Anaerobic Decomposition of DDT in Soil

Acceleration by Volatile Components of Alfalfa

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Ground alfalfa or glucose has been shown to accelerate the anaerobic disappearance of DDT from soil. A steam distillate from alfalfa will also accelerate anaerobic disappearance of DDT. When volatile components of the steam distillate were compared with glucose, the following order of effectiveness was found: acetaldehyde = isobutyraldehyde > ethanol > glucose ≫ methanol. The anaerobic disappearance of DDT, inhibited by autoclaving the soil, was reestablished by inoculating with a small amount of viable soil or by inoculation from air. Two percent oxygen inhibited anaerobic disappearance of DDT. With total disappearance of DDT, DDD in an amount equivalent to about 26% of the added DDT was the only breakdown product detected. Both DDD and DDE were stable under anaerobic conditions, indicating that the DDT was disappearing by some route other than *via* DDD or DDE.

Long-term field studies have shown that DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane] disappears very slowly from soils, suggesting that it resists decomposition by soil microorganisms (Clore *et al.*, 1961; Nash and Woolson, 1967). Guenzi and Beard (1968), however, found that DDT disappeared relatively rapidly from an anaerobic soil, especially when the soil was amended with alfalfa meal. Ko and Lockwood (1968) confirmed the accelerating effect of alfalfa on the disappearance of DDT from anaerobic soil and found that glucose-protein mixtures were also effective. In both studies (Guenzi and Beard, 1968; Ko and Lockwood, 1968) DDT was stable in aerobic soil despite amendment with alfalfa. In anaerobic soil, DDD [1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethane] was the main identifiable product, with a considerable fraction of the disappearing DDT not accounted for as any of the other known dechlorination products of DDT. Guenzi and Beard (1968) were able to demonstrate with ^{14}C tagged DDT that part of the missing radioactivity was not extracted from the soil.

The above results from soil studies are similar to those from other work wherein yeast cells (Kallman and Andrews, 1963) and certain bacteria from the intestinal tracts of rats (Braunberg and Beck, 1968; Mendel and Walton, 1966), mice (Barker *et al.*, 1965), and flies (Stenersen, 1965) dechlorinated DDT relatively rapidly to DDD with a significant fraction of the disappearing DDT unaccounted for. Wedemeyer's work (1967) with intact cells and cell-free extracts of *Enterobacter aerogenes* is exceptional in that he accounted for essentially all the added DDT in the form of DDD and other degradation products beyond DDD. DDE [1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethylene], like DDD, a first-step degradation product of DDT, was formed but was not further degraded. DDE has also been shown to be stable when fed to animals (Hayes, 1964). Wedemeyer (1967) depicted a degradation sequence from DDT *via* DDD by stepwise reactions to DBP (4,4′-dichlorobenzophenone). All the intermediates were degraded by his cell-free preparations.

Inhibition of dechlorination of DDT by autoclaving a soil as found by Guenzi and Beard (1968) and Ko and Lockwood (1968) is not final evidence that the process is microbial. Soils, as nonbiological entities, may react with DDT since they contain reactive free radicals and iron and other metals subject to redox changes. Autoclaving may quench free radicals and may bring about other chemical as well as physical changes in soil. However, restoration of the ability of a soil to dechlorinate DDT by inoculation with a small amount of viable soil would demonstrate that microorganisms growing in soil can dechlorinate DDT.

The respiratory activities of soils can be greatly increased by amending them with an aqueous solution of volatiles obtained by steam or vacuum distillation from an alfalfa-water slurry (Menzies and Gilbert, 1967). These results suggest that the acceleration of DDT conversion to DDD by alfalfa might be at least partly explained by the stimulating effect of alfalfa volatiles on the activities of soil microorganisms.

The major objective of this study was to test the ability of alfalfa volatiles, active in increasing soil respiration, to accelerate the anaerobic decomposition of DDT in soil. Other objectives were to determine the effect of small amounts of oxygen on DDT decomposition in soil, to determine in a definitive way whether or not the process was microbial, and to test the possibility that DDD or DDE could be intermediates in the decomposition of DDT in anaerobic soil.

