Extensive Microbial Degradation of DDT *in Vitro* and DDT Metabolism by Natural Communities

Frederic K. Pfaender[1] and Martin Alexander*

Extracts of *Hydrogenomonas* sp. cells converted 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), 1-chloro-2,2-bis(*p*-chlorophenyl)ethane (DDMS), 4,4′-dichlorobenzophenone (DBP), and several other products under anaerobic conditions. *p*-Chlorophenylacetic acid was formed when whole cells and oxygen were subsequently added, thereby demonstrating that enzymes of a single organism can convert DDT to ring-cleavage products. A strain of *Arthrobacter* grew on *p*-chlorophenylacetic acid, thus showing that action of two bacteria leads to extensive biodegradation of DDT. The *Arthrobacter* formed *p*-chlorophenylglycolaldehyde and other products from *p*-chlorophenylacetic acid. The major metabolites formed by microbial communities of sewage and fresh water containing sediment were DDD and DBP, but small amounts of DDMS and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene and no *p*-chlorophenylacetic acid accumulated. Added *p*-chlorophenylacetic acid was rapidly decomposed by the sewage microflora.

The persistence in natural ecosystems of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and structurally related metabolites produced from DDT has been attributed to the inability of microorganisms to degrade such molecules. Evidence exists to show that microorganisms can catalyze modest changes in the DDT molecule, however, converting it to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) in culture (Barker *et al.*, 1965; Johnson *et al.*, 1967; Ledford and Chen, 1969) and in soil (Guenzi and Beard, 1968; Parr *et al.*, 1970). Further steps in DDT metabolism have been noted (Langlois *et al.*, 1970; Matsumura *et al.*, 1971; Wedemeyer, 1967), but microbial degradation to the extent of ring cleavage has not been demonstrated.

A recent report by Focht and Alexander (1970) disclosed that bis(*p*-chlorophenyl)methane (DDM), a product of the microbial metabolism of DDT, could be degraded by a strain of *Hydrogenomonas*. On the basis of this finding, a study was initiated to determine whether this bacterium was capable of metabolizing the insecticide itself and cleaving one or more of its benzene rings. In addition, based on studies of the pathway of decomposition *in vitro*, an attempt was made to establish how the pesticide would be transformed in samples from natural ecosystems containing highly heterogeneous microbial communities.

MATERIALS AND METHODS

Organisms. After 2 yr of growth in organic media following its initial isolation from sewage, the strain of *Hydrogenomonas* had lost the ability to develop autotrophically with H_2 as the sole energy source. For most experiments, the bacterium was grown in a medium containing 0.4% (v/v) of diphenylmethane (DPM), 10 mmol of Na_2HPO_4, 4 mmol of KH_2PO_4, 10 mmol of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.5 mg of $FeSO_4$, and 0.5 mg of $Ca(NO_3)_2$ per liter of distilled water. *Arthrobacter* sp. was isolated from sewage by enrichment cultures using *p*-chlorophenylacetic acid (PCPA) as a sole source of carbon, and this bacterium was grown in the medium described above with 1.0 g of PCPA in place of DPM and 50 μg of yeast extract/ml.

Graduate Field of Microbiology and Laboratory of Soil Microbiology, Cornell University, Ithaca, New York 14850.
[1] Present address: Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, Chapel Hill, North Carolina 27514.

Cell-free extracts of the bacteria were prepared from 24–36 hr cultures grown in the DPM medium. The cells were harvested by centrifugation, washed three times in 0.1 *M* phosphate buffer, pH 7.0, and disrupted by a 10-min exposure to the Sonifier sonic oscillator (Heat Systems Corp., Plainview, N.Y.). The resulting mixture was centrifuged at 8000 × *g* for 10 min to remove intact cells and large cell fragments. Cell suspensions were prepared by resuspending the washed cells in 0.1 *M* phosphate buffer, pH 7.0, to an optical density of 20 at 545 nm.

Incubation Conditions. The cell-free extract containing 5 mg of protein/ml was incubated in 30 ml of 0.1 *M* phosphate buffer, pH 7.0, amended with 10 μg of ^{14}C-DDT/ml. After 4-day incubation at 30°C under an atmosphere of nitrogen, half of the flasks were removed, and freshly harvested cells grown on DPM were added to give a final absorbance of 4.0 at 545 nm. The flasks were then incubated at 30°C on a rotary shaker for 24 hr. Controls consisting of DDT added to 0.1 *M* phosphate buffer, pH 7.0, were subjected to the same anaerobic and aerobic treatments as the biological material. No more than 5% of the added DDT was transformed nonbiologically, and the values reported herein have been corrected for nonbiological changes in the substrate and products.

