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Effects of Pentachlorophenol on Methanogenic Fermentation of Phenol

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According to the U.S. Environmental Protection Agency, pentachlorophenol (PCP) and creosote are the two largest volume pesticides in use in the United States (Chemical and Engineering News 1978). It has been estimated that there are over five hundred wood preserving operations in the United States that collectively utilize over 36 million kg of PCP and 454 million kg of creosote annually (Cirelli 1978).

Creosote is a complex mixture of chemical compounds and typically consists of about 85% polynuclear aromatic compounds (PAH), 2-17% phenolic compounds, and the remainder consisting of nitrogen and sulfur containing heterocyclic compounds. Phenolic compounds are more soluble than the other components of creosote; therefore, when creosote is mixed with water two separate phases result: (1) an aqueous phase rich in phenolic compounds and (2) a dense hydrocarbon phase rich in PAH's. When ground water moves through an area contaminated with creosote, the water soluble phenols dissolve and are transported downgradient in the flow.

PCP is usually applied to wood products as a 5% solution in such solvents as mineral spirits, No. 2 fuel oil, and kerosene or sometimes as aqueous solutions of the sodium salt (NaPCP). The solubility of PCP in water is 20-25 mg/L, while the solubility of NaPCP in water is 5-7 g/L (Bailey and White 1965). It has been estimated that there are over 200 sites in the United States that use both PCP and creosote (Cirelli 1978). At many of these sites, contamination of the ground water with PCP and phenolic compounds from creosote has occured as a result of improper storage and disposal of these compounds. As these compounds migrate in the ground water, they are susceptible to biodegradation.

The biodegradation pathways of phenols, chlorophenols and PCP in anoxic environments have been studied individually (Ehrlich et al. 1982; Godsy et al. 1983; Boyd and Shelton 1984; Ide et al. 1972); but there have been few if any studies relating possible interactions when both materials are present together in this type of environment.

The purpose of this study was to investigate the interactions of

PCP and phenol, the major water soluble anaerobically biodegradable component of creosote, in anaerobic laboratory digestors. Results of this study demonstrated that PCP was inhibitory to the methanogenic fermentation of phenol, and this inhibition could be relieved if the PCP concentration was low enough for itself to undergo biodegradation.

MATERIALS AND METHODS

The study was conducted in 16 butyl rubber capped 125 mL serum bottles with aluminum crimp seals. All operations were carried out in an anaerobic glove box under an atmosphere of 90% Ar and 10% H_2.

Each bottle contained 100 mL of a mixture of mineral salts solution and settled sludge from a bench scale anaerobic digestor (10:1, v/v). Phenol was added to 14 of the bottles to give a final concentration of 200 mg/L (2.1 mM). PCP was added in duplicate to bottles containing phenol solution to produce final concentrations of 0.01 mg/L (0.04 mM), 0.05 mg/L (0.19 mM), 0.1mg/L (0.38 mM), 0.5 mg/L (1.9 mM), 1.0 mg/L (3.8 mM), and 5.0 mg/L (19 mM). The serum bottles were maintained for 150 days at ambient temperature (19-22^{o} C) with once daily shaking.

The mineral salts solution contained per liter: KH_2PO_4, 0.75 g; K_2HPO_4, 0.9 g; MgCl $6H_2O$, 0.36 g; NH_4Cl, 0.9 g; trace metal solution (Zeikus 1977), 9.0 mL; and vitamin solution (Wolin et al. 1963), 5.0 mL. After the pH was adjusted to 7.2 with 1 N NaOH, the solution was boiled to remove dissolved O_2 and cooled under O_2- free Ar. The solution was then placed into the glove box and allowed to equilibrate with the glove box atmosphere for a minimum of 2 days. Just prior to use, 0.5 g of Na_2S was added as a reducing agent.

Volatile fatty acids (VFA) in the mineral salts solution - sludge mixture were determined by high performence liquid chromotography (HPLC) using the method described by Ehrlich et al. (1981). The initial biomass in the serum bottles was determined by drying a measured volume of the mineral salts solution - sludge mixture to a constant weight at 105^{o} C (Gerhardt et al. 1981). Each serum bottle contained 0.2 mg/L formate, 5.9 mg/L acetate, 11.6 mg/L propionate, and 727 mg/L biomass. Gas production in the serum bottles was measured by displacement of the plunger in a water - wetted 10 mL glass hypodermic syringe. Methane concentration in the head space was determined by the gas chromatographic method described by Godsy et al. (1983).

