentration (Alpaslan Kocamemi and Çeçen, 2005). Later (Alpaslan Kocamemi and Çeçen, 2007), we focused on the kinetics of the inhibitory effect of TCE on nitrification, which directly influences the sustainability of TCE degradation in nitrifying systems. The inhibition type and inhibition coefficient of TCE were determined for a broad range of $\text{NH}_4\text{-N}$ and TCE.

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In the present study, which is complementary to our previous study (Alpaslan Kocamemi and Çeçen, 2007), cometabolic TCE degradation was evaluated by a mixed culture enriched for nitrifiers. A batch suspended-growth system was fed with $\text{NH}_4\text{-N}$ as the growth-supporting substrate. The dependence of the process on various factors, such as the $\text{NH}_4\text{-N}$ utilization rate and the relative initial ratio of $\text{NH}_4\text{-N}$ and TCE, was quantitatively analyzed.

2. Methods

2.1. Mixed culture enriched for nitrifiers

The mixed culture was initially taken from the Pasakoy Advanced Biological Sewage Treatment Plant in Istanbul. The culture was then enriched for nitrifiers as described previously (Alpaslan Kocamemi and Çeçen, 2005). After the enrichment period, this mixed culture was continuously fed with $\text{NH}_4\text{-N}$ and mineral solutions only based on the fill-and-draw principle. The sludge samples taken from the stock enriched nitrifier culture were analyzed by Florescent In-Situ Hybridization (FISH) to ensure the enrichment of the mixed culture for nitrifiers. Denaturing gradient gel electrophoresis (DGGE) analyses were also performed to observe whether any changes occurred in the diversity of *amoA* gene sequences 7 months after the enrichment period. The procedures of FISH and DGGE analyses were as described previously (Alpaslan Kocamemi and Çeçen, 2009).

2.2. Experimental procedure

Four sets of experiments were performed in 200 mL capped glass bottles at a constant wastewater temperature of 25 °C and pH range of 7–8. In each set, five runs were performed at constant TCE, but at varying initial $\text{NH}_4\text{-N}$ (25–400 mg/L). The initial TCE was kept at 40, 110, 325, 845 $\mu\text{g/L}$ in the first, second, third and fourth set of experiments. In all experiments, the stock synthetic $\text{NH}_4\text{-N}$ solution [37.75 g/L $(\text{NH}_4)_2\text{SO}_4$ and 95 g/L NaHCO_3] and the stock TCE solution [500 mg/L TCE] were used in diluted form to adjust the desired $\text{NH}_4\text{-N}$ and TCE concentrations. The stock mineral solution [2 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/L CaCO_3 , 0.4 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g/L $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and 0.3 g/L K_2HPO_4] was diluted 40 folds. In each experiment, the stock culture was rinsed thoroughly with water and diluted to MLVSS ranging between 210 and 530 mg/L. Experiments were started under oxygen-supersaturated conditions (initial DO of 35–40 mg/L) and ended before the DO in the test bottle dropped below 4 mg/L. Continuous diffused aeration was avoided to prevent the possible risk of TCE volatilization. Complete mixing in the test bottles was maintained by magnetic stirring. Blank experiments were performed to evaluate the abiotic loss of TCE resulting from stirring action. These experiments demonstrated a negligible TCE exchange rate of 0.0028 $\mu\text{g/L/min}$ per initial TCE ($\mu\text{g/L}$) (data not shown, Alpaslan Kocamemi, 2005). In all runs, samples were analyzed with respect to time for $\text{NH}_4\text{-N}$ with the Nessler Method (APHA, 1998) using Hach DR/2000 spectrophotometer and for TCE with HP 5890 Gas Chromatograph equipped with an electron capture detector. The measured TCE concentrations were corrected by the abiotic TCE loss rate. The specific ammonium utilization rate ($q_{\text{NH}_4\text{-N}}$) and the specific cometabolic TCE degradation rates (q_{TCE}) were then calculated from the slopes of $\text{NH}_4\text{-N/VSS}$ versus time and TCE/VSS versus time plottings through linear regression analysis, respectively.

