Reductive Dehalogenations of Halobenzoates by Anaerobic Lake Sediment Microorganisms†

AMIKAM HOROWITZ, JOSEPH M. SUFLITA,‡ AND JAMES M. TIEDJE*

Departments of Crop and Soil Sciences and of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824

Received 29 November 1982/Accepted 4 March 1983

Methane-producing freshwater lake sediment was found to dehalogenate chloro-, bromo-, and iodobenzoates by a reductive reaction in which the halogen was replaced by a hydrogen atom. The identity of the dehalogenated products was confirmed by mass spectrometry, nuclear magnetic resonance, or chromatography. Removal of the halogens to produce benzoate was necessary before mineralization to $\text{CH}_4 + \text{CO}_2$ could occur. The dehalogenation occurred after a lag period which lasted from 1 week to more than 6 months, depending on the chemical. Dehalogenation was not observed in the absence of CH_4 production, and it was inhibited by the addition of 20% O_2 . Once sediment was acclimated to halobenzoate dehalogenation, new additions of the halobenzoate were degraded without lag. Acclimation was observed regardless of whether the parent substrates were eventually mineralized to $\text{CH}_4 + \text{CO}_2$. Sediment acclimated to bromo- and chlorobenzoate degradation generally metabolized bromo- and chlorobenzoates, but sediment acclimated to iodobenzoate degradation only metabolized iodobenzoate. Prior acclimation of sediment to benzoate decomposition did not alter the pattern of dehalogenation, and sediment acclimated to dehalogenation was not concurrently acclimated to benzoate degradation. The presence of this apparent specificity, the lag period, and subsequent acclimation, together with our findings of the absence of dehalogenation in sterile sediments and by sediments previously incubated at $\geq 39^\circ\text{C}$, suggests that this reaction was biologically catalyzed. Apparently, a pathway for the reductive dehalogenation of aryl halides is present in anaerobic microorganisms of this methanogenic sediment.

Halogenated aromatic compounds continue to be important in the production of pesticides, preservatives, and other industrial chemicals. They are also formed during chlorination processes such as the bleaching of wood pulp (1). Many haloaromatic compounds have appeared as environmental pollutants as a result of their quantity, persistence, bioaccumulation, or toxicity. The metabolic fate of these chemicals under aerobic conditions has been widely studied, and the predominant persistence patterns and degradation pathways have been well established. In contrast, an examination of the anaerobic microbial community for metabolism of these chemicals, especially the more persistent ones, has largely been ignored. This is unfortunate since many organohalogen compounds partition to and reside in anaerobic sediments. In a previous report, we showed the reductive dehal-

ogenation of the aromatic ring (8). This reaction is different from previously reported cases of anaerobic dehalogenation since in those cases the halogen is removed from saturated alkyl structures such as DDT (dichlorodiphenyltrichloroethane) and lindane and not from the aromatic ring (8). In this report, we present the chemical evidence for aryl dehalogenations and show characteristics of the reaction which suggest that it is enzymatic.

MATERIALS AND METHODS

Sediment collection and incubation of substrates. Sediment was sampled from the pelagic zone of hypertrophic Wintergreen Lake (Kalamazoo County, Mich.) with an Eckman dredge and was stored in the dark at 4°C in Mason jars which were filled to capacity. Sediment storage time ranged from a few days to weeks and did not correlate with the length of the lag time before the onset of dehalogenation. Sediment was mixed with filtered anaerobic hypolimnion water and transferred as 100-ml slurries (50 to 65 mg [dry weight] ml^{-1}) to 160-ml serum bottles inside an anaerobic glove box; the bottles were then sealed with butyl rubber septa 1 cm thick. The initial headspace gas composition was 90% N_2 and 10% H_2 with 1 ml of Ne

† Published as journal article no. 10664 of the Michigan Agricultural Experiment Station.

