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SOIL MICROCOSM STUDIES FOR AEROBIC COMETABOLISM OF 1,1,1-TRICHLOROETHANE, 1,1-DICHLORETHYLENE, TRICHLOROETHYLENE AND OTHER CHLORINATED ALIPHATIC HYDROCARBONS BY BUTANE-OR PROPANE-UTILIZING MICROORGANISMS

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Abstract : Microcosm studies were performed to determine the potential of microorganisms grown on butane or propane to cometabolize chlorinated aliphatic hydrocarbon (CAH) mixtures and to evaluate nutrient addition effects on primary substrate degradation and CAHs transformation rates. Chloroform, chlorinated ethane mixtures [1,2-dichloroethane (1,2-DCA), 1,1-dichloroethane (1,1-DCA), 1,1,1-trichloroethane (1,1,1- TCA) and 1,1,2-trichloroethane (1,1,2-TCA)], chlorinated ethylene mixtures [vinyl chloride (VC), cis-1, 2-dichloroethylene (*c*-DCE)], and mixtures of 1,1,1-TCA and 1,1-dichloroethylene (1,1-DCE) were transformed by butane-utilizers. Propane utilizers showed ability to transform a broader range of CAHs than butane-utilizers, especially trichloroethylene (TCE) and trans-1,2-dichloroethylene (*t*-DCE). Nutrient addition (yeast extract) resulted in the increase of both butane and propane utilization and CAHs transformation rates. Nutrient addition increased microbial activity, resulting in the effective transformation of CAHs mixtures. These results indicate that butane- and propane-utilizers can transform problematic CAHs mixtures, including 1,1-DCE.

Key Words : aerobic cometabolism, butane-utilizing microorganisms, chlorinated aliphatic hydrocarbons, propane-utilizing microorganisms, soil microcosm

INTRODUCTION

Chlorinated aliphatic hydrocarbon (CAH) mixtures (i.e. chlorinated methanes, ethenes, and ethylenes), rather than single CAH usually contaminate groundwater. CAH mixtures are difficult to remediate through aerobic cometabolism, since few microorganisms have been known to simultaneously and effectively transform CAH mixtures. Phenol-, and toluene-oxidizing microorganisms effectively transform chlorinated ethenes [e.g. trichloroethylene (TCE)], however, chlorinated methanes [e.g. chloroform (CF)] and ethanes [e.g. 1,1-dichloroethane (1,1-DCA) and 1,1,1-trichloroethane (1,1,1-TCA)] are not effectively transformed.[1)] Methanotrophs expressing soluble methane monooxygenase (sMMO) effectively transformed

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chlorinated methanes and ethanes and less effectively transformed chlorinated ethenes, while those expressing particulate methane monooxygenase (pMMO) are not effective.[2] Thus, finding aerobic systems for effective transformation of CAH mixtures is of interest. In this study the potential of butane or propane as cometabolic growth substrates to transform CAH mixtures was evaluated.

In microcosm studies[3,4] butane- and propane-utilizing microorganisms, enriched from aquifer solids from DOE Hanford Site in Washington, showed the ability to effectively cometabolize CF and 1,1,1-TCA. In microcosm studies presented here the potential of microorganisms grown on butane or propane to cometabolize CAH mixtures was determined. The studies evaluated four different mixtures: a mixture of chlorinated ethanes (1,1-dichloroethane [1,1-DCA], 1,2-dichloroethane [1,2-DCA], 1,1,1-TCA, and 1,1,2-trichloroethane [1,1,2-TCA]), chlorinated ethylenes (perchloroethylene [PCE], trichloroethylene (TCE), cis-1, 2-dichloroethylene [*c*-DCE], trans-1,2-dichloroethylene [*t*-DCE], and vinyl chloride [VC]), a mixture of 1,1,1-TCA and 1,1-dichloroethylene [1,1-DCE], and a mixture of 1,1-DCE and TCE.