MATERIALS AND METHODS

DDT and Metabolites. The *p,p′* forms of DDT, DDD, DDE, and DDA [2,2-bis-(*p*-chlorophenyl)acetic acid] as manufactured by Aldrich Chemical Co., Inc., Milwaukee, Wis., were used throughout this study.

Soil Used. Drummer silty clay loam, pH 5.9, with a field waterholding capacity of 38.4%, was collected in a moist state and passed through a 4-mm screen, then brought to field capacity with distilled water and stored in 1-kg lots in polyethylene bags at −20° C. The soil was allowed to thaw and stand for 4 days in the bags before experimental use.

Application of DDT, Glucose, and Alfalfa. A 100-g subsample of the soil was taken from a bag and air dried. The DDT (1.00 to 2.00 mg) was applied in 20 ml of hexane to the subsample. After evaporation of hexane and mixing of the soil and residual DDT, the subsample was mixed into the rest of the soil. Glucose or alfalfa, when used, was applied by mixing into the same air-dry subsample of soil used for applying the DDT.

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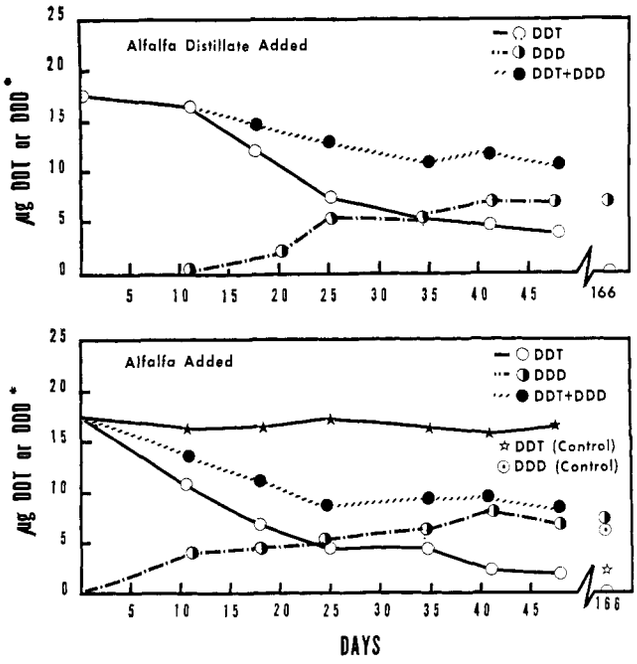

Figure 1. DDT decomposition in anaerobic Drummer soil as influenced by alfalfa or the steam distillate from alfalfa. One milliliter of distillate was added per 20 g of soil. One kilogram of alfalfa was used to obtain 20 ml of distillate. *DDD plotted in DDT-equivalent μg

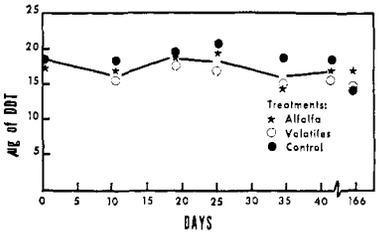

Figure 2. Aerobic decomposition of DDT in Drummer soil as influenced by alfalfa or the steam distillate from alfalfa. One milliliter of the distillate was added per 20 g of soil. One kilogram of alfalfa was used to obtain 20 ml of distillate

Preparation of Soil Sample for Incubation. After treatment, samples of soil (24.6 g) were weighed into 100-ml milk-dilution bottles. For anaerobic incubation, the bottles were degassed and filled with N_2 through hypodermic needles inserted into the self-sealing stoppers used to plug the bottles. This procedure was repeated three times with each sample. For incubation under varying oxygen concentration, oxygen was added to the degassed bottles through the hypodermic needles. The desired partial pressure was established with an attached mercury manometer, then the bottle was filled with nitrogen. For aerobic incubation, the bottles were placed unstoppered in a large desiccator containing free water to maintain humidity. All samples were incubated at 28 ± 1° C in a constant temperature room.