‡ Present address: Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019.

injected by syringe as an internal gas standard. This marl-based sediment was adequately buffered at neutrality. After 1 day of preincubation, substrates were injected into the bottles to achieve the desired concentration (typically 100 and 820 μ M). The bottled sediment was incubated at 20°C in the dark, and all treatments were carried out in duplicate. All bottles were incubated statically, except in the aerobiosis and cross-acclimation experiments, in which the bottles were shaken at 120 rpm.

Substrate and metabolite analyses. The degradation of parent substrate and the appearance of dehalogenated metabolites were monitored by high-pressure liquid chromatography (HPLC). Periodic samples of sediment slurry were taken from the serum bottles by syringe and passed through a 0.45- μ m membrane filter, and the filtrate was stored until analysis at 10°C for short periods or at -10°C for longer periods. The HPLC separations were performed on either an Altex model 153 or a Varian 5000 HPLC system. A C-18 reversed-phase column (25 cm by 4.6 mm [inside diameter]; LiChrosorb, Altex Scientific, Inc., Berkeley, Calif.) and a mobile water-methanol-acetic acid phase (7:4:1.3) was used. For some substrates, increased proportions of mobile-phase methanol were required to achieve efficient peak resolution. Compound detection was by UV absorbance with either fixed-wavelength or variable-wavelength detectors operated at 254 or 280 nm or at the wavelength maximum of the parent substrate.

Methane and CO₂ production were monitored in the headspace atmosphere of the serum bottles by gas chromatography with Ne as an internal gas standard. Methane and CO₂ produced from bottles without substrate were subtracted from those with substrate. Gaseous end products were separated with a 1-m column of Poropak Q in a Carle model 8515 gas chromatograph equipped with a microthermister detector and with He used as the carrier gas (30 ml/min).

Identification of metabolites. The identity of the metabolite produced during the incubation of 4-amino-3,5-dichlorobenzoate with anaerobic sediment was determined by gas chromatography-mass spectrometry of the corresponding trimethylsilyl derivative, and by nuclear magnetic resonance (NMR). To isolate the metabolic product, sediment was acidified to pH 2.0 with 50% H₂SO₄ and extracted with diethyl ether (1:1 [vol/vol]), and the ether phase was concentrated to 0.1 ml. The metabolite was derivatized with *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and injected on the column while the temperature was held at 75°C for 2 min before programming to 250°C at 10°C min⁻¹. Gas chromatography was done on a Varian 3700 instrument with an SE-30 glass capillary column (30 m by 0.25 mm) and a He carrier flow of 1 ml/min; detection was by flame ionization. An electron impact mass spectrum was obtained with a Finnigan model 4023 mass spectrometer equipped with an INCOS data system and operated at 70 eV.

The metabolite was isolated for NMR similarly, except that the ether extracts were combined and concentrated twofold before the addition of activated charcoal. The charcoal was subsequently removed by filtration, and the ether extracts were dried over anhydrous Na₂SO₄. Further purification was achieved by frequent charcoal additions and recrystallizations from hot aqueous methanol. The proton NMR spec-

trum was taken on a Varian EM-360 60-MHz instrument with a sweep width of 10 ppm. The spectrum was recorded with the sample dissolved in dimethyl sulfoxide-*d*₆.

Benzoate was identified by cochromatography with authentic material by means of the reversed-phase HPLC system, normal-phase HPLC, ion-exchange HPLC, and gas chromatography. Normal and ion-exchange HPLC were done with columns of Partisil PXS 10/25 PAC and SAX (Whatman, Inc., Clifton, N.J.), respectively. The mobile phase was methanol-water-acetic acid (8:3:1) for the normal-phase separation; 100 mM H₃PO₄ and KH₂PO₄ buffered at pH 3.0 were used with the anion-exchange column. The extraction and gas chromatography of benzoate were the same as that noted for the 4-amino-3,5-dichlorobenzoate, except that an SP-2100 capillary column was used.

Other intermediates were identified by cochromatography with authentic compounds by using the reversed-phase HPLC system. This system could separate various multisubstituted and positional isomers within a halobenzoate series.