3. Results and discussions

The FISH analysis of the stock culture revealed that the dominant members of the microbial community consisted of *Nitroso-*

Table 1

The estimated first-order TCE degradation constant at various $\text{NH}_4\text{-N}$ levels.

Initial $\text{NH}_4\text{-N}$ concentration (mg/L)	First-order TCE degradation rate constant (L/g VSS h)	Goodness-of-fit of linear regression (R^2)
25	0.26	0.98
50	0.31	0.94
100	0.48	0.95
200	0.73	0.95
400	1.03	0.93

monas species. Photomicrographs of these analyses were shown in a former paper (Alpaslan Kocamemi and Çeçen, 2007). Further, as shown by DGGE analyses (Alpaslan Kocamemi and Çeçen, 2007), no changes had occurred in the diversity of *amoA* gene sequences during the period of about 7 months after enrichment. Since a shift in microbial population was not the case, the rates in the present study are not expected to change during the experimental period.

Evaluation of the cometabolic degradation rate of TCE (q_{TCE}) with respect to initial TCE concentrations (Alpaslan Kocamemi, 2005, data not shown) showed that at each initial $\text{NH}_4\text{-N}$ concentration studied, q_{TCE} increased linearly with TCE concentration. The first-order cometabolic TCE degradation rate constants obtained from the linearization of the graphs are summarized in Table 1. The cometabolic TCE degradation followed first-order kinetics because the bulk TCE concentrations (0–825 $\mu\text{g/L}$) were very small. If higher bulk TCE concentrations were present, TCE degradation rate would most probably reach the saturation level. However, it is very likely that also in real remediation systems, such as groundwater, TCE degradation follows first-order kinetics in the concentration ranges below 1000 $\mu\text{g/L}$.

The first-order TCE degradation rate constants in the present study are considerably lower in comparison to those reported for pure *N. europaea* species as 30.8 L/g VSS h and 42.5 L/gVSS h for the initial TCE concentrations of 0–3300 and 2100 $\mu\text{g/L}$, respectively (Alvarez-Cohen and Speitel, 2001). However, in those studies performed with pure cultures, the culture consisted of pure nitrifier species only and the reported values based on the assumption that the dry cell mass consisted of 50% protein (Alvarez-Cohen and Speitel, 2001). On the other hand, in the present study, the mixed culture still contains cells other than nitrifiers although it was enriched for nitrifiers. Therefore, the values expressed on VSS basis are quite lower compared to those reported in pure culture studies.

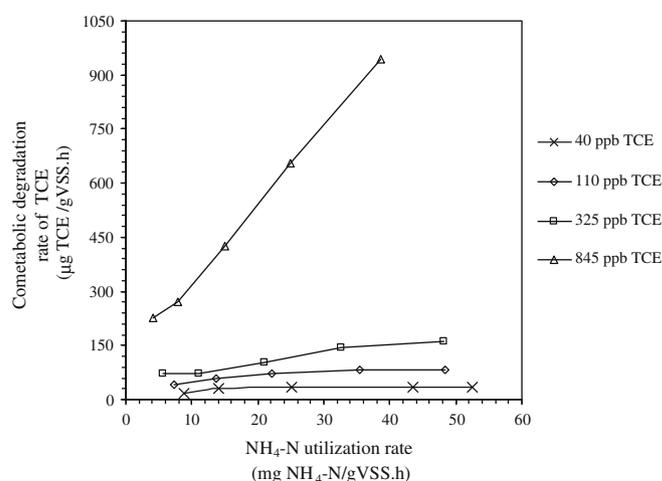


Fig. 1. Cometabolic degradation rates of TCE (q_{TCE}) with respect to the $\text{NH}_4\text{-N}$ utilization rates ($q_{\text{NH}_4\text{-N}}$).

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