Application of Alfalfa Volatiles. The aqueous alfalfa-volatile solution was obtained by distillation from an alfalfa-water slurry as described elsewhere (Owens *et al.*, 1969). One kilogram of alfalfa was used to produce 20 ml of distillate. For treatment, 1 ml of this distillate and 3 ml of water were added per soil sample by hypodermic injection through the serum stopper of each bottle to be incubated anaerobically. Four milliliters of distilled water were added to the control samples. The main active components of the distillate, acetaldehyde, isobutyraldehyde, ethanol, and methanol, as determined by Owens *et al.* (1969) for stimulation of soil microbes, were also applied in 4 ml of water in the same way. Purity of the commercial reagents was checked by gas chromatography (Owens *et al.*, 1969).

Analytical Procedures. A 3-hr Soxhlet extraction with 225 ml of 3 to 1 (v/v) glass-distilled hexane-isopropyl alcohol solution was used to remove the DDT and its metabolites from the soil samples. The samples (20-g, soil oven-dry basis) were extracted in a moist condition. An alumina column was used to clean up the extractant. After cleanup, the isopropyl alcohol was partitioned off by three shakings with 100 ml of distilled water. The remaining hexane containing DDT and its metabolites was made to 250 ml volume for analysis on a Micro-Tek gas chromatograph equipped with an Ni^{63} electron-capture detector. The column was a 0.4-cm i.d. × 180-cm glass tube packed with 10% DC-200 on Gas-Chrom Q kept at 195° C. The flow rate of the carrier gas (95% argon–5% methane) was 145 ml per min.

For detection of DDT metabolites that might be present in extremely low concentrations, a 100-ml portion of the hexane was concentrated to 1 ml in a Kuderna-Danish apparatus. To detect DDA, the hexane-isopropyl alcohol extractant of some of the samples was made to 250 ml with 3 to 1 hexane-isopropyl alcohol before cleanup and a 50-ml sample was removed. This sample was evaporated to dryness and the residue was taken up in 2 ml of ether and esterified with ethereal diazomethane (Woolson and Harris, 1967). Recovery of authentic DDA added to soil samples to test the procedure was about 75%—not highly quantitative but sufficient to detect small quantities of DDA.

Sterilization. The soil was autoclaved at 121° C and 15 psi for 30 min. The soil was prepared for autoclaving in two ways. The bulk soil was spread out to a 1-cm layer and autoclaved before DDT was applied, DDT was applied, and the soil was weighed into the sample bottles and autoclaved. The second procedure was used to avoid microbial reinoculation that apparently occurred from the air when the first procedure was used.

RESULTS AND DISCUSSION

The acceleration of the anaerobic degradation of DDT produced by adding alfalfa to a Drummer soil was also produced by adding volatiles obtained from alfalfa by steam distillation (Figure 1). For the anaerobically incubated soil treated either with alfalfa or volatiles, most of the decomposition occurred before the 25th day of incubation. For the anaerobic control soil, essentially no DDT had disappeared by the 46th day. However, the next analysis (166th day) showed DDT had almost completely disappeared from the control as well as from the treated soil samples. The only detectable product remaining in all cases was DDD in an amount equivalent to about 26% of the DDT initially determined at 0 time. No other decomposition products of DDT, including DDA, were detected to account for the missing 74%. In the aerobically incubated samples, no decomposition of DDT occurred in the controls or the treated samples (Figure 2).

In anaerobic metabolism by microorganisms, DDT may be converted directly to either DDD or DDE as initial products (Braunberg and Beck, 1968) DDE not being a precursor of DDD (Stenerson, 1965). Both of these compounds appear to be stable in Drummer soil, treated with volatiles from alfalfa, and incubated aerobically or anaerobically for 31

days (Table I). Therefore, it seems apparent that the DDT not recovered in these experiments has not been converted to unidentified compounds through DDD or DDE as intermediates.