Sterile controls and effect of aerobiosis. After the initial quantity of xenobiotic material was completely dehalogenated, the sediment was considered acclimated to the degradation of that compound; subsequent substrate additions were always metabolized without a lag. To determine whether dehalogenation was a biologically catalyzed reaction, substrate depletion was monitored in sterile and nonsterile sediment acclimated to the metabolism of 4-amino-3,5-dichlorobenzoic acid. Sterilization was by autoclaving (121°C, 150 kPa, 1 h), gamma irradiation (1 Mrad), or formaldehyde treatment (final concentration, 2% [vol/vol]). To ensure that sterile sediment was also a highly reducing environment, we amended the gamma-irradiated control with 0.5 mM titanium citrate (11) on day 21 and incubated the sediment for 4 additional weeks.

The effect of aerobiosis was investigated by flushing the serum bottle headspace of acclimated sediment with air, maintaining the level of O₂ at 20%, and monitoring substrate disappearance by HPLC. Anaerobic acclimated sediment was treated similarly, but it was flushed with oxygen-free N₂.

Cross-acclimation. Sediment previously acclimated to the metabolism of a single substrate was dispensed into serum bottles inside the anaerobic glove box. It was confirmed by HPLC that the acclimated sediment had completely degraded the previous amount of substrate. Then, different aromatic substrates were added to the acclimated sediment, and primary substrate degradation was monitored with time. The lag time before degradation and the time required for the complete disappearance of the new substrate were compared with the lag time of the compound in unacclimated sediment.

Chemicals. 3-Bromobenzoic acid and 3-iodobenzoic acid were obtained from Eastman Organic Chemicals (Rochester, N.Y.) and 3,5-dichlorobenzoic acid was purchased from Aldrich Chemical Co. (Milwaukee, Wis.). 4-Amino-3,5-dichlorobenzoic acid and 2,3,6-trichlorobenzoic acid were obtained from Pfaltz and Bauer Chemical Co. (Stamford, Conn.). Chemicals that were not of sufficient purity, as determined by HPLC, were purified by charcoal addition, subsequent filtration, and recrystallization before use.

RESULTS

Metabolism of haloaromatic substrates. Incubation of various halobenzoates in anoxic sediment resulted in the depletion of the parent substrate and the appearance of intermediates and gaseous end products. The patterns of biodegradation are illustrated by the examples in Fig. 1 for a substrate completely mineralized (Fig. 1A), for one mineralized after prior exposure to the same substrate (Fig. 1B), and for one only partially metabolized (Fig. 1C).

After a 3-week lag, 2-bromobenzoate was metabolized to benzoate, which accumulated to >70% of that theoretically possible (Fig. 1A). The benzoic acid intermediate was subsequently mineralized to CH_4 and CO_2 . The exposure to 2-bromobenzoate did not simultaneously acclimate the sediment to benzoate degradation since the normal acclimation period of benzoate at similar substrate concentrations is 2 to 7 days. Lower concentrations of benzoate (a few micromolar) were metabolized by sediment without lag. The higher concentrations of benzoate formed here (or if added as substrate) required an acclimation period of 2 to 7 days before rapid removal of this quantity of substrate occurred. Once sediment was acclimated to halobenzoate mineralization, subsequent additions resulted in immediate substrate disappearance and gas production (Fig. 1B), with only trace amounts of an intermediate detected. This is illustrated for 3-iodobenzoate, but is also true of other haloben-

zoates metabolized to gas (Fig. 2).

Acclimation to the metabolism of nonmineralized substrates was also observed, as exemplified with 4-amino-3,5-dichlorobenzoate (Fig. 1C). HPLC analysis indicated that sediment could quantitatively convert the substrate to a single compound identified as 4-amino-3-chlorobenzoic acid. The latter chemical persisted in sediment. The lag period was eliminated after the second addition of primary substrate.

