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Microbial degradation of DDT and its residues—a review

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Abstract Microbial degradation of DDT residues is one mechanism for loss of DDT from soil. In this review pathways for biodegradation of DDT, DDD, and DDE by bacteria and fungi are described. Biodegradation of DDT residues can proceed in soil, albeit at a slow rate. To enhance degradation in situ a number of strategies are proposed. They include the addition of DDT-metabolising microbes to contaminated soils and/or the manipulation of environmental conditions to enhance the activity of these microbes. Ligninolytic fungi and chlorobiphenyl degrading bacteria are promising candidates for remediation. Flooding of soil and the addition of organic matter can enhance DDT degradation. As biodegradation may be inhibited by lack of access of the microbe to the contaminant, the soil may need to be pre-treated with a surfactant. Unlike DDT, little is known about the biodegradation of DDE, and this knowledge is crucial as DDE can be the predominant residue in some soils.

Keywords DDT; DDD; DDE; soil; microbial degradation

INTRODUCTION

1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane (DDT) was used extensively in New Zealand for the control of grass grub (*Costelytra zealandica* White). Although its use ceased in 1970, DDT residues (DDTr) still persist in New Zealand soils, predominantly in the form of DDT, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), and 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane *(o,p'*-DDT) (Fig. 1). Both DDD and DDE are transformation products of DDT (Boul 1995). DDD is formed from DDT through reductive dechlorination, either microbially mediated (Wedemeyer 1966) or as the result of chemical reactions (Castro 1964; Miskus et al. 1965; Glass 1972; Zoro et al. 1974; Baxter 1990) some of which are mediated by biomolecules. DDE is formed from DDT through photochemical reactions in the presence of sunlight (Maugh 1973) and through dehydrochlorination in bacteria (Pfaender & Alexander 1972) and animals (Kurihara et al. 1988). The presence of chlorine substituents on these molecules has been implicated in their persistence. DDT, for example, is recalcitrant, whereas its non-chlorinated analogue diphenylmethane is readily biodegradable (Alexander 1977).

Since DDTr are lipophilic, they tend to accumulate in the fatty tissues of ingesting organisms along the food chain. Although there are no known acute effects of environmental concentrations of DDT or its metabolites on human health, the possibility of chronic effects cannot be ruled out. Some DDTr have estrogenic activity whereas DDE has recently been identified as a powerful androgen receptor (Kelce et al. 1995). DDTr are accumulated by people through consumption of agricultural products grown on contaminated soils (Bates et al. 1994).

DDTr in soil are of concern as their uptake can lead to accumulation in primary products. Their removal from soil is therefore a priority. A review of DDTr in the environment, with particular

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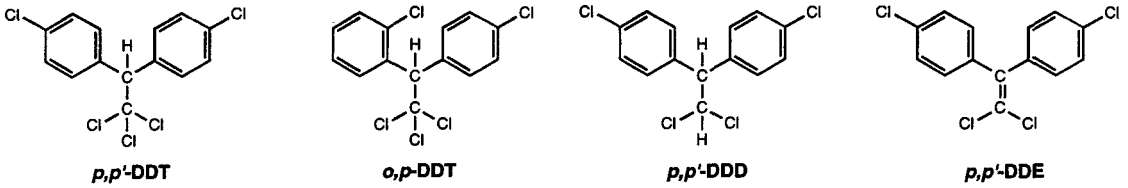

Fig. 1 DDT and its residues DDD, DDE, and *o,p'*-DDT. DDT = 1,1,1-trichloro-2,2-bis(*p*-chloropenyl)ethane; DDD = 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane; DDE = 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; and *o,p'*-DDT = 1,1,1 -trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane.

reference to New Zealand, has been published recently (Boul 1995). Possible mechanisms for DDTr loss from soil include volatilisation, erosion, uptake by plants and animals, and biodegradation. This paper reviews mechanisms for biodegradation of DDT residues by bacteria and fungi, as these are the microbes commonly implicated in the breakdown of pesticides (Bollag & Liu 1990). The enhancement of microbial degradation of DDTr in soil is also discussed.

MICROBIAL DEGRADATION OF DDT RESIDUES

Biodegradation of DDTr largely involves co-metabolism in which microbes growing at the expense of a growth substrate are able to transform DDTr without deriving any nutrient or energy for growth from the process (Bollag & Liu 1990). Mechanisms for microbial attack on DDT have been described in the literature. Most reports indicate that DDT is reductively dechlorinated to DDD under reducing conditions (Johnsen 1976; Essac & Matsumura 1980; Lal & Saxena 1982; Kuhn & Sulflita 1986; Rochkind-Dubinsky et al. 1987). Bacteria and fungi are reported to metabolise DDT in this way and pathways for biodegradation by this route have been determined using both. Recently an alternative route for microbial attack under aerobic conditions has been described, involving strains of chlorobiphenyl-degrading bacteria (Nadeau et al. 1994). In the following section the metabolic pathways are described and the mechanisms are discussed. Unlike DDT, there are few reports of microbial degradation of DDE.