The inhibiting effect of oxygen at normal atmosphere concentration on DDT dechlorination by microbial cultures and in soil has been clearly demonstrated by Barker *et al.* (1965) and other workers, but the concentration at which oxygen becomes inhibiting is not known. As shown in Table II, the presence of 2% oxygen, introduced at the outset of the experiment, was enough to strongly inhibit the dechlorination of DDT. After 64 days of incubation, a small amount of DDD was present in the 2% O_2 treatment, but total recovery (DDT + DDD) came close to accounting for all the DDT present at time 0. For oxygen levels above 2%, recovery was complete without any production of dechlorination products, but for the samples at 0 oxygen, 25% was recovered as DDT, 34% as DDD, and 41% was unaccounted for after 64 days of incubation. Dechlorination of DDT probably occurred in the 2% O_2 treatment only after microbial respiration had essentially removed the O_2.

Table I. Recovery of 30.00 μg of DDD or DDE when Either is Added to a Drummer Soil (20 g, Oven-Dry Basis) Treated With an Aqueous Solution of Alfalfa Volatiles

Incubation	0 days	18 days	31 days
	DDD Recovered (μg)		
Aerobic	27.5	31.2	29.1
Anaerobic	27.5	30.7	31.0
	DDE Recovered (μg)		
Aerobic	27.8	27.9	25.7
Anaerobic	27.8	28.7	26.0

In the experiment summarized in Table III, the autoclaved samples did not remain sterile. DDT was added after autoclaving, and inoculation from the air apparently occurred at this time. The rate of disappearance of DDT was much higher in the autoclaved soil inoculated with viable soil than in the soil apparently inoculated from the air. The influence of the alfalfa volatiles in this experiment was

Table II. Influence of O_2 on Decomposition of 30 μg of DDT in Drummer Soil (20 g, Oven-Dry Basis) Treated With Alfalfa Volatiles

DDT Reported in μg and DDD Reported in DDT-Equivalent μg

	% O_2					
	0.0	2.0	5.0	7.0	10	20
			0 Days			
DDT	30.7	33.9	30.4	30.8	33.8	28.1
DDD	0.0	0.0	0.0	0.0	0.0	0.0
Total	30.7	33.9	30.4	30.8	33.8	28.1
			15 Days			
DDT	32.9	32.8	33.4	31.7	32.9	29.4
DDD	0.0	0.0	0.0	0.0	0.0	0.0
Total	32.9	32.8	33.4	31.7	32.9	29.4
			35 Days			
DDT	25.5	35.0	33.8	32.0	32.3	31.5
DDD	5.0	0.0	0.0	0.0	0.0	0.0
Total	30.5	35.0	33.8	32.0	32.3	31.5
			64 Days			
DDT	8.6	27.2	31.9	30.9	31.1	29.4
DDD	10.5	4.1	0.0	0.0	0.0	0.0
Total	19.1	31.3	31.9	30.9	31.1	29.4

Table III. Influence of Autoclaving and Addition of Alfalfa Volatiles on the Decomposition of DDT (50 μg) in a Drummer Soil (20 g, Oven-Dry Basis) Incubated Anaerobically

Autoclaved Samples Did Not Remain Sterile. DDT Reported in μg and DDD in DDT-Equivalent μg

	Water Added			Volatiles Added		
	Days			Days		
	0	23	41	0	23	41
			Autoclaved			
DDT	46.4	37.0	16.8	53.5	38.5	26.2
DDD	0.0	10.0	21.9	0.0	10.6	18.1
Total	46.4	47.0	38.7	53.5	49.1	44.3
			Autoclaved Inoculated			
DDT	55.8	18.5	6.2	44.1	12.6	6.7
DDD	0.0	19.0	24.8	0.0	19.5	24.5
Total	55.8	37.5	31.0	44.1	32.1	31.2
			Not Autoclaved			
DDT	56.0	54.2	52.4	51.9	28.8	11.0
DDD	0.0	0.0	0.0	0.0	15.3	25.3
Total	56.0	54.2	52.4	51.9	44.1	36.3