In studies of the metabolism of PCPA, *Arthrobacter* sp. was grown for 48 hr at 30°C and harvested by centrifugation. The cells were washed three times in 0.1 *M* phosphate buffer, pH 7.0, and resuspended in the same buffer. In some instances, cell-free extracts were prepared from these bacterial suspensions.

Two-liter portions of sewage and freshwater containing sediment collected from a rural stream were placed in 4-l. glass bottles, and DDT, 40% of which was radioactive, was added to the water-sediment ecosystem to a final concentration of 50 μg/ml. The sewage sample contained 100 μg DDT/ml, with 30% of the DDT being labeled. Samples of sewage and freshwater plus sediment which had been autoclaved were used to show nonbiological changes. Air sterilized by filtration was passed over the surface of the liquid contained in the bottles, and the air leaving the bottles bubbled through isopentyl alcohol and then 15% KOH to trap volatile organic compounds and CO_2. The isopentyl alcohol and KOH traps were collected regularly for analysis and the liquid was replaced.

PCPA and 4,4′-dichlorobenzophenone (DBP) at final concentrations of 1.0 mg/ml and 40 μg/ml, respectively, were

added to 1.0 l. of sewage, and the mixtures were incubated in 2-l. flasks on a rotary shaker. The disappearance of PCPA and DBP was measured by removing duplicate 25-ml samples at regular intervals, acidifying them to pH 2.0 with concentrated H_3PO_4, and extracting continuously.

Chemicals. Uniformly ring-labeled ^{14}C-DDT (1.14 mCi/mmol) was obtained from Tracerlab Inc. (Waltham, Mass.). DDT, DDD, DBP, DPM, PCPA, 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene (DDE), 2,2-bis(*p*-chlorophenyl)acetic acid (DDA), and 4,4′-dichlorobenzhydrol (DBH) were obtained from Aldrich Chemical Co., Milwaukee, Wis. DDM was obtained from Eastman Organic Chemicals, Rochester, N.Y.

DDMU [1-chloro-2,2-bis(*p*-chlorophenyl)ethylene] was prepared by the procedure described by Peterson and Robison (1964), and the infrared spectrum of the product was identical to that reported by Abou-Donia and Menzel (1968). DDOH [2,2-bis(*p*-chlorophenyl)ethanol] was prepared from DDA by reduction with $LiAlH_4$ (Nystrom and Brown, 1947). The DDOH prepared by this method gave a single peak on thin-layer chromatograms in a 2% acetone–hexane solvent system and melted at 99°C. Its infrared spectrum (Figure 1) was not identical to that reported by Abou-Donia and Menzel (1968), so confirmation of its identity was sought by mass spectrometry. The mass spectrum (Figure 2) showed a parent ion at *m/e* 266 and the loss of H_2O (18) and CH_2OH (31) from the parent ion, confirming the identity as DDOH.

DDMS [1-chloro-2,2-bis(*p*-chlorophenyl)ethane] and DDNU [2,2-bis(*p*-chlorophenyl)ethylene] were synthesized from DDOH. DDOH tosylate was prepared by mixing 14 g of DDOH dissolved in pyridine with 13.3 g of benzenesulfonyl chloride also dissolved in pyridine. The mixture was allowed to stand for 24 hr. The crystals that resulted when the solution was poured over ice gave a single spot on thin-layer chromatograms in a 2% acetone–hexane solvent system and showed the presence of a sulfonyl peak in the infrared spectrum. DDOH tosylate (219 g) was refluxed for 24 hr with 2.5 g of KCl in *N,N′*-dimethylformamide. The oily crystals that resulted when the reaction mixture was poured over ice revealed four peaks in gas chromatograms. The four compounds were separated by column chromatography on a 0.5 × 15-cm silica gel column using 10% chloroform–hexane as the solvent. The fractions (10 ml) were collected and examined by gas chromatography. DDNU emerged in the early fractions (150–200 ml), while DDMS was eluted in later fractions (350–400 ml). The tubes containing the separated compounds were pooled and evaporated. After recrystallization from methanol, both compounds gave infrared spectra identical to those of Abou-Donia and Menzel (1968).