Reductive dechlorination

Under reducing conditions reductive dechlorination is the major mechanism for the microbial conversion of both the *o,p'*-DDT and *p,p'*-DDT isomers of DDT to DDD (Fries et al. 1969). The reaction involves substitution of an aliphatic chlorine for a hydrogen atom (Fig. 2).

Early investigations on the fate of DDT in rodents demonstrated that the indigenous microflora of animals were responsible for the conversion of DDT to DDD (Barker et al. 1965; Mendel & Walton 1966). Since then a number of microbes have been shown to be capable of converting DDT to DDD in pure culture. These include the bacteria *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Bacillus* sp., "*Hydrogenomonas*", and the fungi *Saccharomyces cerevisiae*, *Phanerochaete chrysosporium*, and *Trichoderma viridae* (Johnsen 1976; Lal & Saxena 1982; Subba-Rao & Alexander 1985; Sharma et al. 1987; Beunink & Rehm 1988). As the biotransformation of DDT to DDD by these micro-organisms requires the presence of an alternative carbon (C) source, the process is co-metabolic.

DDT-metabolising microbes have been isolated from a range of habitats including animal faeces, soil, sewage, activated sludge, and marine and freshwater sediments (Johnsen 1976; Lal & Saxena 1982; Rochkind-Dubinsky et al. 1987). There are reports of aerobic biotransformation of DDT to DDD. Rochkind-Dubinsky et al. (1987) proposed, however, that reducing conditions would have developed in the culture flasks during these experiments. Mechanisms for reductive dechlorination, with transition metals and metal complexes acting as reductants, have been reviewed by Hollinger & Schraa (1994). In most instances the process involves single electron transfer, removal of a chlorine ion, and formation of an alkyl radical. In micro-organisms transition metal complexes are associated with the active centres of electron transport molecules. Reductive dechlorination of DDT by *E. coli,* for example, required flavin-

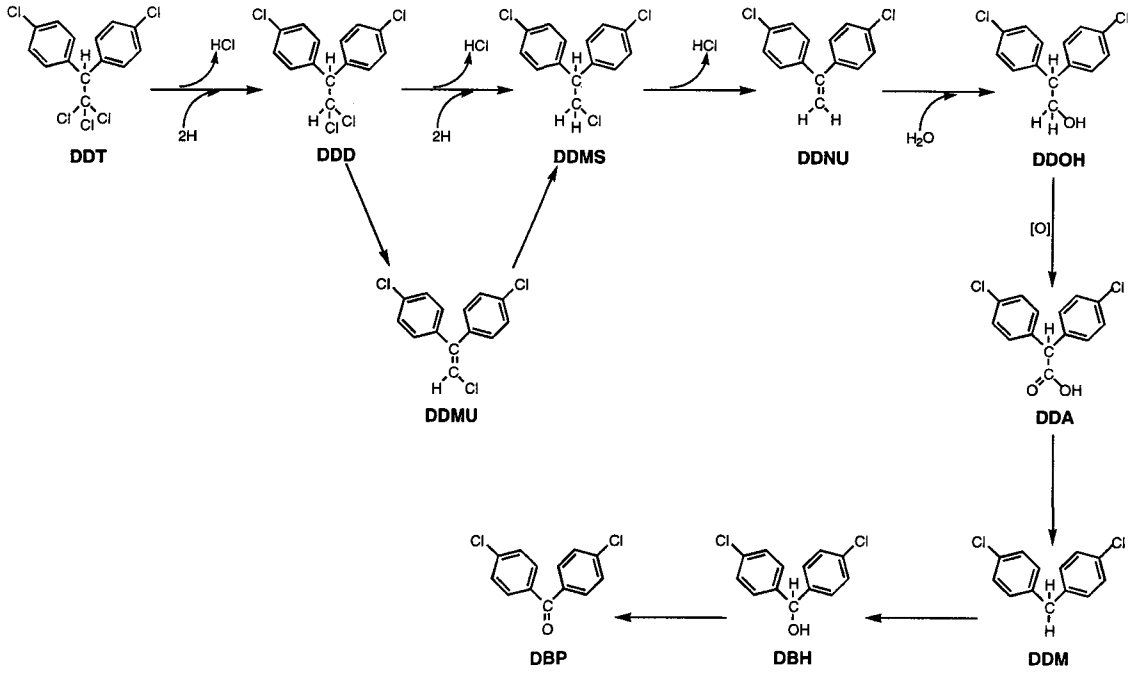

Fig. 2 Proposed pathway for bacterial metabolism of DDT via reductive dechlorination. DDT = 1,1,1-trichloro-2,2-bis(*p*-chloropenyl)ethane; DDD = 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane; DDMU = 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene; DDMS = 1-chloro-2,2-bis(*p*-chlorophenyl)ethane; DDNU = 2,2-bis(*p*-chlorophenyl)ethylene; DDOH = 2,2-bis(*p*-chlorophenyl)ethanol; DDA = bis(*p*-chlorophenyl)-acetic acid; DDM = bis(*p*-chlorophenyl)methane; DBH=4,4'-dichlorobenzhydrol; DBP = 4,4' dichlorobenzophenone; and PCPA = *p*-chlorophenylacetic acid. Adapted from Wedemeyer (1967), Langlois et al. (1970), and Pfander & Alexander (1972).

adenine dinucleotide (FAD) and anaerobic conditions (French & Hoopingarner 1970) whereas in *E. aerogenes* cytochrome oxidase (Wedemeyer 1966) was implicated.

