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Cometabolic degradation of TCE in enriched nitrifying batch systems

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Abstract

The aim of this study was to evaluate the effect of the trace pollutant trichloroethylene (TCE) on the nitrification process and to assess its cometabolic degradation. Nitrification was accomplished in batch suspended growth systems containing an enriched nitrifier culture. The presence of TCE resulted in both the inhibition of specific oxygen uptake rate (SOUR) and specific ammonium utilization rate (qNH_4–N). In both SOUR and qNH_4–N a 50% decrease was observed in a TCE concentration range of 1000–2000 ppb. TCE was cometabolically degraded by this enriched nitrifier culture. The cometabolic degradation of TCE was found to be dependent on initial TCE concentration. The results may be applicable in the treatment of TCE containing industrial wastewaters and contaminated groundwaters and soils.
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1. Introduction

Trichloroethylene (TCE) is widely used in various industrial applications such as degreasing of fabricated metal parts, industrial dry-cleaning, printing, production of printing-ink and paint, extraction processes and textile printing. As a result of improper disposal, TCE enters the environment and causes contamination of groundwater and soil. Therefore, different treatment techniques such as air stripping, carbon adsorption and chemical oxidation have been developed to limit the TCE discharges into the environment and to remediate TCE-contaminated subsurface media. However, air stripping and carbon adsorption simply transfer TCE from one medium to another without any significant reduction in volume or toxicity [1] while the chemical oxidation techniques are energy intensive and expensive [2]. Therefore, biological treatment techniques have received much attention as a cost-effective alternative. They may provide partial or full degradation of TCE rather than transferring it from one medium to another.

TCE is usually resistant to biodegradation because it cannot be used as an energy and growth substrate by microorganisms. However, it can be biodegraded through either anaerobic or aerobic cometabolic processes. Cometabolism is the biological transformation of a non-growth substrate by bacteria through the catalysis of non-specific enzymes synthesized in the presence of a growth substrate. In an anaerobic environment, various types of methanogens, sulfidogens and homoacetogens can reductively dechlorinate TCE. The aerobic cometabolism of TCE is known to be initiated by a variety of non-specific oxygenase enzymes of microorganisms grown on a variety of substances like methane, propane, toluene, phenol and ammonia. To date, the cometabolic degradation of TCE is most widely studied in the presence of methanotrophs, propane degraders, toluene degraders and phenol degraders [3–7]. The cometabolic degradation of TCE by ammonia oxidizing bacteria in a nitrification system was first demonstrated by Arciero et al. [8]. Since then, a limited number of studies [9–14] have been conducted to investigate the TCE transformation through the catalysis of the ammonia monooxygenase (AMO) enzyme in nitrifier cells. Also, inhi-

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bition and inactivation of nitrifiers was investigated when this TCE transformation took place. In these studies, the emphasis was on the inhibitory effect of TCE on nitrifiers. All of these studies, except the studies of Yang [13] and Yang et al. [14], have been conducted with pure *Nitrosomonas Europaea* cultures. Therefore, there is still a lack of information about the cometabolic removal of TCE with enriched nitrifier cultures. This type of a culture represents a more realistic case since in real biological treatment and bioremediation systems pure nitrifying cultures are hardly employed.

In this study, the cometabolic removal of TCE and the inhibitory effect of TCE on the nitrification process were investigated in batch suspended growth systems, which contained an enriched nitrifier culture.

2. Materials and methods

2.1. Enrichment of a mixed culture for nitrifiers

A mixed liquor was taken from the aeration tank recycle line of the Istanbul Pasakoy Advanced Biological Sewage Treatment Plant and enriched for nitrifiers in a 16 L batch reactor for 4 months. A synthetic feed stock solution consisting of 37.75 g/L $(NH_4)_2SO_4$, 97.8 g/L $NaHCO_3$ and a synthetic mineral stock solution consisting of 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 were prepared. The culture was daily fed based on fill-and-draw principle with these stock solutions and the initial ammonium (NH_4–N) concentration was kept at 200 mg/L. The dissolved oxygen (DO) concentration in the reactor was maintained above 4 mg/L at an air flow rate of 5.5 L/min. During the enrichment period, NH_4–N and volatile suspended solids (VSS) measurements were periodically performed from which the specific ammonium utilization rate (qNH_4–N) of the culture was calculated.