Analytical Procedures. All extractions were done for 6 hr with continuous extractors (Ace Glass Co., Vineland, N.J.) using anhydrous ethyl ether as the solvent. Thin-layer chromatography was performed using 20- × 20-cm plastic sheets coated with Silica Gel S containing a fluorescent indicator (Brinkmann Instrument Co., Westbury, N.Y.). The solvent systems used were 2% acetone–hexane and 10% chloroform–methanol. Spots were visualized by viewing the plates under uv light at approximately 270 nm.

Gas–liquid chromatography was performed with an Aerograph gas chromatograph, Model 1740-20, equipped with electron capture and hydrogen flame detectors. The 0.3- × 183-cm Pyrex glass column used for separating DDT and the products formed from it anaerobically contained 10% DC-200 on acid-washed Chromosorb W and was connected to the

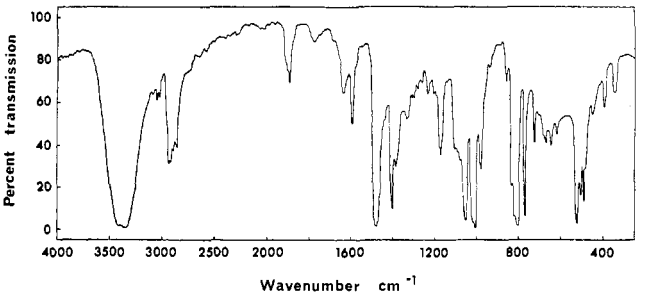

Figure 1. Infrared spectrum of synthetic DDOH

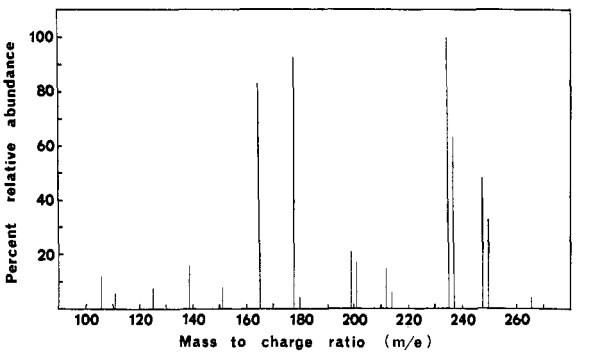

Figure 2. Mass spectrum of DDOH

electron capture detector. This column was operated at 180°C. PCPA and its metabolites were separated with the same packing material in a 0.3- × 183-cm stainless steel column connected to the hydrogen flame detector. The operating temperatures were 195°C for the detector and 205°C for the injector. The flow rates were 110 and 30 ml/min for the glass and stainless steel columns, respectively. Before analysis, the samples were treated with 10% BCl_3–methanol (Applied Science Laboratories, State College, Pa.) for 10 min at 100°C.

Radioactivity on thin-layer chromatograms was counted using an Actigraph II Radiochromatography Strip Counter (Nuclear Chicago, Des Plaines, Ill.). The samples from the natural ecosystems were counted using a Packard Tricarb Liquid Scintillation Spectrometer (Downers Grove, Ill.). Aqueous samples and the material from the KOH traps were counted in Bray's solution (Bray, 1960). Nonpolar products and the material from the isopentyl alcohol trap were counted in a toluene solution containing 0.30 mg of POPOP and 5.0 mg of PPO/ml. To calculate the DDT recovery, a known amount of ^{14}C-DDT was added to each solution, and the counts corresponding to 1 μmol were determined.

Ultraviolet absorption spectra were determined with a Beckman double beam spectrophotometer, Model DB-G. Infrared spectra were obtained on a Beckman IR-10 double beam infrared spectrophotometer using KBr pellets. Mass spectra were obtained with a Perkin-Elmer gas chromatograph–mass spectrometer, Model 270. Catechol was measured by the method of Evans (1947). Phenol was detected as a green to blue color, resulting when aqueous 1% $FeCl_3$ was added to the sample. Chloride release was measured by adding 1.0 ml of 6 *N* HNO_3 and 0.1 ml of 0.1 *M* $AgNO_3$ to 1.0 ml of sample and measuring the turbidity at 545 nm. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Manometric techniques were those described by Umbreit *et al.* (1964).

