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Effect of Perchloroethylene (PCE) on Methane and Acetate Production by a Methanogenic Consortium

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ABSTRACT

The effects of perchloroethylene (PCE) concentration in the range of 0–100 mg/L on methane and acetate production by a methanol-enriched methanogenic consortia were investigated at 17°C. The results indicate that PCE is more inhibitory to methanogenesis than to acetogenesis. At concentrations as low as 10 ppm, PCE affects the methanogenic activity of the consortium, and has completely inhibited this activity at 100 ppm. Conversely, PCE does not begin to inhibit acetogenic activity until the concentration is above 10 ppm, and has not completely inhibited it even at a PCE concentration of 100 ppm.

Index Entries: Methanogenic; acetogenic; PCE; inhibition.

INTRODUCTION

Perchloroethylene (PCE) is one of the priority pollutants found in the groundwater at many contaminated sites. This compound was used as a degreasing solvent, but improper disposal and/or operating practices have led to subsurface contamination. Hence, technologies are needed to remediate this contaminant from the groundwater. One technology that is being developed to treat such sites is *in situ* bioremediation. Several researchers have reported that sequential reductive dechlorination of PCE to lower chloroethenes can be accomplished under methanogenic conditions *(1–3)*. Others have reported complete dechlorination to nontoxic end products, such as ethylene *(4–6)*, ethane *(7)*, and carbon dioxide *(8)*.

Previous studies have demonstrated that the activity of the dechlorinating culture may be inhibited by the chlorinated solvent. For example, Renard et al. *(9)* found that a mixture of polychlorinated aliphatic compounds inhibited methanogenesis. These authors also found that the degree of inhibition increased as the concentration of the chlorinated solvent increased and that complete inhibition was achieved at 100 mg/L of total solvents. Additionally, it was reported that individual contaminants were more toxic than the mixed contaminants. In another study, Blum

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and Speece *(10)* reported that PCE, trichloroethylene (TCE), and 1,2-DCE caused 50% inhibition of methanogenesis at 22, 13, and 19 mg/L, respectively. However, Trevors *(11)* showed that any investigation of the toxic effects of pollutants on environmental processes should not rely on any single measurement, such as methane production rate, to describe microbial activity. This conclusion was based on their observation that methylene chloride caused instabilities in the electron transport system of fresh water sediment microbes and inhibited CO_2 evolution. However, they also found that it stimulated oxygen uptake.

Chou et al. *(12)* correlated molecular structure to toxicity. They demonstrated that chlorine substitution, aldehydes, double bonds, and benzene rings were toxic to unacclimated methanogenic cultures. Additionally, it was found that as the number of hydroxyl groups increased, and as the carbon chain length increased, the toxicity decreased. Finally, these authors report that acclimation to a toxic compound can substantially reduce its toxicity, particularly for aldehydes and compounds containing double bonds and benzene rings.

To date, there have been few reports that demonstrate the effects of chlorinated solvent concentration on the multiple metabolic activities that are present in a typical anaerobic consortium. In this article, such experimental results are presented. Here, the effect of PCE concentration on the activity of acetogenic and methanogenic bacteria in a mixed culture is presented. Such qualitative information provides the background needed to design quantitative experiments from which the kinetic expressions that describe the microbial degradation of these contaminants can be developed. These kinetic expressions can then, in turn, be used in the numerical prediction of contaminant fate and transport in the subsurface.

MATERIALS AND METHODS

Microbial Inoculum

Water-saturated, anaerobic sediments were obtained from the top 25 cm of an uncontaminated portion of the Yakima River delta in southeastern Washington state. These sediments were subsequently cultivated anaerobically with methanol as described by Skeen, et al. *(13)*. A stock culture was prepared for these experiments by adding 20 g of sediment and 40 mL of culture media with 3 g/L methanol to a 100-mL serum bottle under anaerobic conditions. The culture was continuously maintained by exchanging 20 mL of culture solution with fresh media and methanol every 3 wk. To grow the inoculum for this experiment, the culture was revived by adding 20 mL of fresh anaerobic media to the serum bottle, which was then incubated in the dark at 37°C. After 3 d the bottle was shaken, the soil was allowed to settle, and the supernatant was then used to inoculate two 125-mL serum bottles containing 90 mL of fresh anaerobic medium. These cultures were incubated at 37°C for 7 d and were used as the inoculum source for the experiments. Separate growth tests indicated that the culture was in exponential growth after this time period.