2.2. Experimental procedure

First, the TCE range was studied which caused inhibition of nitrification. For this purpose, oxygen uptake rate (OUR) experiments were carried out in eight sets in 300 mL capped glass bottles. The wastewater temperature was kept at 20 °C and complete mixing condition was provided by magnetic stirring. In each set of experiment, the stock enriched nitrifier culture was rinsed and diluted by deionized water to a VSS concentration of 100–150 mg/L and aerated till saturation (7–8 mg/L DO). The culture was then fed with the stock feed and mineral solution such that the initial NH_4–N concentration at the start of the run was 40 mg/L. The initial TCE concentration at the start of the run was varied in the range of 50–50,000 ppb. A control experiment was also carried out in the absence of TCE. In each set of experiments, the DO concentrations were recorded at 15 s intervals over a 10 min test period. The VSS concentrations were also measured at the end of each test. From these measurements, the specific oxygen uptake rates (SOUR) of the culture were calculated by dividing OUR through VSS.

Based on these preliminary OUR experiments, six sets of batch experiments were performed in the selected TCE concentration range of 50–4500 ppb. The aim was to investigate the inhibitory effect of TCE on ammonium oxidation and cometabolic degradation rate of TCE in the presence of ammonium. Since TCE was highly volatile under diffused aeration conditions, aeration with diffusers was not used and the culture was initially supersaturated with pure oxygen gas to DO = 30–35 mg/L. The experiments were performed in 2 L capped glass bottles with an enriched nitrifier culture which was kept in suspension by magnetic stirring. The wastewater temperature was kept constant at 20 °C. In each set of experiments, three bottles were run over a test period of 3–4 h as described in Table 1. The first bottle was used as a blank to calculate qNH_4–N and SOUR of the culture in the absence of TCE by performing hourly NH_4–N, pH, DO and VSS measurements. For this purpose, the stock enriched nitrifier culture was rinsed and diluted with deionized water to a VSS concentration of 131–135 mg/L and initially supersaturated with pure oxygen gas to DO = 30–35 mg/L. The culture was then fed with the stock synthetic feed and mineral solutions such that the initial NH_4–N was 40 mg/L. The second bottle was used as a blank to determine the transfer rate of TCE from the liquid phase into the head space of the capped bottle due to magnetic stirring by performing hourly TCE measurements. The bottles were filled with deionized water and TCE solution only so that the final TCE concentrations ranged between 50 and 4500 ppb. The third bottle was used to investigate the

Table 1
Operating conditions in batch experiments designed to investigate specific ammonium utilization rates (1st bottle), TCE volatilization rates (2nd bottle) and specific ammonium utilization rates and TCE cometabolic degradation rates at various TCE concentrations (3rd bottle)

Exp. no.	1st bottle (blank)			2nd bottle (blank)			3rd bottle		
	VSS (mg/L)	NH_4–N (mg/L)	TCE (ppb)	VSS (mg/L)	NH_4–N (mg/L)	TCE (ppb)	VSS (mg/L)	NH_4–N (mg/L)	TCE (ppb)
1	135	40	–	–	–	50	123	40	50
2	131	40	–	–	–	100	134	40	100
3	131	40	–	–	–	500	130	40	500
4	131	40	–	–	–	1000	137	40	1000
5	135	40	–	–	–	2000	133	40	2000
6	135	40	–	–	–	4500	144	40	4500

qNH4–N in the presence of TCE and the cometabolic degradation rate of TCE (qTCE) by performing hourly NH4–N, pH, DO, TCE and VSS measurements. For this purpose, the stock enriched nitrifier culture was rinsed and diluted by deionized water to VSS concentration of 123–144 mg/L and initially supersaturated with pure oxygen gas to DO = 30–35 mg/L. The culture was fed such that the initial NH4–N was 40 mg/L while the initial TCE ranged from 50 to 4500 ppb.