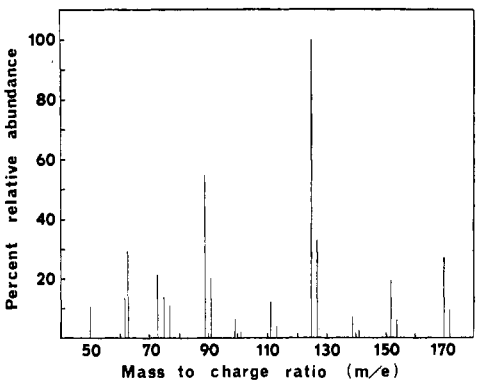

Figure 3. Mass spectrum of metabolite formed from *p*-chlorophenylacetic acid by *Arthrobacter* sp.

Table I. Products of DDT Metabolism by *Hydrogenomonas* sp. in the Absence and Presence of Oxygen

Compound	Compound recovered, nmol: Anaerobic incubation	Compound recovered, nmol: Anaerobic, then aerobic incubation
DDT	108	94
DDD	388	405
DDE	9	19
DDMU	17	<1
DDMS	24	17
DDNU	<1	<1
DDA	<1	<1
DDM	<1	0
DBH	<1	11
DBP	113	78
PCPA	0	78

RESULTS

Metabolism of DDT by Pure Cultures. When 1.42 μmol of ^{14}C-DDT was incubated with cell-free extracts of DPM-grown *Hydrogenomonas* sp. under anaerobic conditions, a number of products were formed. The compounds were separated by gas–liquid chromatography, and they were identified by comparing their retention times with those of the authentic compounds. DDA and PCPA were determined as the methyl esters. The yields of the various metabolites were calculated by comparing the peak areas of the biological products with the peak areas of known amounts of the same compounds. The quantities of the various compounds were corrected for the amount observed in sterile samples, and all data represent averages of triplicate samples.

The data in Table I demonstrate that DDT was metabolized by the bacterial extracts anaerobically, and products with the chromatographic characteristics of DDD, DBP, DDMS, DDMU, and DDE were generated. These are the same as the intermediates in the pathway proposed by Wedemeyer (1967). The radioactivity unaccounted for was present in the remaining extracted reaction mixture.

Because ring-cleavage reactions typically require O_2, the reaction mixture was exposed to aerobic conditions after these metabolites were formed, on the assumption that no further metabolism would occur unless O_2 were available. Furthermore, inasmuch as the 4-day anaerobic incubation probably led to denaturation of some of the enzymes, fresh DPM-grown cells were added. The data in Table I demonstrate that the metabolites generated anerobically were further transformed in air to give at least one additional metabolite. The products were separated on thin-layer sheets in a 2% acetone–hexane solvent system, the compounds being identified by eluting the spots and characterizing them by gas chromatography. The spot at R_f 0.14 was radioactive, as determined on the thin-layer strips. When eluted from the strips and injected into the gas chromatograph, the compound with an R_f value of 0.14 gave the same retention time as authentic PCPA. A sample containing the same metabolite was injected into the gas chromatograph–mass spectrometer, and the spectrum obtained corresponded exactly to that of authentic PCPA. Thus, PCPA is apparently generated as a result of cleavage of one of the rings of a metabolite already formed from DDT.

Arthrobacter sp. cells grown in Trypticase Soy Broth and in the PCPA medium were examined for their ability to oxidize PCPA. When tested by manometric techniques at 30°C with air as the gas phase, intact cells grown on the former medium consumed O_2 after a 30-min lag period, but extracts of the cells showed no O_2 uptake on PCPA. On the other hand, PCPA-grown cells consumed O_2 rapidly and without a lag, but extracts of such cells likewise failed to consume O_2 when PCPA was the substrate; chloride release was not observed under these conditions. In these studies, the Warburg flasks contained 1.0 μmol of PCPA and either intact cells at an absorbance of 4.0 or bacterial extract equivalent to 5.0 mg protein/ml.