Chemicals

HPLC-grade PCE, having a purity of 99.9% or better, was purchased from Sigma Chemical Co. (St. Louis, MO). Trichloroethylene (TCE), with a purity of 99.6% was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). trans-1,2-dichloroethylene (trans-1,2-DCE), with a purity of 98% and cis-1,2-dichloroethylene

(cis-1,2-DCE), of 97% purity were obtained from Aldrich Chemical Co. (Milwaukee, WI). Methane standards were obtained from Scott Speciality Gases and Equipment (Fremont, CA). All other chemicals were obtained from J. T. Baker Chemical Co.

The media used in these experiments was the same as that employed by Skeen et al. *(13)* and was composed of (per liter of deionized water): KH_2PO_4, 270 mg; K_2HPO_4, 350 mg; NH_4Cl, 530 mg; $CaCl_2 \cdot 2H_2O$, 75 mg; $MgCl_2 \cdot 6H_2O$, 100 mg; $FeCl_2 \cdot 4H_2O$, 20 mg; $NaHCO_3$, 1200 mg; $Na_2S \cdot 9H_2O$, 250 mg; $HO_2CCH_2N(CH_2CO_2Na)_2$ (NTA), 10 mg; $MnCl_2 \cdot 4H_2O$, 0.5 mg; H_3BO_3, 0.5 mg; $ZnCl_2$, 0.5 mg; $CoCl_2 \cdot 6H_2O$, 0.5 mg; $NiSO_4 \cdot 6H_2O$, 0.5 mg; $CuCl_2 \cdot 2H_2O$, 0.3 mg; $NaMoO_4 \cdot 2H_2O$, 0.1 mg; $Na_2SeO_2Na_2$, 0.17 mg; $WO_4 \cdot 2H_2O$, 0.29 mg; and resazurin, 1.0 mg. In addition, the medium was supplemented with the following vitamins per liter *(14)*: pyridoxine-HCl, 0.1 mg; riboflavin, 0.1 mg; thiamine-HCl, 0.1 mg; cyanocobalamine, 0.1 mg; lipoic acid, 0.06 mg; pantothenic acid, 0.05 mg; *p*-Aminobenzoic acid (PABA), 0.05 mg; biotin, 0.02 mg; and folicin, 0.02 mg.

Experimental Setup

Microbial growth was accomplished in 25-mL headspace vials (Kontes Glass Co., Vineland, NJ), which had been fitted with Teflon™-lined rubber septa and aluminum crimp seals (Fisher Scientific, Pittsburgh, PA). Ten milliliters of sterile, anaerobic medium were first added to each of these vials. After mixing, the media and vessel were autoclaved at 121°C. Immediately after it was removed from the autoclave, the headspace of the serum bottles was sparged with an 80:20 mixture (v/v) of N_2/CO_2 (Liquid Air Corp., Walnut Creek, CA) while the solution cooled. To ensure that no oxygen was present in this purge gas mixture, it was passed through a hot copper catalyst heated to 350°C in a tube furnace (Barnstead/Thermolyne Corp., Dubuque, IA). Once cooled, the medium was supplemented by a previously prepared sterile, anaerobic, concentrated stock solution containing Cysteine-HCl, $Na_2S \cdot 9H_2O$, $NaHCO_3$, and vitamin mixtures to give 0.0025 g/L cysteine-HCl and 0.0025 g/L $Na_2S \cdot 9H_2O$.

Appropriate amounts of PCE in methanol were then added to each of the vials to obtain the desired concentration of 0, 10, 50, or 100 ppm in the aqueous phase. The amount of PCE to be added was determined using Henry s law *(15)*. Finally, additional methanol was added to each vial to bring the methanol concentration in the medium to 25 mM. Except for the controls, each vial was then inoculated with 10% (v/v) methanogenic consortium, which had been grown as described above using the same type of medium, but without PCE. All vials were incubated upside down without mixing at 17°C.

