Journal of Environmental Sciences Vol. 16, No. 3, pp. 487—489, 2004

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)03-0487-03 **CLC number**: X131 **Document code**: A

Cometabolic microbial degradation of trichloroethylene in the presence of toluene

SUI Hong[1, 2], LI Xin-gang[1], XU Shi-min[1, *]

(1. National Engineering Research Center for Distillation Technology, Tianjin 300072, China. E-mail: xingli@tju.edu.cn; 2. School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China)

Abstract: Trichloroethylene (TCE), a common groundwater pollutant, was cometabolized by microorganisms in the presence of toluene as a growth substrate. The effect of concentrations of toluene and TCE and temperature on biodegradation was discussed. Acclimated microorganisms degraded TCE after a lag period of 5 to 22 h depending on toluene concentrations. Approximately 60%, 90% and 64% of TCE were degraded at toluene to TCE concentration ratios of 23 : 1, 115 : 1 and 230 : 1, respectively. At a TCE concentration of 1.46 μg/ml, 80% of TCE and 98.4% of toluene were removed. But less degradation of TCE and toluene was observed when TCE concentration was above 48.8 μg/ml. The lag time of TCE decreased and the TCE biodegradation rates increased with the increase of temperature.

Keywords: cometabolism; microbial degradation; toluene; trichloroethylene

Introduction

Improper storage and waste management practices have left a legacy of contaminated soil and aquifers, threatening drinking water supplies in many areas. Chlorinated ethenes, particularly trichloroethylene(TCE), are among the frequently detected soil and groundwater contaminants(Barbash, 1986). Chlorinated ethenes in general do not serve as substrates for aerobic growth. Under aerobic conditions, microbial degradation of TCE(non-growth substrate) occurs only in the presence of a growth substrate or another transformable compound.

Laboratory investigations(Folsom, 1990; Rasche, 1991; Lu, 1998) have identified a number of aerobic microorganics capable of transforming chlorinated ethenes aerobically. Aerobic cometabolism requires the addition of oxygen as the electron acceptor and an appropriate electron donor such as methane, phenol, toluene, ammonia and propane and so on. To date, few reports are available on the effect of TCE concentration and temperature on the cometabolic degradation of TCE.

In this work, toluene was used as a growth substrate for biodegradation of TCE. The effects of concentrations of TCE and toluene as well as temperatures on the biodegradation process were investigated so as to find favorable conditions for the experiment of TCE cometabolic degradation in the aquifer.

1 Materials and methods

1.1 Microorganisms and chemicals

The microorganisms were initially obtained from Dagang Oil Field, Tianjin, China and acclimated for about two months. Culture compositions and domesticated conditions are listed in Table 1. Other conditions include: pH 6.8—7.0, temperature 20 ± 1℃, dissolved oxygen 2.7 ± 0.5 mg/L.

Table 1 Culture compositions and domesticated conditions

Culture composition, mg/L	< 15 d	15—30 d	> 30d
$(NH_4)_2SO_4$	2000	2000	1000
Glucose	2000	500—1000	0
Toluene	0	0—515	515
KH_2PO_4	500	500	300
K_2HPO_4	500	500	300
$MgSO_4$	30	30	30
$Fe_2(SO_4)_3$	30	30	30
$MnSO_4 \cdot H_2O$	40	40	40
$ZnSO_4 \cdot H_2O$	50	50	50

The mineral medium for cometabolism consisted of $(NH_4)_2SO_4$ 2 g/L, KH_2PO_4 1 g/L, K_2HPO_4 0.5 g/L, $CaCl_2 \cdot H_2O$ 0.03 g/L, $Fe_2(SO_4)_3$ 0.03 g/L, $ZnSO_4 \cdot H_2O$ 0.034 g/L, $MnSO_4 \cdot H_2O$ 0.039 g/L, and $MgSO_4 \cdot 7H_2O$ 0.046 g/L.

All chemicals used were of analytical grade and obtained commercially.

1.2 Degradation experiments

Degradation experiments were conducted in 300 ml reactors. Each flask has two gas sampling ports and one liquid sampling port. Aerated nutrient-containing solutions were added to the sealed flasks from the liquid port. Each reactor contained 30 ml suspended microorganism solution. TCE and toluene were injected into the sealed flasks with a gastight syringe and the reactors were placed on a rotary shaker(140 r/min) at 20, 25 and 30℃, respectively. In each experiment, there are replicate flasks in order to insure the experimental results.

1.3 Analytical methods

Biodegradation of TCE and toluene was measured by gas

Foundation item: The National Natural Science Foundation of China(No. 20276048); * Corresponding author

chromatographic analysis of headspace concentrations over time. 0.2 ml of headspace was removed with a gastight syringe and injected into an Autosystem XL gas chromatograph (Perkin Elmer) equipped with a capillary column(30 m × 0.53 mm i.d. × 1 μm) and a Turbochrom 4.1 workstation and detected by a flame ionization detector. N_2(1 ml/min) was used as the carrier gas. Other conditions were: injection temperature 250℃, oven temperature 150℃, detector temperature 300℃, H_2 flow rate 15 ml/min, air flow rate 300 ml/min, split ratio 10:1.