At the conclusion of the experiment, 20.0 mL of the mixed suspension from each serum bottle was centrifuged at 10,000 x g for 5 min. The supernatant fluid was removed and filtered

through a 0.4 um polycarbonate filter (Nucleopore Corp., Pleasanton, Calif.)[1]. Concentrations of phenol and chlorophenols in the filtrate were determined by HPLC using the method described by Goerlitz (1982). The cell pellet was mixed with 2.0 mL of methanol - KOH solution (50.0 g KOH/L methanol) and digested at 70° C for 1 hr. Five ml of water was then added and the tube was heated at 100° C for 15 min to remove the methanol. Cell debris was removed by centrifugation. The supernatant was removed and the pH was adjusted to 7.0 with glacial acetic acid. The neutralized solution was filtered through a 0.4 um polycarbonate filter before HPLC analysis for phenol and chlorophenols. Finally, the serum bottles were emptied and their inner surfaces were rinsed with 4.0 mL of the methanol - KOH solution. Ten mL of water was added to the wash solution and the methanol was removed by heating as above. After pH adjustment and filtration the concentrations of phenol and chlorophenols were determined by HPLC analysis.

RESULTS AND DISCUSSION

The mean rates of gas evolution from the duplicate serum bottles are shown in figure 1. Individual values of gas production from the duplicate serum bottles were within 5% of the average values shown. Methane production in the early part of the experiment resulted largely from the VFA's introduced with the sludge. The rates of methanogenesis were unaffected at PCP concentrations of 0.1 mg/L or less. Marked reductions occurred at levels of 0.5 and 1.0 mg/L and methanogenesis virtually stopped at 5.0 mg/L.

Methanogenesis from phenol also began to occur at the same time as the VFA's but at a slower rate. This was verified by weekly analysis of phenol concentrations and of CH_4 in the head space. The methanogenic fermentation of phenol was inhibited longer in the presence of low PCP concentrations than the methanogenic fermentation of the VFA's. Interestingly, once methanogenesis began the rate was not affected by the presence of PCP. Methanogenesis of phenol did not occur at PCP concentrations greater than 1.0 mg/L.

Results of the HPLC analysis of the culture solutions are given in table 1. For these experiments, solutions were prepared containing phenol at a fixed concentration of 200 mg/L and PCP at concentrations ranging between 0.0-5.0 mg/L. After 150 days, phenol, PCP and other chlorophenols were not found if the initial PCP was less than 0.5 mg/L. At a starting PCP concentration of 1.0 mg/L there was no apparent decrease in phenol, but the PCP disappeared and several chlorophenols that were not present in the starting mixture then appeared. After 150 days, the PCP

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1. The use of brand names is for identification only and does not constitute an endorsement by the U. S. Geological Survey.

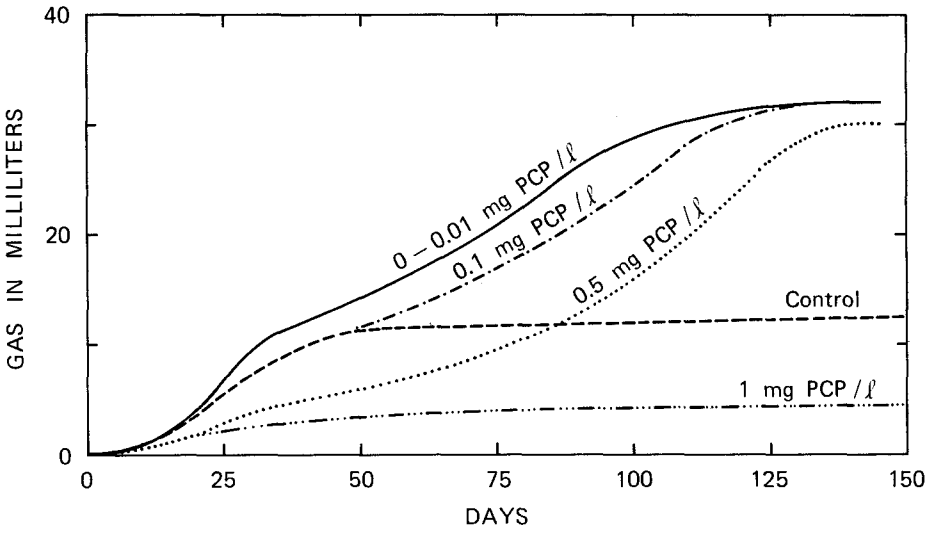

Figure 1. Effect of pentachlorophenol upon rate of phenol degradation.

concentration in the serum bottle that originally was at 5.0 mg/L was reduced to 3.4 mg/L.