To ensure anaerobic conditions, all transfers were made inside an anaerobic chamber (Forma Scientific model 1025, Marietta, OH). Additions of supplements and inocula were made using disposable plastic syringes and 26-gage monoject hypodermic needles (Sherwood Medical, St. Louis, MO). PCE/methanol solutions were added using gas-tight glass syringes (Dynatech, Baton Rouge, LA), which were fitted with 26-gage sterile disposable monoject hypodermic needles.

At each sample time, a total of 14 culture vessels were sacrificed. Of these, 12 were the result of three repeats at each of the four PCE concentrations employed. Additionally, two abiotic controls, each containing 50 ppm of PCE, were sacrificed at each sample time. One set of sample culture vials was sacrificed every other day for a total of 22 d of operation.

Analytical Procedures

The methane concentration in the headspace of the culture vessel, and the methanol concentration in the aqueous phase were both analyzed using a Hewlett Packard 5840 gas chromatograph that was equipped with a flame ionization detector. A packed metal column, 8 ft × 1/8 in., 80/100 Hayesep P HP (Supelco, Inc., Bellefonte, PA) was used to accomplish the separation. In both cases, the oven temperature was set at 80°C for 4 min, and was then ramped to 180°C at 10°C/min.

A 0.25-mL headspace sample was employed for methane analysis. This sample was collected into a 1-mL disposable plastic syringe fitted with a 26-gage monoject hypodermic needle (Sherwood Medical, St. Louis, MO). Total headspace volume was measured by allowing the headspace to expand in a Micro-mate interchangeable hypodermic glass syringe (Popper & Sons, Inc., New Hyde Park, NY) to atmospheric pressure. These volumes, coupled with the determined methane concentration, were used to quantify the amount of methane produced.

To determine the methanol concentration in the aqueous phase, the solution was first filtered through a 0.2-μm syringe filter to remove cellular materials. Then, 1 μL of the resulting, cell-free solution was manually injected onto the column using a 7000 series microliter syringe (Hamilton Co., Reno, NV). Calibration was performed prior to sample analysis.

Acetate concentrations were determined from filtered samples using a Dionex DX 500 ion chromatograph system equipped with a CD 20 conductivity detector, a GP 40 gradient pump, a 4-mm ASRS autosuppressor, and an AS3500 autosampler (Dionex, Sunnyvale, CA). A Dionex 4-mm AS12 column, and a 4-mm AG12 guard column were used to accomplish the separation. The eluent employed was 1.35 m*M* $NaCO_3$ and 0.15 m*M* $NaHCO_3$ fed at 3 mL/min. The run was controlled, and data were collected and analyzed using Peaknet version 4.1 (Dionex, Sunnyvale, CA).

Chlorinated hydrocarbons PCE, TCE, trans-DCE, and cis-DCE were assayed by first extracting the chlorinated compounds from the aqueous sample into hexane. These extractions were made by withdrawing 0.5 mL of aqueous sample into a clean 10-mL series A-2 glass syringe (Dynatech, Baton Rouge, LA), which had been previously filled with 2.5 mL of hexane. The resulting solution was dispensed into a glass centrifuge tube containing an additional 8 mL of hexane. This centrifuge tube was then capped with Teflon-lined cap, and the chlorinated ethylenes were extracted by vortexing for 30 s and then centrifuging at 5000 rpm for 20 min using a bench-top centrifuge (Fisher Scientific, Pittsburgh, PA). The organic phase was transferred into 2 mL autosampling vials and crimp sealed. The resulting hexane solution was then injected with an autosampler into a Hewlett Packard 5890 Series II GC, which was equipped with an electron capture detector (Hewlett Packard, Palo Alto, CA). A 30 m × 0.53 mm id, DB-624 column (J&W Scientific, Fulsom, CA) was used to for separation. Carrier and make-up gases were ultrapure helium and ultrapure nitrogen (Liquid Air Corp.) at 5.4 and 57 mL/min, respectively. The injector, detector, and column temperatures were, 200, 260, and 50°C, respectively.