Figure 2 summarizes the degradation pathway found for several halobenzoates in anaerobic sediment and is based on the sequence and identity of intermediates and final products. Removal of Br, Cl, or I substituents on the benzoate ring always preceded further metabolism of the intermediates, and complete dehalogenation was required before substrate carbon could be mineralized to CH_4 and CO_2 . When 3,5-dichlorobenzoate was degraded, 3-chlorobenzoate was produced. After a second and longer lag period, the latter compound was transformed to benzoate and then was mineralized (Fig. 2). The only transformation of 2,3,6-trichlorobenzoate and 4-amino-3,5-dichlorobenzoate was the loss of a *meta*-substituted chlorine. No transformation of 2- or 4-chlorobenzoates was observed, even after a year of incubation. Lag periods of various lengths are typical of the degradation pattern associated with all halobenzoates in unacclimated sediment (Fig. 2). The lag periods varied according to the type and position of the aryl halide. The sub-

FIG. 1. Patterns of anaerobic degradation of halogenated benzoates by lake sediments. (A) Degradation of 2-bromobenzoate by fresh sediment. (B) Degradation of 3-iodobenzoate by sediment previously acclimated to 3-iodobenzoate degradation. (C) Degradation of 4-amino-3,5-dichlorobenzoate by fresh sediment and accumulation of its partially dehalogenated product, 4-amino-3-chlorobenzoate.

FIG. 2. Summary pathways for anaerobic metabolism of several halobenzoates. Lag periods (in weeks): a, 1 to 3; b, 0.2 to 1; c, 2 to 3; d, 30–52; e, 12 to 52.

strate concentration also seemed to have an effect on the length of the lag for 4-amino-3,5-dichlorobenzoate; low concentrations (13 to 26 μM) of substrate were not degraded after 21 weeks, whereas the standard concentration used in these studies (820 μM) was degraded within 4 weeks (Fig. 1).

Evidence for dehalogenation. The metabolite produced when 4-amino-3,5-dichlorobenzoate was incubated with anaerobic sediment was identified by gas chromatography-mass spectrometry of the trimethylsilyl derivative and NMR of the isolated compound. The gas chromatography-mass spectrometry (Fig. 3) shows a parent ion at m/z 243, major fragmentation peaks at m/z 228 and 184, and a base peak at m/z 154. A clear one-chlorine isotopic abundance ratio is observed at each of these mass spectral features.

FIG. 3. Mass spectral fragmentation pattern used to identify the derivatized product resulting from the anaerobic metabolism of 4-amino-3,5-dichlorobenzoic acid.

The mass fragments at m/z 228, 184, and 154 correspond to the progressive losses of the trimethylsilyl group from the derivatized structure. These results confirm that the two-chlorine parent substrate was converted in anaerobic sediment to the corresponding monochloroamino benzoate by replacement of one chlorine by a hydrogen atom.

The structure was further defined by NMR analysis (Fig. 4). The proton H_b (Fig. 4) was a doublet, demonstrating that it was *ortho* to another proton, H_a . Its chemical shift suggests that it was likely to be *ortho* to an amino group (3). The apparent absence of a *meta* splitting effect, as observed for H_a , indicated that there were no protons *meta* to H_b . Protons H_a and H_c are given the assignments shown in Fig. 4 because the chemical shift values were consistent with protons *ortho* to a carboxyl group (3) and because only one (H_a) was split by an *ortho* proton. *Meta* splitting of H_a was observed (Fig. 4, arrow), but some of these features were obscured by the large shift corresponding to H_c . Presumably, the expected *meta* splitting of H_c was obscured by the richness of the spectrum in this region. The carboxyl and amino protons (H_d) are likely freely exchangeable in dimethyl sulfoxide- d_6 , and this could account for the broad tailing feature at 6.15 ppm. This assignment probably reflects an averaging of the amino- and carboxyl-proton chemical shifts. The only structure consistent with all of the above NMR features is 4-amino-3-chlorobenzoic acid.

Characterization of dehalogenation. Dehalogenation activity was inhibited in acclimated

FIG. 4. NMR spectrum used to identify 4-amino-3-chlorobenzoic acid as the product resulting from the anaerobic metabolism of the dichlorinated parent material. Spectrum taken in dimethyl sulfoxide- d_6 .

sediment by autoclaving, gamma irradiation, or formaldehyde treatment relative to nonsterile sediment (Fig. 5). In addition, no abiotic transformation of 4-amino-3,5-dichlorobenzoic acid was observed when the reductant titanium citrate was added either to sterile sediment (Fig. 5) or sterile 0.1 M phosphate buffer (pH 7.0).