Changes in the culture medium during growth of *Arthrobacter* sp. on PCPA were assessed by measuring changes in uv absorbance, by determining possible phenol and catechol formation, and by examining ether extracts by means of the gas chromatograph. Although neither catechol nor phenol was evident, a large decrease in uv absorption at 234 nm occurred as the organism utilized PCPA. Gas chromatograms showed the formation of a compound with a retention time of 345 sec which appeared 6 hr after inoculation of the *Arthrobacter*, the concentration reached a maximum at 12 hr, and it had disappeared by 24 hr. At the time of its maximum concentration, this compound accounted for 6.4% of the total material recovered, as determined by the areas of the peaks in the gas chromatograms.

This compound passed through the DC-200 column without methylation, suggesting that it was not a carboxylic acid. The unmethylated ether extract from the 12-hr culture was injected into the gas chromatograph–mass spectrometer equipped with a DC-200 column. In the resulting mass spectrum, the parent ion at *m/e* 170 and the peak at 172 were in a 3:1 ratio, suggesting that the compound contained one chlorine (Figure 3). The peaks at 152 and 139 correspond to the loss of water and a further loss of CH. The peak at 125 could represent the loss of a carboxyl group from the parent ion, but since the unesterified compound passed through a DC-200 column, a polar group is probably absent; the peak could also represent the loss of carbonyl and hydroxyl groups. The peak at *m/e* 111 corresponds to the further loss of CH_2 and apparently leaves a chlorinated phenyl ring. The remaining peaks are the same as those observed in the mass spectrum of PCPA and probably are associated with the degradation of the aromatic ring.

To characterize further this product of PCPA metabolism, the extract was applied to preparative thin-layer plates and developed in a 10% chloroform–methanol solvent system. On these plates, the product ran in a band ahead of PCPA. It also gave a single peak when examined in the gas chromatograph. The metabolite was removed from the plates, mixed with KBr, and its infrared spectrum measured. The spectrum shows what appears to be carbonyl absorption at

Table II. Compounds Formed by Freshwater-Sediment Community and Sewage Community Incubated with DDT

Weeks	Products recovered, μmol											
	DDT	DDD	DDE	DDMU	DDMS	DDNU	DDA	DDM	DBH	DBP	PCPA	Total
Freshwater community												
0	5.96	0.064	0.068	0.0	0.015	0.019	0.14	<0.001	<0.001	0.017	0.0	6.15[a]
1	5.23	0.761	0.230	0.0	0.101	<0.001	<0.001	0.0	0.0	0.044	0.0	6.36
3	5.15	0.792	0.110	0.0	0.029	<0.001	0.0	<0.001	<0.001	0.005	0.0	6.10
6	5.04	0.604	0.0	0.0	0.0	0.47	<0.001	0.035	<0.001	0.012	0.0	5.74
12	4.15	1.19	0.0	0.0	0.055	0.0	0.0	0.100	0.162	0.538	0.0	6.19
24	2.32	3.36	0.446	0.062	0.048	0.049	0.0	0.072	0.065	0.832	0.0	7.25
Sewage community												
0	6.70	0.319	0.130	0.0	0.0	0.0	0.0	0.0	0.0	0.205	0.0	7.35[b]
1	4.86	1.88	0.168	<0.001	0.0	0.014	0.0	0.0	0.0	2.94	0.0	9.86
3	4.57	3.24	0.029	<0.001	0.0	0.017	0.0	0.0	0.0	3.17	0.0	11.02
6	3.80	4.40	0.040	0.132	0.004	0.013	0.0	0.066	0.0	6.15	0.0	12.60
12	3.68	4.37	0.0	0.0	0.0	0.093	0.0	0.090	0.001	3.35	0.0	11.58
24	3.13	4.75	0.0	0.0	0.094	0.063	0.0	<0.001	0.064	3.69	0.0	11.79

[a] A total of 7.1 μmol of DDT was originally added. [b] A total of 14.2 μmol of DDT was originally added.

1690 cm^{-1} and aldehyde C–H bending at 1400 cm^{-1} (Figure 4). The broad peak near 3000 cm^{-1} could represent the OH stretching of an alcohol. The peaks at 790, 825, and 1000 cm^{-1} correspond to a 1,4-disubstituted aromatic ring (Silverstein and Bassler, 1967). Although the infrared spectrum is not too well-defined, it does suggest a probable structure.