Results of the HPLC analysis of biomass extracts and serum bottle wall washings are given in table 2. At low PCP levels there was no apparent uptake of PCP into the cells or sorption to the serum bottle walls. In serum bottles with an initial PCP level of 1.0 mg/L only a fraction of the PCP was recovered from the biomass. At an initial level of 5.0 mg/L appreciable amounts of PCP were recovered from the biomass but the total recovery was only 74% of the amount initially added. We are unable to account for the remainder at this time.

PCP and other halogenated aromatic compounds have been shown to be susceptible to reductive dechlorination and degradation by bacteria under anoxic conditions. Hakulinen and Salkinoja-Salonen (1982) demonstrated that PCP was biodegraded in an anaerobic fluidized-bed reactor treating paper and pulp mill effluents. Ide et al. (1972) reported that PCP was converted to 2,3,4,5- and 2,3,4,6-tetrachlorophenol, 2,4,5- and 2,3,5-trichlorophenol, 3,4- and 3,5-dichlorophenol and 3-chlorophenol after incubation for several weeks in a paddy soil. Boyd and Shelton (1984) investigated the anaerobic biodegradation of mono- and dichlorophenols to CH_4 and CO_2 by anaerobic sewage sludge. Biodegradation was evaluated by monitoring substrate disappearance and, in selected cases, production of $^{14}CH_4$ from labeled substrates.

Table 1. Concentration of phenol and chlorophenols in digestors after 150 days.

Compound	Initial PCP Concentration (milligrams per liter) 0 - 0.5	1.0	5.0
Phenol	ND	200	200
PCP	ND	ND	3.4
Tetrachlorophenols	ND	0.09	ND
Trichlorophenols	ND	TR	ND
Dichlorophenols	ND	0.5	ND
Chlorophenols	ND	0.05	ND

ND = not detected (<0.001 mg/L). TR = trace (~ 0.002 mg/L).

Table 2. Recovery and distribution of PCP in laboratory digestors.

Initial PCP in digestors	Micrograms of PCP Cells	liquid	Glass	Recovery percent
1	ND	ND	ND	0
10	ND	ND	ND	0
50	ND	ND	ND	0
100	0.8	ND	ND	0.8
500	30	340	ND	74

ND = not detected (<0.001 mg/L)

Both Boyd and Shelton (1984) and Horowitz et al. (1983) found that methanogenic fermentation of chlorinated aromatics required complete removal of the halogen as a first step. Our results demonstrate the occurrence of dechlorination of PCP at initial levels below 5 mg/L PCP. Presumably at higher levels, PCP is toxic to the dechlorinating organisms. The fact that PCP was partially dechlorinated in the digestors with an initial PCP

concentration of 1.0 mg/L and the fact that phenol, PCP or other chlorophenols were not found in digestors with an initial concentration of PCP at or below 0.5 mg/L demonstrates that PCP was totally biodegraded to CH_4 and CO_2.

These results suggest that the dechlorinating component of the microbial consortium may be distinct from the components that effect ring reduction, ring cleavage and the other intermediate steps incident to production of one carbon compounds, acetate, H_2, and CO_2 - the immediate precursors for CH_4.

The time required before the onset of phenol biodegradation in digestors with initial PCP concentrations of 0.1 and 0.5 mg/L is the time required to effect the dechlorination of PCP to a non-toxic level. At this time methanogenic fermentation of phenol can begin.

The operation of this effect in ground water contaminated with both PCP and phenol would be to enlarge the areal extent of contamination when the PCP concentration was greater than 5.0 mg/L. In areas where the PCP was ~ 1 mg/L or less, dechlorination of the PCP to a non-toxic level will allow for complete bioconversion of phenol and PCP to CH_4 and CO_2 - if there are no other limiting environmental factors.

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