2 Measurement of Henry constant

Henry constant was measured as follows: Sterilized water(400 ml) was added to two 700 ml sterilized glass vials sealed with latex tube and two clamps. 100 μl TCE and 100 μl toluene were injected into the sealed vials by gastight syringes. The two vials were placed on a rotary shaker at 100 r/min and room temperature for 24 h. Then samples of headspace solution were removed for gas chromatography analysis. Henry constant was calculated from the concentration ratio of headspace to solution(Table 2).

Table 2 Henry constants(H) of TCE and toluene

Chemical	Headspace concentration, μg/ml	Solution concentration, μg/ml	H (Calculated)	H (Literature)
TCE	37.78	91.31	0.41	0.40
Toluene	61.54	212.19	0.29	0.26

Henry constants of 0.41 and 0.29 were calculated for TCE and toluene, respectively. These values are in agreement with the literature data (Yaws, 1991). The equilibrium concentration of TCE and toluene in the solution were calculated based on the measured Henry constants.

3 Results and discussion

3.1 Effect of toluene concentration

Fig.1a shows that no TCE degradation occurred in the absence of toluene. The lag period for TCE degradation was the shortest when toluene to TCE concentration ratio was 115:1. This is in disagreement with the results of Der and Scow (Der, 1994) who reported that the lag period for degradation TCE and toluene increased with increasing toluene concentration. Approximately 60%, 95%, and 64% of TCE was degraded at initial toluene concentrations of 33.7, 168.6 and 337.2 μg/ml, respectively. The highest removal rate and most significant degradation of TCE were observed when toluene to TCE concentration ratio was 115:1.

Fig.1b shows that compared with TCE degradation, the lag period for toluene biodegradation decreased greatly as toluene was used as the only source for microorganisms during the late domestication. The no detectable biodegradation of TCE and toluene about 60 h later may be due to the short of dissolved oxygen or nutrients.

The oscillation of TCE and toluene degradation may be ascribed to the competitive inhibition effects that TCE has on toluene as evidenced by less oscillations at higher toluene concentrations.

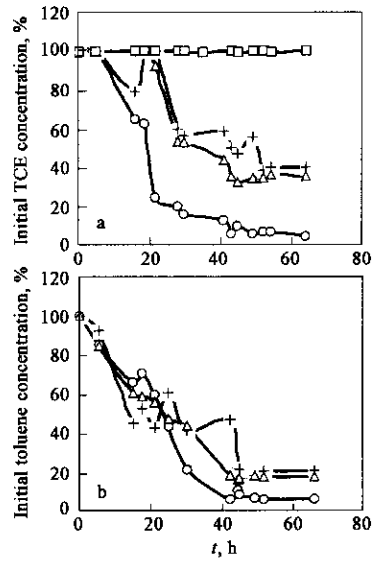

Fig.1 Biodegradation of TCE (a) and toluene (b) at different initial toluene concentrations

—+—toluene to TCE concentration ratio: 23:1; —○—toluene to TCE concentration ratio: 115:1; —△—toluene to TCE concentration ratio: 230:1; —□—no toluene

3.2 Effect of TCE concentration

The effect of initial TCE concentration on the biodegradation of toluene and TCE was measured. Initial concentrations of TCE and toluene used in experiments are listed in Table 3. TCE removal rates decreased and the lag period increased from approximately 4 h to 24 h with increasing initial TCE concentration(Fig.2a). At lower TCE concentrations of 0.49, 0.98 and 1.46 μg/ml, the lag periods of TCE and toluene were almost the same. The data of biodegradation of toluene are shown in Fig.2b. With increases in TCE concentration, the length of the lag period increased greatly. The lag periods preceding toluene degradation were increased from approximately 1.8 to 15 h respectively. The larger the initial TCE concentration was, the less the degradation extents of TCE and toluene were. For example, at a TCE concentration of 1.46 μg/ml, 80% of TCE and 98.4% of toluene were removed. In contrast, only 37% of TCE and 22% of toluene were degraded at a TCE concentration of 488 μg/ml. Higher concentration of TCE may be more toxic to microorganisms. Toxicity of TCE to pure and mixed cultures has been reported (Broholm, 1990; Oldenhuis, 1991).