Figure 5 also illustrates the effect of aerobiosis on dehalogenation. After 5 days of incubation, sediment acclimated to the dehalogenation of 4-amino-3,5-dichlorobenzoic acid was divided into two portions, one aerobic and one anaerobic. Only the anaerobically incubated sediment continued to metabolize the substrate and to produce the dehalogenated product.

Temperature also exerted a strong influence on aryl dehalogenations. When sediment was incubated with 3-bromobenzoate at temperatures ranging from 10 to 60°C, dehalogenation activity ceased at $\geq 39^\circ\text{C}$.

Figure 6 is a semilogarithmic plot illustrating that dehalogenation of aromatic substrates in anaerobic sediments followed first-order decay kinetics. The first-order dehalogenation rates for 4-amino-3,5-dichlorobenzoate varied from 0.070 h^{-1} to 0.015 h^{-1} in different experiments but generally averaged about 0.05 h^{-1} . Other substrates (Fig. 2) exhibited comparable rates of dehalogenation in this sediment.

Sediment that dehalogenated either 4-amino-3,5-dichlorobenzoate or 3-bromobenzoate cross-acclimated to the degradation of some other bromo- and chloro-halogenated benzoates and exhibited a similar substrate specificity (Table 1). In contrast, sediment acclimated to the degradation of benzoate or 3-iodobenzoate showed no ability to immediately degrade any compound other than the original acclimation substrate

FIG. 5. The effect of various sterilization procedures and the addition of 20% oxygen on the dehalogenation of 4-amino-3,5-dichlorobenzoic acid in acclimated sediment.

FIG. 6. First-order dehalogenation rate (k_1) of 4-amino-3,5-dichlorobenzoate in anoxic acclimated sediment (60.2 mg [dry weight] ml^{-1}).

(Table 1). Of the substrates tested for cross-acclimation, only 3-chlorobenzoate could not be degraded. For those substrates that were metabolized, depletion of $>90\%$ was observed in a time less than or equal to the lag time found for their degradation in unacclimated sediment.

DISCUSSION

These data demonstrate that several halogenated benzoates can be degraded under the anaerobic conditions found in freshwater sediments. This finding is encouraging because many problem environmental contaminants are halogenated aromatic compounds which often reside in anoxic environments like sediments, groundwaters, land fills, waste treatment facilities, and flooded soils.

Our evidence indicates that the removal of ring-substituted halogens represents the initial degradative reaction for the model halobenzoates used in this study. We have thoroughly characterized the dehalogenated products of 4-amino-3,5-dichlorobenzoate by gas chromatography-mass spectrometry and NMR, and we conclude that the reaction was a one-chlorine replacement by a proton and that the chlorine had not shifted position on the ring. Intermediates and products from other halogenated substrates were identified by cochromatography with a variety of chromatographic systems. All were found to be at least one halogen less than the parent compound. Complete dehalogenation was required before a substrate could be mineralized to CH_4 and CO_2 . In a previous report, we showed that *meta*-substituted halogens were preferentially removed from the benzoate ring (8).

Reductive dechlorination of aromatic xenobiotics has been noted previously for two compounds. Pentachlorophenol was shown to be degraded in anaerobic soil to isomeric mixtures of partially dehalogenated intermediates (4, 7),

TABLE 1. Cross-acclimation of anaerobic sediment microorganisms to halobenzoate decomposition