MATERIALS AND METHODS

Soil Microcosm Preparation

In previous soil microcosm studies,[3,4] indigenous butane- or propane-utilizing microorganisms were stimulated with groundwater (50 mL), aquifer solids (25 g), and headspace air (60 mL) through repeated additions of butane or propane into soil microcosms (125 mL) in the presence of CF or 1,1,1-TCA. A suspension (1-mL) from these microcosms was used as inoculum for these studies. The new microcosms were constructed as described previously,[4] but were sterilized by exposure for 11 hr to a Cobolt 60 gamma irradiation source prior to inoculum addition. The soil microcosms were incubated on a rotary shaker (200 rpm) at room temperature, and no nutrient was initially added into them.

The microcosms were operated as described by Kim et al.[4] The total mass of propane or butane added was 0.09 μmole for each incubation, representing aqueous concentration of 51 and 31 μmole/L, respectively. Initial average aqueous concentrations of chlorinated ethanes and chlorinated ethylenes were 0.6 (standard deviation [SD] $\pm$0.12) mole/L and 0.8 (SD $\pm$0.14) mole/L, respectively, representing total masses addition ranging from 0.03 to 0.09 μmole.

Analysis

Headspace concentrations of propane and butane were analyzed on a HP5890A series gas chromatograph (GC) using 3.2-mm $\times$ 1.2-m HayeSep D80/100-mesh, packed column (Alltech Associates, Deerfield, IL) and a flame ionization detector (FID). CAH analysis was conducted by injecting liquid samples onto a purge & trap and using HP 5890 series II GC with 0.25-mm $\times$ 30-m HP-624 capillary column and Model 5220 electrolytic conductivity detector (OI analytical, College station, TX).

RESULTS AND DISCUSSION

Transformation of CAH Mixtures in Propane-fed Microcosms

The ability of propane-utilizers to transform mixtures of chlorinated ethylenes and chlorinated ethanes is shown in Figure 1, respectively. Prior to the addition of trace nutrients (yeast extract, 25 mg/L), slower propane degradation and transformation of CAHs was observed. Propane-utilizers transformed all chlorinated ethylenes except PCE, with *c*-DCE being most rapidly transformed followed by VC, TCE and *t*-DCE. Propane-utilizers also transformed all chlorinated ethanes tested, with 1,2-DCA being most rapidly transformed followed by 1,1-DCA, 1,1,2-TCA and 1,1,1-TCA. These results demonstrate a broad range of CAH transformation abilities of propane-utilizers. These results are consistent with those

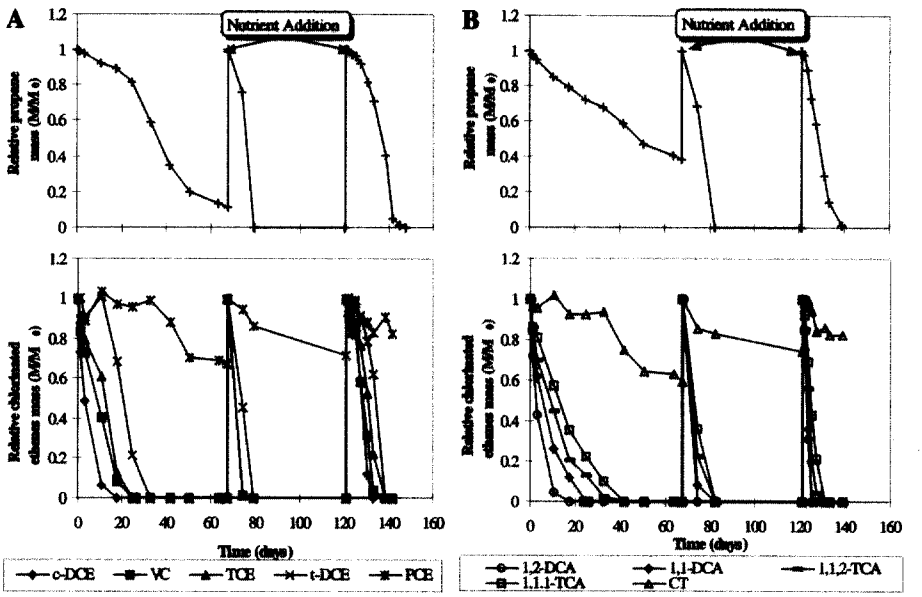

Figure 1. Propane degradation and transformation of mixtures of chlorinated ethylenes (A) and chlorinated ethanes (B) in propane microcosms.

of Tovanabootr and Semprini[5] who found propane-utilizing microorganisms enriched from the subsurface of McClellan AFB were able to transform mixtures of CF, 1,1,1-TCA, and TCE. Thus microorganisms grown on propane appear to have good potential to transform CAH mixtures.