RESULTS AND DISCUSSION

Figure 1 shows the temporal changes in the amount of methane produced as a function of the PCE concentration in the bottles. Statistical analysis of the results using Student's t-test indicates that, at the 95% confidence level, the methane concentration achieved at the end of the experiments is a function of the PCE concen-

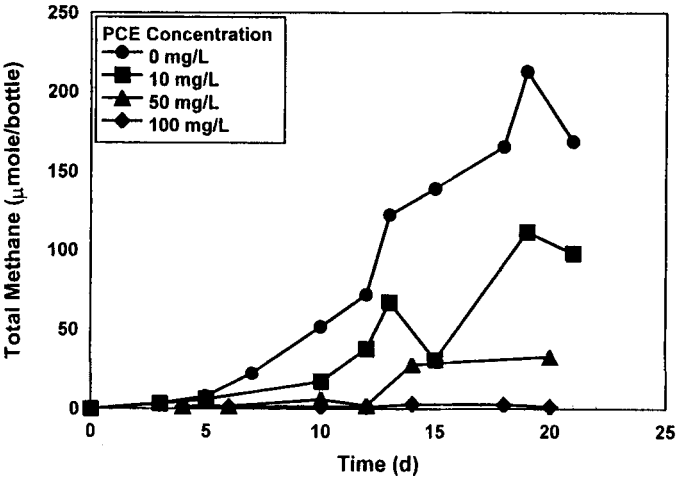

Fig. 1. Total moles of methane as a function of time at various PCE concentrations. Each data point represents the average of three replicate experiments.

tration in the bottles. That is, in all cases, the mean methane concentrations are different from one another. These data demonstrate there is an increase in the lag time before the onset of methane production with increasing PCE concentration. In addition, there is a decrease in both the total amount of methane and the maximum methane production rate in the presence of PCE. The maximum methane production rate was 28, 19, 4.7, and 0.9 μmol/d for 0, 10, 50, and 100 mg/L PCE, respectively. Based on these maximum rate data, the IC_{50} for methane production is estimated to be 10 mg/L. This IC_{50} value is similar to the value reported by Blum and Speece *(10)* for a methanogenic culture.

Temporal changes in the amount of acetate present in each experimental system are represented in Fig. 2. Comparison of the acetate data suggests that acetogenic activity in these cultures is less affected by PCE than methanogensis. At the 95% confidence level, Student s t-test indicates that after 5 d of incubation, the acetate level achieved in the bottles to which 10 ppm PCE were added was the same as that achieved in the bottles to which no PCE was added. However, this acetate concentration was statistically different from that achieved in the bottles to which 50 and 100 ppm of PCE were added. However, analysis of the data at the end of the experiment indicated that the acetate level in all bottles was, from a statistical standpoint, the same. Hence, the acetate production is unaffected by 10 mg/L PCE and only partially inhibited by the higher two levels of the chlorinated ethylene. In addition, the increase in lag time observed for methane production is not apparent in acetate production at any concentration of PCE. Comparison of both the acetate and methane data for 0 and 10 mg/L PCE suggests that slowing of methane formation is not caused by differences in acetate production rates. For these two conditions, acetate production was identical, whereas the methane production was significantly lower with 10 mg/L PCE.

The temporal changes in the substrate (methanol) as a function of PCE concentration added to the system are represented in Fig. 3. Statistical analysis of these results indicates that, throughout the course of the experiment, the amount of methanol consumed in the bottles to which 10 ppm PCE were added was not significantly

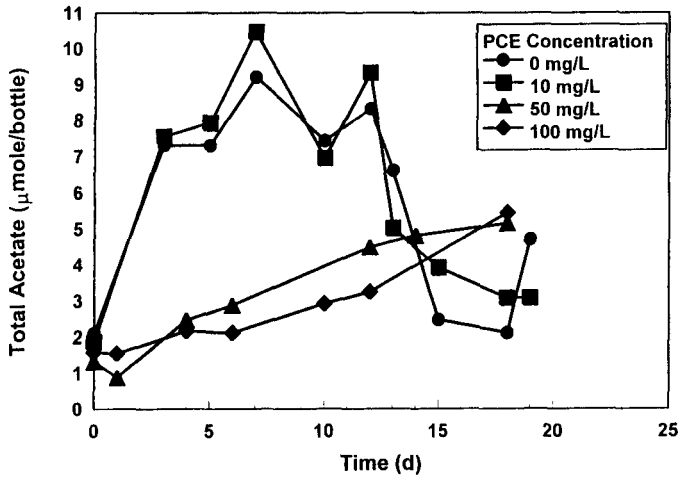

Fig. 2. Total moles of acetate as a function of time at various PCE concentrations. Each data point represents the average of three replicate experiments.