Under anaerobic conditions DDD is able to undergo further metabolism. Pure culture studies with *E. coli* and *E. aerogenes* resulted in the isolation of DDD as a major metabolite and trace amounts of 1-chloro-2,2-bis(*p*-chlorophenyl)ethylene (DDMU), 1-chloro-2,2-bis(*p*-chlorophenyl)ethane (DDMS), 2,2-bis(*p*-chlorophenyl)ethylene (DDNU), 2,2-bis(*p*-chlorophenyl)ethanol (DDOH), bis(*p*-chlorophenyl)-acetic acid (DDA), and 4,4' dichlorobenzophenone (DBP) (Langlois et al. 1970). Using metabolic inhibitors together with changes in pH and temperature, Wedemeyer (1967) found that discrete enzymes were involved in the metabolism of DDT by *E. aerogenes*. The suggested pathway for the anaerobic transformation of DDT by bacteria is shown in Fig. 2. Degradation proceeds by successive reductive dechlorination reactions to yield DDNU, which is then oxidised to DDOH. Further oxidation of DDOH yields DDA which is decarboxylated to bis(*p*-chlorophenyl)methane (DDM). DDM is metabolised to DBP or, alternatively, may undergo cleavage of one of the aromatic rings to form *p*-chlorophenylacetic acid (PCPA). Under anaerobic conditions DBP was not further metabolised (Pfaender & Alexander 1972). Through an investigation of the co-metabolism of DDT metabolites by a number of fungi, Subba-Rao & Alexander (1985) were able to substantiate the pathway proposed by Wedemeyer (1967) for the degradation of DDT by the bacterium *E. aerogenes*. There has been one report describing the conversion of DDE to DDMU by a bacterium (Masse et al. 1989).

Alternating anaerobic and aerobic conditions promote reductive dechlorination and ring cleavage

reactions respectively. Their effect on the degradation of DDT was investigated by Pfaender & Alexander (1972). Under anaerobic conditions, cell-free extracts of "*Hydrogenomonas*" sp. metabolised ^{14}C-DDT to DDD, DDMS, DDNU, and DBP. The reaction mixture containing these metabolites was subsequently exposed to aerobic conditions along with a fresh culture of "*Hydrogenomonas*" sp. A metabolite identified as PCPA was formed as a result of ring cleavage. This acid was further metabolised by an *Arthrobacter* sp. to *p*-chlorophenylglycoaldehyde. Since no PCPA was formed under anaerobic conditions, these in vitro studies suggest that ring cleavage reactions required O_2. DDT was not co-metabolised by the "*Hydrogenomonas*" sp. under aerobic conditions.

A detailed study of the pathway for DDT degradation by *P. aeruginosa* 640×, which was isolated from DDT-polluted soils of the Crimean region, also yielded much information (Golovleva & Skryabin 1981). This bacterium was observed to degrade DDT either completely or to the nonchlorinated compounds phenylacetic, phenylpropionic, and salicylic acids. Only the first step in the process, that is the reductive dechlorination of DDT to DDD, took place without an additional substrate. All other degradative reactions, until the formation of benzhydrol, proceeded exclusively under co-metabolic conditions. The degree of DDT degradation by strain 640× depended on the nature of the co-substrate and on the aeration conditions. Anaerobic conditions were necessary for dechlorination of the aliphatic fragment and the aromatic rings of DDT. The addition of nitrate as an electron acceptor and of calcium lactate as a C source further activated the process. Using genetic engineering this bacterium was used to construct a strain *P. aeruginosa* BS 827 which has an enhanced capability of degrading DDT (Golovleva et al. 1982).

Mineralisation of DDTr by ligninolytic fungi

Ligninolytic fungi have been shown to possess biodegradative capabilities for a broad spectrum of environmentally persistent compounds, including DDT. This capability has been attributed to their ability to degrade lignin (Barr & Aust 1994).