Nutrient Addition Effects on Degradation/ Transformation of Propane, Butane, and CAHs

Table 1 presents the first order rates of degradation of butane, propane, and transformation of CAH mixtures with and without trace nutrient addition (yeast extract, 25 mg/L). With trace nutrient addition, rates of propane and butane utilization increased by factors 3 to 8 and 8 to 20, respectively, while CAH transformation rates increased by factors of 2 to 13 and 4 to 22. Tracer nutrient addition had a greater impact on the butane-utilizers. No CAH transformation was observed with yeast extract addition in the absence of butane, indicating yeast extract provided nutrients, and likely not an energy source (Data not presented). A positive correlation between CAH transformation rates and primary substrate utilization rates was observed in both the butane and propane microcosms. Tovanabootr and Semprini[5] reported that a positive correlation between TCE transformation rates and growth substrate utilization rates in both methane and propane-fed microcosms. This correlation may result from enhanced total activity or an increase in the microbial population. The large increase suggests enhanced total activity results from nutrient addition.

In butane-fed microcosms, all the chlorinated ethanes tested were effectively transformed with the same sequential removal as observed in propane-fed microcosm. Here again the more chlorinated ethanes were transformed more slowly. Similar results were obtained in resting cell studies[6] with butane-grown cells. Butane-utilizers were less effective in transforming chlorinated ethylenes than propane-utilizers (Table 1). VC was most rapidly transformed followed by *c*-DCE. Some TCE transformation was observed, while *t*-DCE was not transformed. Butane-utilizers more effectively transformed VC than *c*-DCE, while

Table 1. Comparison of the first order rates of degradation/transformation of butane, propane, and CAH mixtures before and after yeast extract (YE, 25 mg/L) addition in butane and propane microcosms

CAH mixture	Compound	First order rates (per day)[a]					
		Butane microcosm			Propane microcosm		
		Before YE addition	After YE addition	Factor of rate increase[c]	Before YE addition	After YE addition	Factor of rate increase
CF only	CF	0.01	0.04	4	0.14	1.01	7
	Butane	0.02	0.40	20	NA	NA	NA
	Propane	NA[b]	NA	NA	0.02	0.15	8
Chlorinated ethylenes	VC	0.09	0.69	8	0.09	1.20	13
	c-DCE	0.01	0.20	20	0.25	2.41	10
	t-DCE	≈0	≈0	NT[d]	0.02	0.11	6
	TCE	≈0	0.05	75	0.05	0.67	13
	PCE	≈0	≈0	NT	≈0	≈0	NT
	Butane	0.03	0.25	8.3	NA	NA	NA
	Propane	NA	NA	NA	0.01	0.04	4
Chlorinated ethanes	1,2-DCA	0.02	0.30	15	0.32	1.01	3
	1,1-DCA	0.01	0.22	22	0.12	0.37	3
	1,1,2-TCA	0.01	0.15	15	0.08	0.22	3
	1,1,1-TCA	0.01	0.11	11	0.07	0.15	2
	CT	≈0	≈0	NT	≈0	≈0	NT
	Butane	0.02	0.15	8	NA	NA	NA
	Propane	NA	NA	NA	0.01	0.06	6
1,1-DCE, TCE, and 1,1,1-TCA	1,1-DCE	0.03	0.12	4	0.04	0.32	8
	TCE	NA	NA	NA	0.10	1.01	10
	1,1,1-TCA	0.01	0.07	7	NA	NA	NA
	Butane	0.02	0.16	8	NA	NA	NA
	Propane	NA	NA	NA	0.01	0.03	3

[a] The first order rates represent overall mass degradation rates including known headspace and liquid volume and Henry's law constant. The first order rates of each compound were calculated based on these assumptions: 1) aqueous concentration of each compound is much smaller than half-saturation coefficient; 2) inhibition constant is much larger than aqueous inhibitor concentration. [b] NA indicates compounds are not added. [c] Through nutrient addition. [d] NT indicates no uptake observed with and without YE addition.

propane-utilizers more effectively transformed *c*-DCE than VC. In these microcosms, propane-utilizers were capable of transforming a broader range of CAH than butane-utilizers.