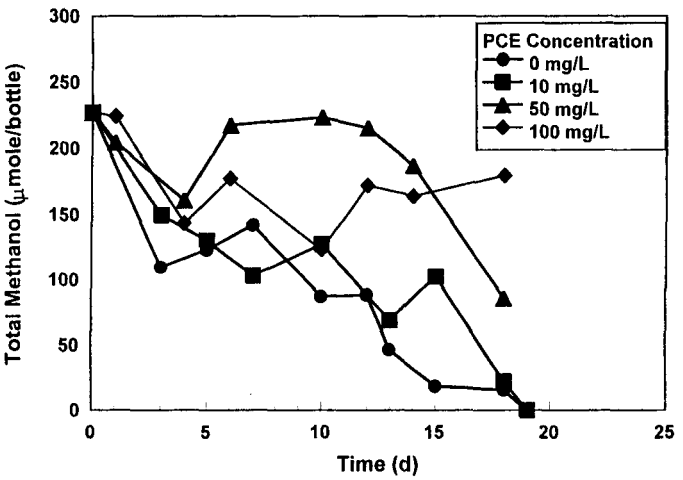

Fig. 3. Total moles of methanol as a function of time at various PCE concentrations. Each data point represents the average of three replicate experiments.

different from that consumed in the bottles to which no PCE had been added. However, these methanol levels were significantly different from those measured in the bottles to which 50 and 100 ppm PCE were added. These results indicate that, as observed for acetate production, the amount of methanol consumed by the culture with 0 and 10 mg/L PCE were similar. In contrast, methanol consumption is slowed at the higher two PCE levels.

When compared to the abiotic control, in all cases, the amount of PCE degraded was less than the detection limit of the gas chromatograph. Hence, no significant loss of PCE was observed in any of the cultures. TCE, the primary PCE

degradation product, was detected in all the cultures that contained PCE, except the abiotic controls, however. The decrease in the PCE concentration that would correspond to this measured TCE concentration would have been less than the instrument's detection limit. Hence no measurable change in the PCE concentration should have been observed. In addition, in contrast to the results presented recently by Skeen et al. *(13)*, who observed 1,1-DCE, but not 1,2-DCE from this culture, trace amounts of cis-1,2-DCE were detected in all PCE-exposed cultures. Although these results differ from those reported by Skeen et al., they are in agreement with others observations using similar consortia. This difference might be caused by cysteine-HCl, which was present in the growth media used in this study, but not in the growth media reported by Skeen et al. Further work is in progress to determine the cause of this product variation. In addition, to cis-1,2-DCE, trace amounts of trichloroethane were detected in cultures grown in the presence of 10 mg/L PCE.

CONCLUSION

This study focused on the effect of PCE concentration on acetogenic and methanogenic activity in a methanol-enriched methanogenic consortia. The data presented here demonstrate that there is a decrease in both the total amount of methane and the maximum methane production rate in the presence of PCE. An IC_{50} of 10 mg/L PCE was determined for this system based on estimates for the maximum rate of methane production for each level of PCE.

PCE was observed to have less of an inhibitive effect on acetogeneis. Acetate production did not seem to be affected in those cultures that were exposed to 10 mg/L of PCE. Higher concentrations of PCE resulted in only partial inhibition of the activity. These results indicate that because methanogenic activity was not inhibited at the same PCE levels as was acetogenic activity, both must be monitored in future experiments that seek to quantify the kinetics of the destruction of chlorinated solvents, such as PCE by such methanogenic consortia.

During these experiments, a small amount of reductive dechlorination was observed since TCE, TCA, and cis-1,2-DCE were detected in all cultures that were exposed to PCE, but not in the abiotic controls or cultures where PCE was not added. However, as noted by Skeen et al. *(13)*, such activity may not be sufficient to accomplish *in situ* remediation in an economical fashion.

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