The majority of work on DDTr degradation by ligninolytic fungi has been carried out by Aust and co-workers using a white rot fungus *P. chrysosporium* (Bumpus & Aust 1987; Fernando et al. 1989; Aust 1990; Shah et al. 1992; Bumpus et al. 1993). Bumpus & Aust (1987) described degradation of ^{14}C-labelled DDT over 30 days in cultures of *P. chrysosporium* deficient in nutrient nitrogen (N). Approximately 50% of the DDT was transformed during this period with around 10% being mineralised and the remainder appeared as metabolites including dicofol, FW-152, and DBP. These results led to the proposal of a DDT degradation pathway involving oxidation to dicofol followed by dechlorination to FW-152 and subsequent breakdown via DBP (Fig. 3). This pathway was thought to be controlled by the ligninase system of the fungus, as mineralisation and dicofol production were observed only after a lag phase during which ligninase production was established. DDD was the only product during this initial phase and was subsequently degraded. It was therefore concluded that DDD was produced by a mechanism distinct from the ligninase system, but nevertheless degraded by it. DDE was not produced. Other white rot fungi were found to mineralise ^{14}C-DDT, but were less effective than *P. chrysosporium*. The brown rot fungus *Gleophyllum trabeum* did not mineralise DDT to any significant extent. In further work (Fernando et al. 1989) it was discovered that the C source greatly influenced the extent of DDT mineralisation by *P. chrysosporium*. Starch and cellulose supported much greater mineralisation of ^{14}C-DDT than other complex carbohydrates or sugars. Mineralisation only occurred in the presence of a utilisable C source and ^{14}C-CO_2 release stopped when available carbohydrate was exhausted. It was also found that while 100% O_2 atmosphere had been used for many of these experiments, there was no difference in DDT mineralisation if air was used.

Using one of the isolates of Bumpus & Aust (1987), plus some additional strains, Katayama et al. (1992) also observed ^{14}C-DDT degradation. In their experiments, however, mineralisation was very low and DDE was a significant (1–9%) product. Dicofol production was also observed. High levels (up to 61% of added ^{14}C) of unidentified water-soluble products were formed when fungi were grown in cultures that were either limited or rich in N. This observation appears to argue against the involvement of the ligninase system. Examination of the limited description of the methods used, however, suggests that the "aqueously" soluble fraction may have contained appreciable amounts of acetone in which case the "aqueous" fraction would have been overstated.

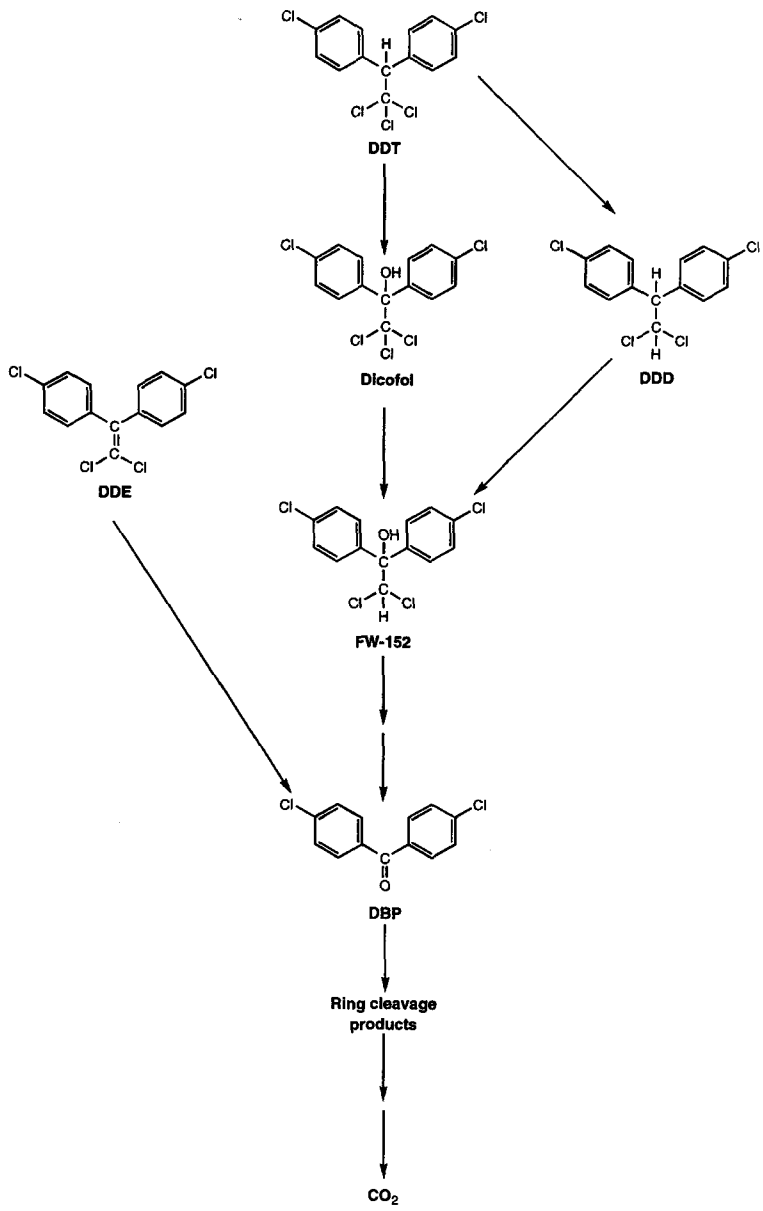

Fig. 3 Proposed pathway for DDTr degradation by *Phanerochaete chrysosporium*. Modified from Bumpus & Aust (1987).