Substrate tested for degradation	Lag time in unacclimated sediment (wk)	Time (wk) for complete degradation with substrate used to acclimate sediment:			
		4-amino-3,5-dichlorobenzoate	3-bromobenzoate	3-iodobenzoate	Benzoate
4-amino-3,5-dichlorobenzoate	3-8	2-3	2-3	>1 ^a	ND ^b
3,5-dichlorobenzoate	2-3	<1	<1	>1 ^a	ND
3-iodobenzoate	2-3	2-3	2-3	<1	>4 ^a
3-chlorobenzoate	32-40	>6 ^a	>6 ^a	>1 ^a	>6 ^a
3-bromobenzoate	0.4-4	<1	<1	>1 ^a	>2 ^a

^a Lag before the onset of degradation.^b ND, Not determined.

and Kirkpatrick et al. (5) recently showed that the bactericide *N*-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorophthalamic acid is metabolized in the tetrachlorophthalamic acid moiety to two or possibly more monodechlorinated products. In these studies, the reaction was characterized only by the identification of dechlorinated products. However, our results on the several halobenzoates together with the above two cases suggest that aryl dehalogenation may not be restricted to one class of haloaromatics.

The aryl dehalogenation reaction was shown to require anaerobic conditions since the introduction of oxygen immediately inhibited the degradation of 4-amino-3,5-dichlorobenzoic acid (Fig. 5). We never observed aryl dehalogenation unless vigorous CH₄ production was occurring.

For the previous anaerobic alkyl dehalogenations, it has never been well established that the reaction was catalyzed by a specific enzyme since electron carriers such as flavin mononucleotide, reduced iron porphyrins, and boiled cell constituents (6, 10) can catalyze some dechlorinations. This does not seem to be the case with the aryl dehalogenations reported here. The following points argue for a specific biological mechanism. First, sediments sterilized by gamma irradiation, autoclaving, or formaldehyde treatment showed little or no ability to dechlorinate 4-amino-3,5-dichlorobenzoate. However, sterile sediment would not necessarily have the electron donors maintained at the low potential that the nonsterile sediment possessed. Therefore, the reductant titanium citrate was added to the gamma-irradiated sediment. This redox buffer did not cause a dehalogenation reaction in either sterile phosphate buffer or sterilized acclimated sediment, thus indicating no abiotic transformation of the parent substrates under conditions of low redox potential.

Second, the long lag period followed by a phase of rapid degradation and the acclimation of the sediment by prior exposure to substrate both indicate a specific biological reaction. During the lag phase, low-potential electron donors were being produced, as evidenced by a con-

stant background rate of CH₄ production, yet dehalogenation did not occur.

Third, loss of the dehalogenation reaction at temperatures above 39°C also supports the contention that dehalogenation is a biologically catalyzed reaction. Temperature maxima similar to this have been reported as optimal for methanogenesis in other freshwater sediments (12).

Finally, the cross-acclimation experiments indicate a degree of substrate specificity among dehalogenating microorganisms (Table 1). The fact that microbial populations are simultaneously acclimated to the degradation of more than one halobenzoate suggests that compounds of similar structure are degraded by similar pathways. One type of dehalogenating activity exhibited a broader substrate range; several, but not all, chloro- and bromo- substrates were degraded without lag by sediments acclimated to either 3-bromobenzoate or 4-amino-3,5-dichlorobenzoate. However, when sediment was acclimated to 3-iodobenzoate decomposition, a different dehalogenating capacity was expressed which did not cross-acclimate to any of the chloro- or bromo- substrates. Thus, at least two types of dehalogenating activity are present in anaerobic sediment, each with a different substrate specificity. This specificity would not be expected of an abiotic reaction. The specificity conveyed by enzymes seems required to explain the above results. It is also worth noting that acclimation to benzoate did not stimulate the dehalogenation, suggesting that dehydrogenation of the ring (first step in the benzoate pathway [2]) was also not responsible for the dehalogenation.

For substrates that are completely mineralized to gaseous end products, acclimation is expected since energy is released for microbial growth. However, certain substituents on the ring appear to block ring cleavage and presumably the yield of energy to the organism. Since no carbon or energy is released with these substrates, significant increases in microbial growth are unlikely. Nevertheless, the sediment microorganisms were observed to acclimate to

the dehalogenation of a nonmineralized substrate (Fig. 1C).