Table IV. Influence of Autoclaving on the Anaerobic Decomposition of DDT (60 μg) in Drummer Soil (20 g, Oven-Dry Basis)

Autoclaved Samples Remained Sterile Throughout the Incubation. DDT Reported in μg and DDD in DDT-Equivalent μg

	Days of Incubation				
	0	11	27	47	82
			Not Autoclaved (Alfalfa Volatiles)		
DDT	58.8	42.0	18.3	8.4	1.9
DDD	0.0	3.7	16.3	24.5	21.4
Total	58.8	45.7	34.4	32.9	23.3
			Autoclaved		
DDT	47.2	39.9	43.4	47.0	43.6
DDD	0.0	0.0	0.0	0.0	0.0
Total	47.2	39.9	43.4	47.0	43.6
			Autoclaved Inoculated		
DDT	44.5	30.7	15.0	7.7	2.6
DDD	0.0	4.4	14.1	19.2	17.7
Total	44.5	35.1	29.1	26.9	20.3

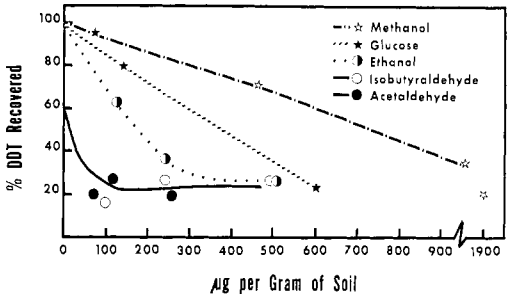

Figure 3. DDT remaining in anaerobic Drummer soil as influenced by concentrations of acetaldehyde, isobutyraldehyde, ethanol, glucose, and methanol. The aldehyde and alcohol concentrations were equal to and at 2× and 4× their concentrations in the alfalfa distillate applied to soils in Figure 1. The glucose concentrations were arbitrary

obscured by autoclaving. Autoclaving seems to have released stimulants just as effective as the alfalfa volatiles in accelerating anaerobic dechlorination and disappearance of DDT.

The process of dechlorination and disappearance of DDT was shown to be biological by experiments wherein DDT was added before autoclaving so that the soils did not need to be exposed to inoculation from the air (Table IV). When inoculation from the air was prevented, autoclaving inhibited the degradative process. The possibility that this inhibition might have been a result of chemical alteration of the soil was eliminated because inoculation of the sterilized soil sample with a small amount of viable soil (0.5 g added to 20 g) restored the ability to degrade DDT.

Isobutyraldehyde and acetaldehyde are the components of the aqueous solution of alfalfa volatiles that produce the most respiratory activity when added to soils (Owens *et al.*, 1969). As shown in Figure 3, they are also the most effective of the volatiles tested in inducing disappearance of DDT. Surprisingly these two compounds, along with ethanol, are more effective than glucose. Methanol is effective but much less so than the other four substances.

CONCLUSIONS

The anaerobic breakdown of DDT in soils is accelerated by glucose and by volatiles present in ground alfalfa. The order of effectiveness of the substances is acetaldehyde = isobutyraldehyde > ethanol > glucose ≫ methanol. The degradative process, highly sensitive to oxygen, is definitely microbial, at least in the conversion of DDT to DDD. DDE did not appear and, like DDD, was found to be stable relative to DDT. Therefore, the process by which DDT disappears does not seem to involve either a first step conversion to DDD or DDE. It may well be that the first step in the conversion of DDT to DDD or DDE involves a complex which reacts with microbial protoplasms or other components of the soil mineral or organic matter.

Our findings suggest that additions of isobutyraldehyde, acetaldehyde, ethanol, and other short-chain aldehydes or alcohols followed by imposing anaerobic conditions may be useful for decontaminating soils of DDT.

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