DDT degradation by New Zealand ligninolytic fungi has also been observed. During a 35-day incubation with cultures of *Phanerochaete cordylines*, 80–90% of added DDT disappeared (Walter 1992). This was somewhat greater than degradation by *P. chrysosporium* in parallel experiments. Putative metabolites at low levels were detected including DDD, DDE, and 9 unidentified compounds. Preliminary results from our own work (unpubl. data) suggest that a number of strains of native white rot fungi are able to degrade ^{14}C-DDT to ^{14}C-CO_2 and labelled water-soluble compounds.

Bumpus et al. (1993) considered the degradation of DDE by *P. chrysosporium*. Mineralisation of ^{14}C-DDE was slower than that observed for DDT, reaching around 6% of added label after 60 days. DBP and an unidentified compound were found to

be intermediates. As mineralisation of DDE was far greater in cultures that were nitrogen (N)-limited than in those where N was not limiting, the involvement of the ligninase system was inferred.

The issue of the involvement of the ligninase system is important as it suggests organisms and conditions that may facilitate degradation. Kohler et al. (1988) argued against its involvement on the ground that DDT degradation (measured as DDT disappearance) occurred under conditions where nutrient N is not limiting. This finding, however, did not contradict previous work, since DDT was transformed to DDD under these conditions (Bumpus & Aust 1987). Aust (1990) further showed that timing of DDT mineralisation coincided with ligninase production. If DDT was added to ligninolytic culture, mineralisation began immediately but if cultures were initiated from spores, the onset of degradation and ligninolytic activity coincided after about 4 days. Inhibitors of ligninase such as EDTA and tetramethylethylenediamine also inhibit DDT mineralisation. Although some xenobiotic compounds are oxidised by purified lignin peroxidases, this does not happen with DDT, indicating that DDT is co-oxidised by a mediator molecule such as veratryl alcohol rather than by the enzyme directly (Barr & Aust 1994).

Bacterial attack on DDT under aerobic conditions

It is often difficult to isolate from the environment, microbes which attack a compound co-metabolically. A technique known as analogue enrichment is then adopted (Bartha 1990) in which a structural analogue is substituted for the compound of interest. The DDT-metaboliser "*Hydrogenomonas*", for example, was isolated from sewage when diphenylmethane, a structural analogue of DDT, was supplied for growth (Focht & Alexander 1970). This organism grew on diphenylmethane and was able to co-metabolise DDT. Using this technique, bacteria known to degrade 4-chlorobiphenyl, another structural analogue of the DDTr, were screened for their ability to metabolise DDTr (Masse et al. 1989; Nadeau et al. 1994; Parsons et al. 1995). Some of these bacteria have since been shown to metabolise DDTr by novel mechanisms. The bacterium strain B-206 produced a number of phenolic metabolites from DDT, DDE, and DDD (Masse et al. 1989). No DDTr ring cleavage products, however, were observed in these experiments. Nadeau et al. (1994) subsequently reported that *Alcaligenes eutrophus* A5 could metabolise both *o,p'*- and *p,p'*-DDT isomers when incubated at high cell density in resting cell cultures. The mechanism for attack presumably involves the same enzymes that are specific for 4-chlorobiphenyl degradation, that is DDT appears to be oxidised by a dioxygenase to yield a dihydrodiol-DDT derivative that undergoes *meta* cleavage, ultimately yielding 4-chlorobenzoic acid (Fig. 4). The dihydrodiol-DDT intermediate and 4-chlorobenzoic acid were isolated from resting cell incubations.

We have recently isolated a Gram-positive bacterium from a DDT-contaminated soil in the South Island, New Zealand, capable of attacking DDE (unpubl. results). The bacterium was enriched in minimal medium using biphenyl as a C source in the presence of DDT, DDD, and DDE. After 3 months incubation, a yellow product, indicative of *meta*-ring cleavage, accumulated in one of the cultures. Subsequently a bacterium able to attack DDE was isolated from the culture and purified. When the bacterium was inoculated onto nutrient agar and sprayed with 1% DDE, a yellow product formed within 48 h. The disappearance of DDE was confirmed by HPLC analysis. Research is ongoing to identify the bacterium and determine the mechanism by which it attacks DDE. In the long term, we propose to investigate the ability of this bacterium to degrade DDE in the field.

DEGRADATION OF DDTR IN SOILS

In soils the initial attack on DDT appears to be centred on the aliphatic trichloroethyl group of the molecule and proceeds in either one of two directions, depending on the prevailing environmental parameters. Under aerobic conditions, DDT undergoes dehydrochlorination to yield DDE. Under anoxic conditions, transformation of DDT to DDD by reductive dechlorination is considered to be the dominant reaction.