For the calculation of the specific oxygen uptake rates in the first and third bottles of each run, the measured DO concentrations were corrected by an oxygen exchange rate of 0.294 mg/(L h) which was determined between the reaction liquid and the overhead gas through a control experiment (data not shown) as shown in Eq. (1).

$$DO_C = DO_M + 0.294 \times t \quad (1)$$

where DO_C is the corrected DO concentration (mg/L) at time t, DO_M the measured DO concentration (mg/L) and t is time (h).

The SOUR values were then calculated from the initial and final corrected DO concentrations (ΔDO_C) determined during a run carried out in a definite time period (Δt) as shown in Eq. (2).

$$SOUR = \frac{\Delta DO_C/\Delta t}{VSS} \quad (2)$$

For the calculation of the specific ammonium utilization rates (qNH4–N) in the first and third bottles of each run, the initial and final NH4–N concentrations (ΔNH4–N) were taken during a run carried out in a definite time period (Δt) and divided by the present VSS as shown in Eq. (3).

$$q\text{NH}_4\text{–N} = \frac{\Delta\text{NH}_4\text{–N}/\Delta t}{VSS} \quad (3)$$

Under the experimental conditions described above, volatilization of TCE was due to transfer of TCE from the liquid phase into the head space of the capped bottle which was very low compared with the volatilization of TCE under diffused aeration. Since the second blank bottle of each run did not contain microorganisms, the decreases observed directly reflected the volatilization losses. On the other hand, the decreases in the TCE concentration in the third bottle containing microorganisms, TCE and NH4–N were due to both cometabolic degradation and volatilization. Therefore, the measured TCE concentrations in the third bottles were corrected for the volatilized amount of TCE as shown in Eq. (4).

$$TCE_C = TCE_M + r_{vol,TCE} \times t \quad (4)$$

where TCE_C is the corrected TCE concentration (ppb), TCE_M the measured TCE concentration (ppb), $r_{vol,\,TCE}$ the volatilization rate of TCE (ppb/h) and t is the time (h).

The specific TCE cometabolic degradation rates were then calculated from the initial and final corrected TCE concentrations (ΔTCE_C) determined during a run carried out in a definite time period (Δt) as shown in Eq. (5).

$$qTCE = \frac{\Delta TCE/\Delta t}{VSS} \quad (5)$$

2.3. Analytical methods

NH4–N concentrations were analyzed using the Nessler Method with Hach DR/2000 spectrophotometer. The DO concentrations and pH were measured by using WTW OxiLevel-2 DO meter and WTW Inolab-1 pH meter, respectively. VSS analyses were performed using the Method 2540E in Standard Methods [15]. Liquid samples for TCE analysis were extracted into *n*-pentane by EPA Method 502.1. The extracts were analyzed by Hewlett Packard 5890 Gas Chromatograph equipped with a J&W prosteel megabore column (0.53 mm i.d., 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 TCE solution (200 μg/mL) supplied from Crescent Chemical Company Inc., New York.

3. Results and discussions

3.1. Enrichment for nitrifiers

The enrichment for nitrifiers was monitored through the specific ammonium utilization rate (qNH4–N). As seen from Fig. 1, qNH4–N showed an increase with respect to enrichment time and reached a steady value of about 20–25 mg NH_4–N g^{-1} VSS h^{-1} at the end of 129 days. All subsequent experiments were then started with this enriched nitrifier culture.

3.2. The effect of TCE on the specific oxygen uptake rate

The immediate response of enriched nitrifier cultures to an initial TCE concentration in the range of 50–50,000 ppb was assessed by SOUR as shown in Fig. 2. These results correspond to SOUR values in a total experiment period of

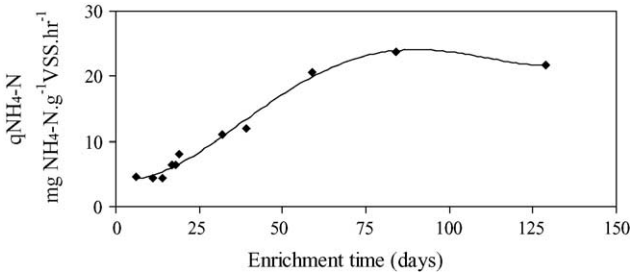

Fig. 1. The calculated specific ammonium utilization rates (qNH4–N) during the enrichment period of nitrifiers.