Transformation of 1,1-DCE and 1,1,1-TCA in Butane-fed Microcosms

The transformation of mixtures of 1,1-DCE and 1,1,1-TCA was evaluated in butane-fed microcosms with and without nutrient addition (Figure 2). A 1,1-DCE/1,1,1-TCA mixture was investigated since they are often observed as groundwater co-contaminants resulting from the abiotic transformation of 1,1,1-TCA to 1,1-DCE. In the absence of nutrient addition complete removal of 1,1-DCE and slow transformation of 1,1,1-TCA was observed in duplicate microcosms. After 86 days of incubation, nutrient was added to one of the microcosms (Figure 2(B)). In microcosm without nutrient addition, 1,1-DCE was transformed slowly and minor 1,1,1-TCA transformation was observed. After 70% removal of

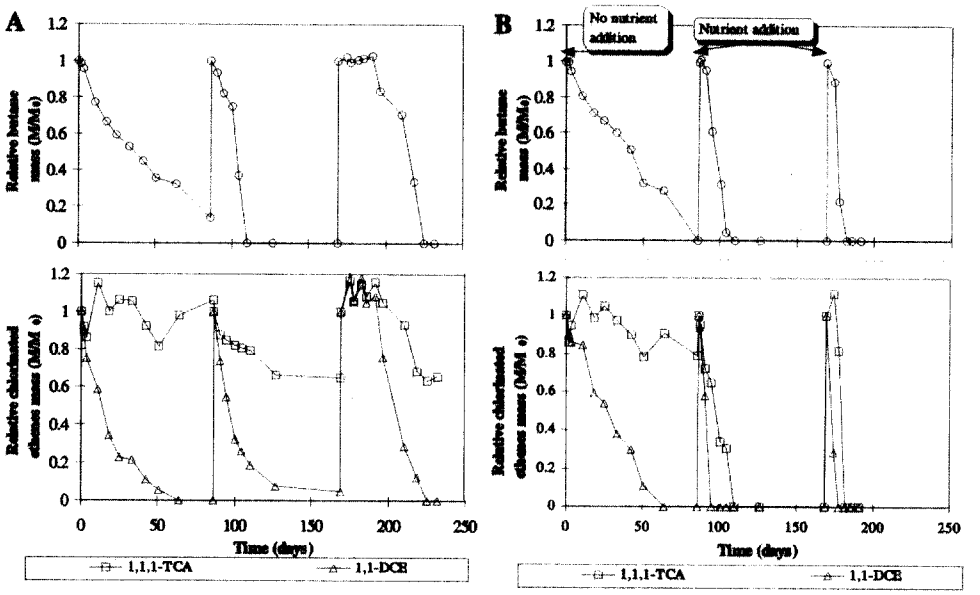

Figure 2. Butane degradation and transformation of mixtures of 1,1,1-TCA and 1,1-DCE without (A) and with (B) the addition of yeast extract (25 mg/L) in the butane microcosms.

1,1-DCE, fairly fast propane uptake was observed, but a minor amount of 1,1,1-TCA transformed. Nutrient addition resulted in a factor of 8 increase in the rate of butane degradation and a factor of 4 increase in the rate of 1,1-DCE transformation. Complete removal of 1,1-DCE and 1,1,1-TCA was observed. Significant butane utilization and 1,1,1-TCA transformation was observed after complete removal of 1,1-DCE, suggesting that 1,1-DCE competitively inhibits the degradation of both butane and 1,1,1-TCA. Butane also appeared to inhibit 1,1,1-TCA transformation. When butane, 1,1-DCE, and 1,1,1-TCA were re-added after 170 days of incubation, similar inhibition was observed. These microcosm results indicate that butane-utilizers have potential for transforming 1,1-DCE and 1,1,1-TCA mixtures.