In New Zealand topsoils, DDTr are less than 0.1 mg/kg in most parts of the country. However, levels of 1–5 mg/kg DDE are not uncommon in agricultural soils in Canterbury where DDT use was high and soils are dry (Holland 1996). DDT residue decline in temperate soils is slow (Boul et al. 1994). In the absence of fresh applications, DDE tends to be the residue at dry sites (Childs & Boul 1995).

Early studies on DDT degradation in soil generally involved the addition of DDT to soil and

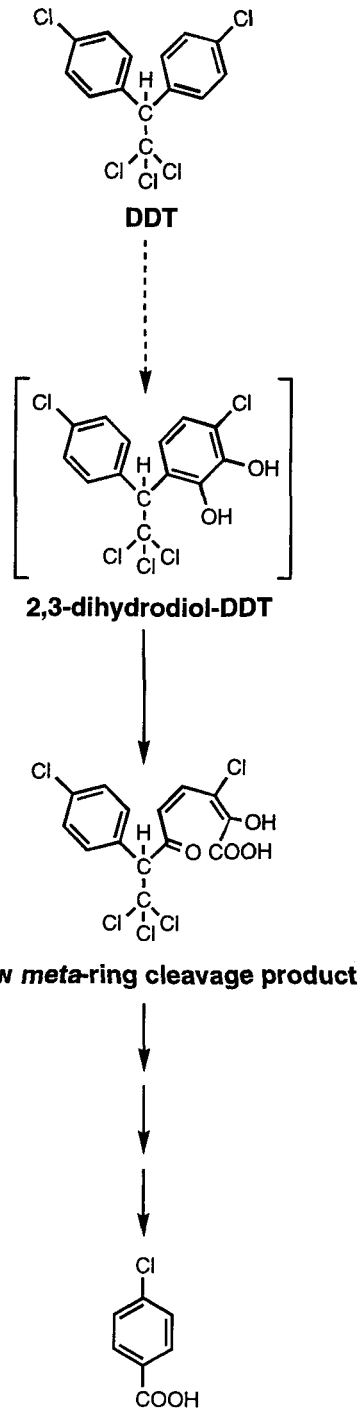

Fig. 4 Proposed pathway for degradation of DDT by *Alcaligenes eutrophus* A5. Modified from Nadeau et al. (1994).

the subsequent analysis of the parent molecule and its residues after various incubation periods (Burge 1971; Castro & Yoshida 1971, 1974). The data, however, gave no information on whether the molecule had been totally mineralised to CO_2 and Cl^-. More recent studies on the fate of DDTr, used ^{14}C-DDT to monitor the production of ^{14}C-CO_2 and obtain a mass balance (Scheunert et al. 1987; Nair et al. 1992; Boul 1996).

The microbial degradation of DDT in soil apparently proceeds by a pathway analogous to that proposed by Wedemeyer (1967) (Fig. 2). Under anoxic conditions the first and major biotransformation product of DDT is DDD with minor levels of DDA, DDM, DDOH, DBP, and DDE being detected (Guenzi & Beard 1967, 1968; Mitra & Raghu 1988; Xu et al. 1994; Boul 1996). Reports of microbial transformation of DDE in soil are rare although Agarwal et al. (1994) have recently reported isolation of DDMU as a biotransformation product of DDE. This indicates that DDE biotransformation in soil may proceed via a pathway similar to that proposed for the bacterial metabolism of DDT (Fig. 2).

Studies on the potential for mineralisation of ^{14}C-DDT in soil (Scheunert et al. 1987; Nair et al. 1992; Boul 1996) have often been conducted under both aerobic and reducing conditions to reflect the complexity of in situ conditions. To generate reducing conditions in the incubation flasks, the soil was either flooded with water (Nair et al. 1992; Boul 1996) or flushed with N (Scheunert et al. 1987). All these studies indicate that rates of DDT mineralisation in soils are very low. In general, less than 3.1% of the added label was released as ^{14}C-CO_2 after 42 days (Scheunert et al. 1987; Nair et al. 1992; Boul 1996) or longer incubations (Guenzi & Beard 1968; Zayed et al. 1994). Furthermore, flooding the soils reduced ^{14}C-CO_2 evolution (Nair et al. 1992; Boul 1996). Boul (1996) monitored the accumulation of ^{14}C-DDD and ^{14}C-DBP and the release of ^{14}C-CO_2 in Lismore silt loam from the AgResearch Winchmore Research Station that had been spiked with ^{14}C-DDT. Although flooding the soil resulted in considerable accumulation of DDD and low levels of DBP, no release of ^{14}C-CO_2 was detected. By contrast, minimal mineralisation (< 0.7% of added label) was detected under non-flooded conditions. Similar rates of mineralisation in soil have been reported for DDE (Zayed et al. 1994; Boul 1996). Laboratory studies using sterile controls (Guenzi & Beard 1968; Boul 1996) indicate that the biotransformation and

ultimately the mineralisation of DDTr to CO_2 in soil are attributed to microbially mediated processes.