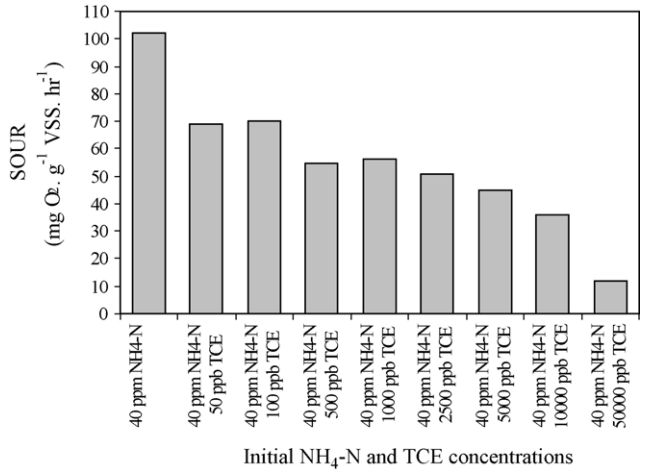

Fig. 2. Decrease in the specific oxygen uptake rate (SOUR) of an enriched nitrifier culture in the presence of TCE [initial NH_4–N = 40 mg/L, initial TCE = 0–50,000 ppb].

10 min. The results showed that SOUR associated with the ammonium oxidation decreased by about 30% at 50–100 ppb TCE, 45% at 500–1000 ppb TCE, 50% at 2500 ppb TCE, 56% at 5000 ppb TCE, 65% at 10,000 ppb TCE and 88% at 50,000 ppb TCE. These results are consistent with the study of Hyman et al. [10] which indicated that increasing TCE led to increased inhibition of O_2 uptake for pure *N. Europaea* cells. The non-specific AMO enzyme, which serves as a catalyzer in the reduction and insertion of an oxygen atom from molecular oxygen into ammonia, is also able to catalyze the oxidation of TCE. TCE affinity for AMO is about four times greater than the NH_4–N affinity for AMO [11]. Therefore, such decreases were observed in the SOUR due to the inhibition of the AMO enzyme by TCE.

3.3. Evaluation of TCE volatilization in batch experiments

As seen from Fig. 3, the volatilization rate of TCE from the liquid phase into the headspace of capped bottles increased linearly with TCE and the first-order rate constant was calculated as 0.0208 h^{-1}. Based on this first-order rate constant, the percent decreases in TCE due to volatilization were determined as 6% in a period of 3 h at 50 ppb TCE and 8% in a period of 4 h at 100, 500, 1000, 2000 and 4500 ppb TCE.

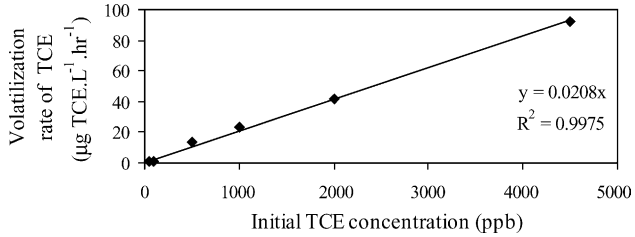

Fig. 3. The volatilization rate of TCE from the liquid phase into the head space with respect to the initial TCE concentration.

The cometabolic degradation rates of TCE were calculated by considering these volatilized amounts.