The mass and infrared spectra are consistent with the hypothesis that the compound produced from PCPA is *p*-chlorophenylglycolaldehyde. As a further test that it was an aldehyde, the compound was mixed with Tollen's reagent, and a precipitate resembling a silver mirror resulted.

Metabolism of DDT by Natural Microbial Communities. To investigate the fate of DDT in natural habitats populated by diverse microorganisms, labeled DDT was added to sewage and to a freshwater-sediment model ecosystem. DDT and nonpolar products of its metabolism were recovered by continuous extraction of aliquots with ethyl ether, and the counts remaining in the samples after extraction were determined. The results were similar in both model ecosystems, and more than 99.9% of the radioactivity was recovered in the ether extract in both model communities at each sampling period. The unextracted liquid contained most of the remainder, possibly as residual DDT. The KOH and isopentyl alcohol traps, through which the air passing over the incubation mixture had been bubbled, had less than 0.01% of the counts; thus, volatile organic compounds or CO_2 were not produced in significant amounts.

The ether extracts were analyzed by gas chromatography. The yield and identity of products found in the freshwater-sediment and sewage model ecosystems are given in Table II. The amounts of products, if any, formed in sterile controls have been subtracted from the quantities shown. The compounds present in the initial samples resulted from impurities in the labeled DDT. The metabolites generated from DDT by these microbial communities were similar to those elaborated by *Hydrogenomonas* sp. *in vitro*.

The major metabolites accumulating were DDD and DBP. Significant quantities of several other bacterial products were noted too, but their levels did not approach those of DDD and DBP. The total recovery increased in the 24-week samples from the freshwater-sediment ecosystem and rose with time in samples from the model sewage ecosystem. This may have resulted from an initial binding of DDT to organic material and microbial cells, the insecticide then possibly being released as the organic materials were subsequently decomposed.

In samples from neither model ecosystem was PCPA found to accumulate. To determine if this intermediate could be degraded as rapidly as it might have been formed, sewage was amended with 1.0 mg of PCPA/ml. The disappearance of PCPA was assessed by regularly measuring the uv absorption at 234 nm and the area of the peak in the gas chromatogram that corresponded to PCPA. As shown in Figure 5, PCPA disappeared rapidly after the fourth week, and almost none was present at 6 weeks. The compound did not disappear from sewage sterilized by autoclaving. The decrease in uv absorbance indicates ring cleavage. Thus, the lack of accumulation of PCPA may be a consequence of the fact that it was metabolized as rapidly as it was formed. On the other

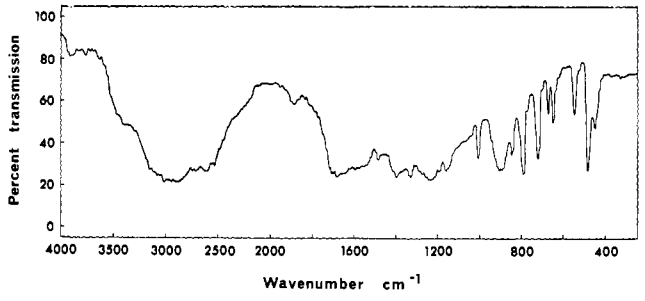

Figure 4. Infrared spectrum of metabolite formed from *p*-chlorophenylacetic acid by *Arthrobacter* sp.

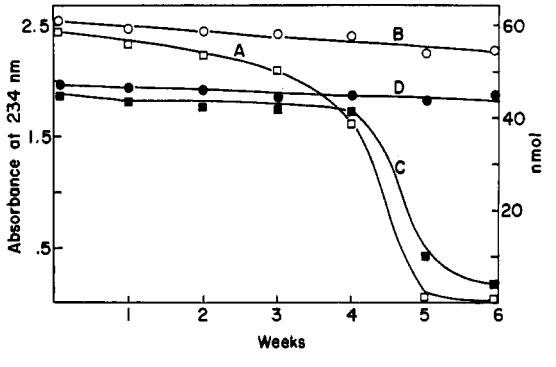

Figure 5. Metabolism of *p*-chlorophenylacetic acid by microorganisms in sewage. Disappearance of the substrate was measured by gas chromatography (A and B) or by changes in ultraviolet absorbancy (C and D). Samples B and D were sterilized

hand, the absence of significant accumulations of radioactive CO_2 in model ecosystems receiving ^{14}C-DDT suggests that little PCPA would have been synthesized inasmuch as it likely would have been completely degraded.