Despite the evidence that microbes with the ability to degrade DDT are resident in soil, DDTr persist. Possible reasons for the slow rates of DDT mineralisation can be gleaned from pure culture studies described earlier. These studies have demonstrated that for DDT to be mineralised to CO_2 a complex set of environmental conditions is required. Thus, anoxic conditions are conducive to dechlorination of DDT, whereas ring cleavage resulting in the release of CO_2 requires the presence of oxygen (Pfaender & Alexander 1973; Golovleva & Skryabin 1981). Additional C for microbial growth is also required for some of these steps to occur.

ENHANCEMENT OF BIODEGRADATION OF DDTr IN SOIL

To enhance biodegradation of DDTr in soils it is necessary to consider the factors that affect degradation of pesticides. For a review of these factors see Torstensson (1987), Fewson (1988), and Bollag & Liu (1990). Of particular relevance to the degradation of DDTr in situ are the presence and numbers of microbes with the ability to metabolise DDTr, and environmental factors which limit both growth and activity of the DDTr-metabolising microbes, and access of the microbes to DDTr.

Useful microbes for DDTr degradation

Degradation of DDTr in soils is dependent on the presence and numbers of microbes in the contaminated soil with the required degradative ability. These microbes may be resident in the soil or they may be isolated from elsewhere and introduced to the soil. Microbes potentially useful for the biodegradation of DDT in soil include the bacteria and fungi that metabolise DDTr via reductive dechlorination, ligninolytic fungi, and the chlorobiphenyl-degrading bacteria which carry out ring cleavage of DDTr under aerobic conditions. Alternatively, microbes with the ability to degrade DDTr can be constructed using molecular techniques and introduced into the contaminated soil (Golovleva et al. 1982).

Although there is evidence for the existence of DDTr-metabolising microbes in soils, and some have been isolated, their prevalence is unknown. From laboratory experiments it is clear that the transformation of DDT to DDD occurs readily in spiked soils under certain conditions. The process may be attributed directly to microbial activity, either bacterial or fungal (Wedemeyer 1966; Subba-Rao & Alexander 1985), or indirectly to the generation of anaerobic conditions and/or the production and release of biomolecules that act as reductants, such as the iron porphyrins (Castro 1964; Zoro et al. 1974). Since DDD accumulates in soil (Guenzi & Beard 1967, 1968; Mitra & Raghu 1988; Xu et al. 1994; Boul 1996) subsequent biotransformation reactions, which lead to mineralisation, occur very slowly. This could mean that microbes or microbial populations able to attack DDT directly and convert it to DBP via DDD are present in very low numbers. Alternatively, environmental conditions may not allow biodegradation of DDD to proceed. It may therefore be necessary to introduce microbes to contaminated soils that are capable of mineralising DDTr. Kearney et al. (1969), for example, were able to demonstrate the disappearance of DDT from soil after heavy inoculation with a DDT-degrading strain of *E. aerogenes*. Although successful introduction of microbes to soil is a contentious issue there are increasing reports of success. For many years commercial strains of *Rhizobium* which fix atmospheric N have been added to soil (Macgregor 1994). Recently a strain of *Serratia entomophila* has been developed in New Zealand for release into soil as a biological control agent for grass grub (Jackson et al. 1992).

The advantages of using ligninolytic fungi for decontamination of soil have been described by Barr & Aust (1994). These fungi have a broad spectrum of activity and are able to mineralise both DDT and DDE. The degradative enzymes are induced by environmental conditions rather than in response to the presence of the contaminant, and being extracellular they degrade very insoluble chemicals. Although ligninolytic fungi can clearly degrade DDT their ability to do so in the environment is equivocal. Fernando et al. (1989) examined the degradation of ^{14}C-DDT in an unsterilised soil/corn cob mixture to which *P. chrysosporium* was added. Over 60 days, 10% of the ^{14}C was evolved as $^{14}CO_2$, 5% was soluble in water, and 18% was unextractable. The extractable label contained DDT (61%), DDE (6.9%), dicofol (4.4%), and DDD/DBP (2.6%), plus unidentified residues more polar than dicofol (22.9%). It may therefore be possible to engineer DDT degradation by the fungus in the field, but this is unlikely to

occur naturally in the absence of a suitable primary growth substrate and inoculum.

A combination of photolytic and biodegradative processes has been proposed for the detoxification of aromatic compounds (Miller et al. 1988). One obstacle to developing this technology is the lack of suitable microbes. To overcome this Katayama & Matsumura (1991) developed a strain of *P. chrysosporium* that was resistant to UV irradiation. Significantly enhanced rates of DDT mineralisation by cultures of these microbes were achieved under UV irradiation.