3.4. Evaluation of ammonium removal in the presence of TCE

At a constant initial ammonium concentration of 40 mg/L NH_4–N, the specific ammonium utilization rate (qNH_4–N) at each initial TCE concentration was calculated. The initial TCE concentrations at the start of runs were in the range of 50–4500 ppb. In the presence of TCE, a relative decrease in qNH_4–N was observed with respect to the base qNH_4–N which was 20–24 mg NH_4–N g^{-1} VSS h^{-1} in the absence of TCE. Fig. 4a shows these decreases indicating the inhibition of ammonium utilization. The degree of inhibition increased with TCE concentration and reached 50% in the TCE concentration range of 1000–2000 ppb.

As seen from Fig. 4b, SOUR values showed a similar trend as in the case of qNH_4–N and a relative decrease was observed with respect to the base SOUR which was about 0.02–0.05 mg O_2 mg^{-1} VSS h^{-1} in the absence of TCE. In terms of SOUR, a 50% decrease was observed in the TCE concentration range of 1000–2000 ppb. This indicated that in these systems oxygen uptake and ammonium utilization were similarly affected by the presence TCE. TCE may exert its effect in two basic ways. Either the TCE may directly cause a toxic effect on the enzyme, or it may be degraded and its by-products may exert a toxicity. As discussed in Section 3.5, TCE measurements clearly showed that TCE was cometabolically degraded. If the by-products formed during TCE degradation exerted a toxicity, a higher decrease would be expected in SOUR compared to those observed in 10-min preliminary SOUR experiments with negligible TCE degradation. However, the decreases observed in SOUR in

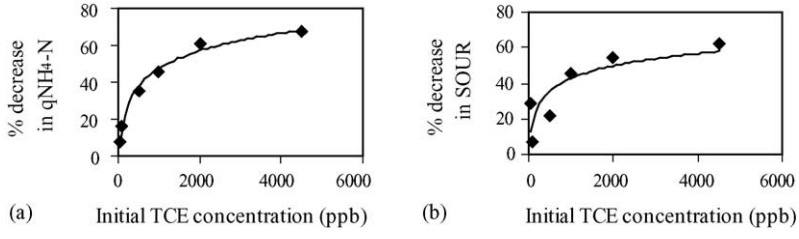

Fig. 4. Effect of TCE on (a) specific ammonium utilization rate (qNH_4–N) and (b) specific oxygen uptake rate (SOUR).

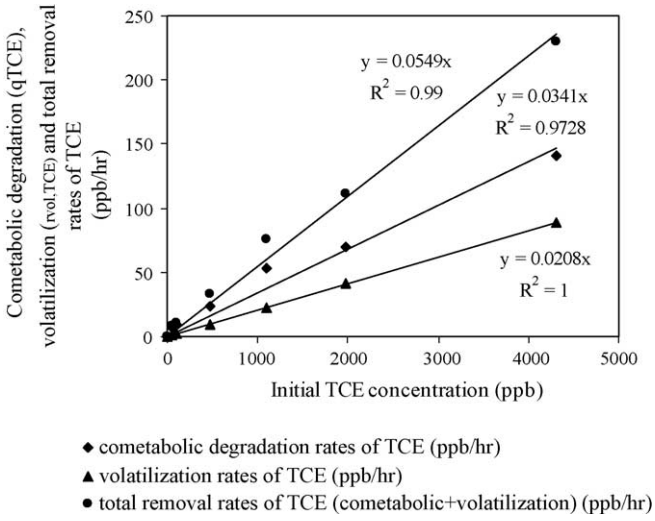

Fig. 5. Dependence of TCE removal rates on the initial TCE concentration.

4 h tests were generally in accordance with those obtained from preliminary SOUR experiments having a duration of only 10 min. Therefore, we may conclude that the toxic effect of TCE on ammonium oxidation was mainly attributable to the inhibition of the AMO enzyme activity when the cells were exposed to TCE rather than the inhibition of the AMO enzyme by short-lived reactive intermediates produced during TCE oxidation. Even at a very high initial TCE concentration (4500 ppb), ammonium oxidation was still possible and showed 68% decrease with respect to the control test. This result is in contrast to a previous study [13] which indicated that in a system containing 4 mg/L NH_4–N and 4.8 mg/L enriched nitrifier culture, ammonium oxidation activity was completely halted when the initial TCE concentration was at 200 ppb. The discrepancy between our results and those of Yang [13] may be attributed to the differences in nitrifier concentrations. Moreover, the mixed culture used in this study and the culture in the study of Yang [13] may contain different nitrifying strains.