Transformation of 1,1-DCE and TCE in Propane-fed Microcosms

Transformation of a TCE and 1,1-DCE mixture was evaluated in a propane-fed microcosm. 1,1-DCE has been a very problematic compound to cometabolize due to its high transformation product toxicity (defined as toxicity resulting from byproducts of CAH transformation rather than CAH itself[7]) and strong inhibition on the transformation of the other chlorinated ethylenes.[6,8,9] Complete removal of both TCE (106 μg/L in aqueous solution) and 1,1-DCE (54 μg/L in aqueous solution) was observed with and without nutrient addition (Figure 3). Propane utilization accelerated after TCE and 1,1-DCE were transformed, indicating TCE and 1,1-DCE competitively inhibited propane utilization. In field test, 1,1-DCE and TCE inhibited phenol utilization.[8] 1,1-DCE also appears to be strong competitive inhibitor on propane. Limited inhibition of 1,1-DCE on TCE transformation was observed after the 1,1-DCE concentration was increased by a factor of 1.5. Although more studies are needed, propane-utilizers are capable of transforming TCE and 1,1-DCE mixtures in the aqueous concentration range of 100 μg/L.

The ability of propane-utilizers to transform both CAHs at concentrations of TCE (264 μg/L) and 1,1-DCE (146 μg/L) was evaluated in the third addition. Slow and nearly constant

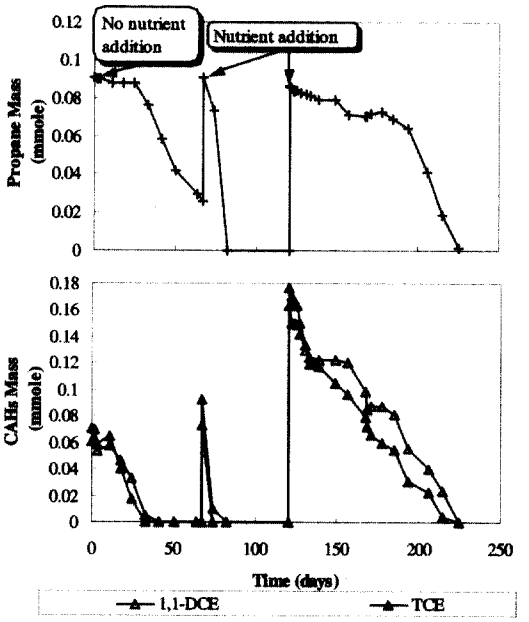

Figure 3. Propane degradation and transformation of TCE and 1,1-DCE with increasing concentrations of both CAHs in a propane-fed microcosm.

transformation of TCE and 1,1-DCE was observed for 103 days. The propane utilization rate was very slow at these high concentrations of TCE and 1,1-DCE. The propane utilization rate in the absence of TCE and 1,1-DCE was about 18 times faster (Data not shown). After 80% of TCE and 60% of 1,1-DCE was transformed, faster propane utilization was observed. This prolonged degradation/transformation of propane, TCE, and 1,1-DCE might be due to transformation product toxicity of TCE and 1,1-DCE and inhibition between the compounds.

CONCLUSIONS

Butane-utilizers have ability to transform chlorinated ethane mixtures (1,2-DCA, 1,1-DCA, 1,1,1-TCA and 1,1,2-TCA), chlorinated ethylene mixtures (VC and *c*-DCE), and mixtures of 1,1,1-TCA and 1,1-DCE. Propane utilizers showed ability to transform a broader range of CAHs than butane-utilizers.

The rates of butane and propane utilization and CAHs transformation were increased through nutrient addition (yeast extract). A positive correlation was also observed between CAH transformation rates and primary substrates utilization rates. Nutrient addition increased microbial activity, resulting in the effective transformation of CAHs mixtures. Butane-utilizers transformed a mixture of 1,1,1-TCA and 1,1-DCE. A mixture of TCE and 1,1-DCE was transformed by propane-utilizers. These results indicate that butane- and propane-utilizers can transform problematic CAHs mixtures, including 1,1-DCE.

Many sites are contaminated with CAH mixtures.[10] Our results indicate butane and propane are effective cometabolic growth substrates for the treatment of CAH mixtures. Microorganisms grown on phenol and toluene effectively transform chlorinated ethylenes, but not chlorinated methanes or ethanes.[1,8] Our work has shown propane as a better substrate for transforming mixtures of chlorinated ethylenes than butane. The results indicate the great potential of bioremediating aquifers contaminated with CAH mixtures using microbes grown on propane or butane.

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