Because DBP accumulations were noted in studies of both bacterial enzymes and model microbial ecosystems, an investigation of its fate in sewage was undertaken. DBP was added to 1.0 l. of raw sewage at a concentration of 40 μg/ml, and the treated sewage was incubated at 30°C on a rotary shaker. Samples were taken at regular intervals, and they were extracted and then analyzed by gas chromatography. In a study with sewage collected in the autumn, DBP disappeared rapidly, and all was gone in 4 weeks. In a second investigation using samples from the same sewage treatment plant collected in the winter, no degradation of DBP was evident in a 6-week period. It is likely that organisms were present that could actively degrade DBP in one but not in the second sewage sample, possibly a result of seasonal fluctuations in the microflora.

DISCUSSION

This study has shown that DDT can be extensively degraded by enzymes of a single microorganism. Moreover, this is the first demonstration of a ring-cleavage product being formed when DDT is the initial substrate for enzymes of a single microbial population. This cleavage is evident in the finding that DDT is metabolized to a series of compounds, one or more of which is then apparently converted to PCPA. Although it is conceivable that the carboxyl of PCPA came from the ethylene moiety rather than from one of the rings, this seems quite unlikely in view of Focht and Alexander's (1971) finding that the same bacterium converted diphenylmethane to phenylacetic acid and DDM to PCPA; that is, it can cleave the ring.

Furthermore, the action of enzymes of two different bacteria is shown to result in the extensive biodegradation of the pesticide, the enzymes of *Hydrogenomonas* sp. converting DDT to PCPA and those of *Arthrobacter* sp. degrading PCPA. Although only a single product of PCPA metabolism by *Arthrobacter* sp. was isolated, *p*-chlorophenylglycolaldehyde, PCPA is apparently extensively decomposed, as shown by the decrease in uv absorbancy in *Arthrobacter* sp. cultures and sewage.

The freshwater-sediment and sewage ecosystems also metabolize DDT. The compounds formed from DDT by both microbial communities are strikingly similar to those formed by *Hydrogenomonas* and those previously reported to be products of the metabolism of DDT by *Aerobacter aerogenes* (Wedemeyer, 1967). The initial steps in DDT degradation have already been reported to occur under anaerobic conditions (Parr *et al.*, 1970; Wedemeyer, 1967), a finding confirmed in the present investigation. However, the present study has shown that one of the rings is cleaved microbiologically once O_2 is available. Therefore, although the first stages in the reaction sequence will occur in the absence of atmospheric oxygen, the *in vitro* studies suggest that the ring cleavage reactions are O_2-requiring. Whether ring cleavage is only effected by an oxygenase and hence whether complete destruction requires aerobiosis require further investigation.

Inasmuch as the findings herein show extensive biodegradation of DDT *in vitro*, although admittedly necessitating high protein concentrations in bacterial extracts or thick cell suspensions, it is tempting to speculate why the insecticide is so persistent in natural ecosystems. One hypothesis is that complete destruction requires both anaerobiosis and subsequent aerobiosis. Such fluctuations in O_2 status may be infrequent in some ecosystems, but they do commonly take place in soil; for example, following a heavy rain or the incorporation of plant remains in soil, the O_2 level falls, but it rises again when the rate of O_2 diffusion into the soil once again exceeds the rate of its utilization by the indigenous species. The binding of DDT to clay minerals may prevent the movement of the insecticide from anaerobic to aerobic microhabitats, but O_2 may still move to the site where the chemical is bound.

A second and more plausible hypothesis is that the pesticide is degraded by only a very small number of microorganisms, none of which uses the compound as a carbon or energy source. Since they are thus unable to gain energy or carbon from this substrate, they have no selective advantage in environments containing the pesticide and do not proliferate at its expense. Their population densities, therefore, remain low, and the degradation is only effected by the few cells that are able to compete with neighboring species for alternate carbon sources.

If this latter hypothesis is valid, increasing the cell densities of the active species should enhance the rate of DDT destruction. Thus, the observations that the rate of DDT degradation is increased when nutrients are added to soil (Guenzi and Beard, 1968; Ko and Lockwood, 1968; Parr *et al.*, 1970) tend to support the hypothesis proposed.

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