3.5. Evaluation of cometabolic degradation of TCE

The measurements in the test bottles containing both TCE and NH_4–N showed that the percent total decrease in TCE was 40% at 50 ppb TCE, 44% at 100 ppb TCE, 29% at 500 ppb TCE, 29% at 1000 ppb TCE, 22% at 2000 ppb TCE and 21% at 4500 ppb TCE. Considering the percent decreases in Section 3.3 due to volatilization, these removals were much higher. Therefore, it was concluded that a significant portion of TCE was removed by cometabolism. After correction for volatilization into head space, the cometabolic TCE removal percentages were found as 34% in a period of 3 h at 50 ppb TCE; 36% at 100 ppb TCE, 21% at 500 ppb TCE, 21% at 1000 ppb TCE, 14% at 2000 ppb TCE and 13% at 4500 ppb TCE in a period of 4 h.

The total TCE removal rates (without correction for volatilization), the volatilization rates and the cometabolic degradation rates are shown in Fig. 5 at a constant initial ammonium concentration of 40 mg/L NH_4–N. As seen from the figure, the cometabolic degradation rate of TCE increased linearly with TCE concentration and a first-order rate constant was obtained as 0.0341 ppb/h. As seen from Table 1, the VSS concentration in all experiments varied between 123–144 mg/L with an average value of 134 mg/L. Based on this average VSS concentration, the first-order cometabolic TCE degradation rate constant was determined as $0.25\,L\,g^{-1}\,VSS\,h^{-1}$ ($0.0061\,L\,mg^{-1}\,VSS\,d^{-1}$), this value is considerably lower than the values (0.74 and $1.02\,L\,mg^{-1}\,VSS\,d^{-1}$) reported for pure *N. Europaea* species. However, in the studies with pure *N. Europaea* species, the biomass is reported in terms of protein. The reported values of 0.74 and $1.02\,L\,mg^{-1}\,VSS\,d^{-1}$ are based on the assumption that the dry cell mass consists of 50% protein [16]. Moreover, although the sludge used in the present study was enriched for nitrifiers, it certainly contained a high heterotrophic fraction since it originated from a sewage treatment plant. Therefore, in our system, the fraction of nitrifiers in the total VSS expression was much smaller than in pure culture studies and the cometabolic removal rates of TCE in our systems were quite low when the results were expressed on the basis of VSS.

4. Conclusions

Exposure of an enriched nitrifier culture to TCE resulted in both a decrease in specific oxygen uptake rate and specific ammonium utilization rate (qNH_4–N). In both SOUR and qNH_4–N similar decreases were observed which reached 50% in the TCE concentration range of 1000–2000 pbb. The effect of TCE on ammonium oxidation was probably caused by the inhibition of the AMO enzyme by this compound rather than the short-lived toxic intermediates produced during cometabolic TCE oxidation. TCE was found to

be cometabolically degradable by this enriched nitrifier culture; however, the rate constant in the case of cometabolic TCE removal indicated that cometabolic degradation rate of TCE by enriched nitrifier culture was quite low with respect to those observed for pure *N. Europaea* species. The cometabolic degradation rate of TCE was found to be dependent on the initial TCE concentration. These results could fill a gap in the literature about the cometabolic degradation of TCE with enriched nitrifiers. There are hardly any such detailed studies with an enriched nitrifying culture which show the dependence of cometabolic degradation on various factors. However, it is more realistic to use an enriched culture in full-scale biological treatment and bioremediation systems than pure nitrifying strains. These results could be useful in designing any system for the cometabolic removal of TCE using ammonium as an energy and growth substrate. They may be applied to industrial wastewater treatment as well as to remediation of contaminated groundwaters and soils. However, the data presented here do not allow an adequate estimation of kinetic parameters. Therefore, the study is being continued for the purpose of kinetic modeling under various conditions.

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