Table 3 Initial concentrations of TCE and toluene

Reactor	TCE, μg/ml	Toluene, μg/ml
1#	0.49	289.0
2#	0.98	289.0
3#	1.46	289.0
4#	48.8	289.0
5#	488.0	289.0

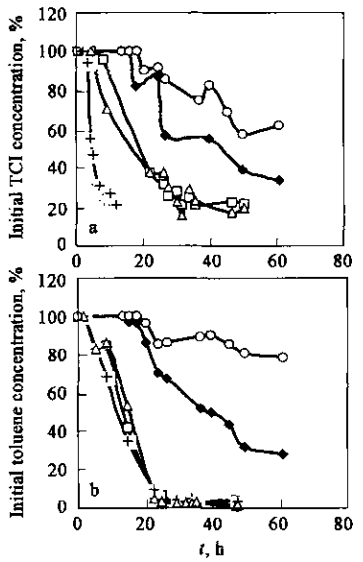

Fig.2 Biodegradation of TCE (a) and toluene (b) at different TCE concentrations

—□—initial TCE concentration 0.49 μg/L; — + —initial TCE concentration 0.98 μg/L; —△—initial TCE concentration 1.46 μg/L; —◆—initial TCE concentration 48.8 μg/L; —○—initial TCE concentration 488.0 μg/L

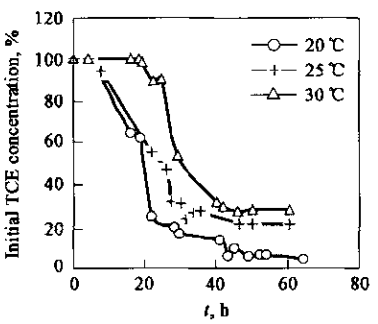

Fig.3 Variation of TCE degradation with temperature

3.3 Effect of temperature

Fig.3 illustrates the influence of temperature on TCL degradation at initial TCE and toluene concentrations of 0.98 μg/ml and 168.6 μg/ml respectively. TCE biodegradation rates increased with the increase of temperature. The maxium specific substrate utilization rates (μ_{max}) obtained by a least-squares fit were 0.055, 0.068 and 0.336 g/(ml · h) respectively for temperature of 20, 25 and 30℃. The lag period preceding TCE degradation was the same at 30 and 25℃ and was longer at 20℃. In the cold regions, low temperature became the main limiting factor and the thermally enhanced biodegradation was required. For example, Filler *et al*. (Filler, 2001) combined mechanical heating with insulation system design to enhance biodegradation of petroleum-contaminated soils in the Actic at Prudhoe Bay, AK.

4 Conclusions

This paper studied the effect of temperature and concentrations of toluene and TCE on cometabolic degradation of TCE. The results showed that TCE degradation occurred only in the presence of a growth substrate, such as toluene. At initial toluene concentrations of 33.7, 168.6 and 337.2 μg/ml, about 60%, 95% and 64% of TCE was degraded, respectively. TCE concentrations above 40 ppm slow down the degradation of both TCE and toluene. In addition, the appropriate temperature will speed the biodegradation of TCE and toluene. All above conclusions can offer useful information for the removal of TCE in groundwater successfully.

References:

Barbash J, Roberts P V, 1986. Volatile organic chemical contamination of groundwater resources in the U.S[J]. J Water Poll Cont Fed, 58: 343—348.

Broholm K, Jensen B K, Christensen T H *et al*., 1990. Toxicity of 1,1,1-trichloroethane and trichloroethene on a mixed culture of methane-oxidizing bacteria[J]. Appl Environ Microbiol, 56: 2488—2493.

Der Y M, Scow K M, 1994. Effect of trichloroethylene (TCE) and toluene concentrations on TCE and toluene biodegradation and population density of trichloroethylene and toluene degraders in soil[J]. Appl Environ Microbiol, 60: 2661—2665.

Filler D M, Lindstrom J E, Braddock J F *et al*., 2001. Integral biopile components for successful bioremediation in the Arctic[J]. Cold Regions Sci and Technol, 32:143—156.

Folsom B R, Chapman P J, Pritchard P H, 1990. Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: kinetics and interactions between substrates[J]. Appl Environ Microbiol, 56: 1279—1285.

Lu C J, Lee C M, Chung M S, 1998. The comparison of trichloroethylene removal rates by methane and aromatic utilizing microorganisms[J]. Wat Sci Tech, 38: 19—24.

Oldenhuis R, Oedzes J Y, van der Waarde J J *et al*., 1991. Kinetics of chlorinated hydrocarbon degradation by methylosinus trichosporium OB3b and toxicity of trichloroethylene[J]. Appl Environ Microbiol, 57: 7—14.

Rasche M E, Hyman M R, Arp D J, 1991. Factors limiting aliphatic chlorocarbon degradation by Nitrosomonas europaea: comrtabolic inactivation of ammonia monooxygenase and substrate specificity[J]. Appl Environ Microbiol, 57: 2986—2994.

Yaws C, Yang H C, Pan X, 1991. Henry's law constants for organic compounds in water[J]. Chem Eng, 11: 179—185.

(Received for review April 2, 2003. Accepted June 15, 2003)