The data presented emphasize the importance of microbially catalyzed dehalogenation reactions of halogenated aromatic compounds in anaerobic environments. Much remains to be learned about the microbiology, ecology, and biochemistry of anaerobic xenobiotic metabolism in general and aryl dehalogenations in particular. It is conceivable that dehalogenating microorganisms or their dehalogenases could eventually be of environmental or commercial value in decontaminating organohalide wastes.

ACKNOWLEDGMENTS

We thank W. D. Reynolds for technical assistance, C. P. Cornell for early analytical work which suggested dehalogenation, B. A. Averill and R. A. Leavitt for mass spectrometry and NMR analysis, respectively, and S. A. Boyd and W. H. Reusch for their help with NMR interpretation.

This study was supported in part by Environmental Protection Agency grant R806569 and Environmental Protection Agency-Office of Toxic Substances subcontract T-6419 (7997)-033.

LITERATURE CITED

1. Bjorseth, A., G. E. Carlberg, N. Gjos, M. Moller, and G. Tveten. 1981. Halogenated organic compounds in spent bleach liquors: determination, mutagenicity testing and bioaccumulation, p. 1115-1130. In L. H. Keith (ed.), *Advances in the identification and analysis of organic pollutants in water*, vol. 2. Ann Arbor Science Publishers, Ann Arbor, Mich.
2. Evans, W. C. 1977. Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments. *Nature (London)* 270:17-22.
3. Gordon, A. J., and R. A. Ford. 1972. The chemist's companion. A handbook of practical data, techniques and references, p. 261-263. John Wiley & Sons, Inc., New York.
4. Ide, A., Y. Niki, F. Sakamoto, I. Watanabe, and H. Watanabe. 1972. Decomposition of pentachlorophenol in paddy soil. *Agric. Biol. Chem.* 36:1937-1944.
5. Kirkpatrick, D., S. R. Biggs, B. Conway, C. M. Finn, D. R. Hawkins, T. Honda, M. Ishida, and G. P. Powell. 1981. Metabolism of *N*-(2,3-dichlorophenyl)-3,4,5,6-tetrachlorophthalamic acid (Techlophthalam) in paddy soil and rice. *J. Agric. Food Chem.* 29:1149-1153.
6. Miskus, R. P., D. P. Blair, and J. E. Casida. 1965. Conversion of DDT to DDD by bovine rumen fluid, lake water, and reduced porphyrins. *J. Agric. Food Chem.* 13:481-483.
7. Murthy, N. B. K., D. D. Kaufman, and G. F. Fries. 1979. Degradation of pentachlorophenol (PCP) in aerobic and anaerobic soil. *J. Environ. Sci. Health Part B Pestic. Food Contam. Agric. Wastes* 14:1-14.
8. Suffita, J. M., A. Horowitz, D. R. Shelton, and J. M. Tiedje. 1982. Dehalogenation: a novel pathway for the anaerobic biodegradation of haloaromatic compounds. *Science (Wash. D.C.)* 218:1115-1117.
9. Williams, P. P. 1977. Metabolism of synthetic organic pesticides by anaerobic microorganisms. *Residue Rev.* 66:90-102.
10. Yoneyama, K., and F. Matsumura. 1981. Reductive metabolism of heptachlor, parathion, 4,4'-dichlorobenzophenone, and carbophenothion by rat liver systems. *Pestic. Biochem. Physiol.* 15:213-221.
11. Zehnder, A. J. B., and K. Wuhrmann. 1976. Titanium (III) citrate as a non-toxic oxidation-reduction buffering system for the culture of obligate anaerobes. *Science (Wash. D.C.)* 194:1165-1166.
12. Zeikus, J. G., and M. R. Winfrey. 1976. Temperature limitation of methanogenesis in aquatic sediments. *Appl. Environ. Microbiol.* 31:99-107.
13. Zoro, J. A., J. M. Hunter, G. Eglinton, and G. C. Ware. 1974. Degradation of *p,p'*-DDT in reducing environments. *Nature (London)* 247:235-236.