Some chlorobiphenyl-degrading bacteria are reported to cleave both *p,p'*-DDT and *o,p'*-DDT, as well as DDE. The advantage of using these microbes for remediating DDT-contaminated soils is that the initial attack on the molecule results in aromatic ring cleavage (Nadeau et al. 1994) considered to be the limiting step for degradation of aromatic compounds. Once the DDTr have been cleaved, further degradation to CO_2 should proceed readily. Chlorobiphenyl-degrading bacteria attack DDTr under aerobic conditions (Masse et al. 1989; Nadeau et al. 1994) which may prevail in DDTr-contaminated soils. These bacteria also need an alternative C source for growth which induces the production of dioxygenase enzymes necessary for ring cleavage. In laboratory experiments these enzymes are induced by biphenyl or 4-chlorobiphenyl. Although the addition of biphenyl to contaminated soils has been shown to enhance degradation of chlorinated biphenyls (Higson 1992), for use in the field other less toxic substrates will be required. Pfaender & Alexander (1973) were able to enhance the numbers of DDT metabolisers in sewage by using the DDT analogue diphenylmethane.

Some biodegradative strains when inoculated into environmental samples are unable to metabolise the pollutant. Among the reasons proposed for this observation is that the low concentration of the substrate limits enzyme induction. For some chemicals there is a threshold concentration below which the biodegradation rate is negligible. This may not be the situation for DDT, however. Katayama et al. (1993) reported the isolation of two strains of bacteria, *Bacillus* sp. B75 and an unidentified Gram-variable rod B116, which degraded DDT at the extremely low level of 10 pg/ml. This concentration is below the aqueous solubility of DDT. Furthermore, they were unable to detect a lower concentration threshold for the induction of DDT degradation. Katayama et al. (1993) propose that degradation of DDT involved two processes: the uptake of DDT into the cell and the transformation of DDT in the cell. The rate of uptake into the cells is unlikely to be important as DDT is extremely hydrophobic; rather, the rate at which the chemical is transformed in the cell would be the rate-limiting step. To our knowledge there are no reports on the rate at which DDTr is transformed in the cell. Microbial accumulation of DDT has been reviewed by Lal & Saxena (1982). Accumulation appears to be a passive process since autoclaved cells sorbed DDT as well as, if not better than, live cells. The binding to microbial cells of hydrophobic compounds such as DDT has been proposed as a mechanism for facilitating transport of DDT from saturated soils to groundwater (Lindqvist & Enfield 1992).

Environmental factors limiting biodegradation of DDTr in soil

As a result of laboratory studies, several strategies for enhancing the biodegradation of DDT in soil have been proposed. These include the addition of extra C to enhance co-metabolic metabolism of DDT, the flooding of soils to create anaerobic conditions, and surfactant treatment to release DDTr from soil.

A number of studies report a rapid rate of reduction of DDT to DDD in soil under reducing conditions when a readily available energy source such as alfalfa, barley straw, or glucose is present (Guenzi & Beard 1968; Burge 1971; Castro & Yoshida 1974). In India, the practice of green manuring in which leaves or whole small leguminous plants are ploughed into flooded soils before rice is planted leads to a decreased persistence of DDT (Mitra & Raghu 1988). Although enhanced degradation of DDT following addition of organic matter to soils indicates the significance of co-metabolic transformations, this effect could also arise because of microbial production and release of porphyrins and/or the generation of anaerobic conditions resulting from enhanced microbial growth (Castro 1964; Zoro et al. 1974).

Some laboratory experiments have shown that flooding enhances the loss of DDT from soil (Guenzi & Beard 1967; Castro & Yoshida 1971; Farmer et al. 1974). Similarly, long-term field trials involving known DDT applications indicate that regular irrigation can achieve a reduction in soil residue levels (Boul et al. 1994). A number of mechanisms might be operative including the

creation of anaerobic micro-environments for microbes able to degrade DDT via DDD, or abiotic reductive dechlorination, and the binding of DDT residues to soil particles. However, subsequent laboratory studies into the effect of various soil moisture regimes on the fate of DDT and DDE indicate that soil flooding can slow down mineralisation (Boul 1996). Xu et al. (1994) have reported similar results. Flooding can also lead to a significant increase in the amount of soil-bound DDT (Nair et al. 1992; Xu et al. 1994; Boul 1996) but not DDE (Boul 1996).

To date, most DDT experiments in soil have examined freshly incorporated DDT. Over time, however, DDT residues in soil may bind to either soil organic matter or to clay surfaces (Boul 1995). In soils, DDT has been demonstrated to have a high affinity for soil organic matter, and very little is known about the binding of DDT to clay (Boul 1995). The binding of DDT to soil is a matter of some ecological and toxicological importance. Once bound to soil; it appears that DDT residues are detoxified and lose their activity (Peterson et al. 1971). However, it is not known if detoxification is permanent or temporary. Certainly DDTr in soil are still accumulated into animals many years after application (Boul 1995). Although soil microbes can play an important role in the binding of pesticide residues to soil organic matter (Bollag & Lui 1990), their contribution to the formation and degradation of soil-bound DDT residues is largely unknown. Boul (1996) observed decreased binding of DDTr in flooded soils after autoclaving and proposed that microbial activity may enhance binding to soil.

The most important mechanisms for binding of DDT to soil are considered to be hydrophobic interactions with, and subsequent trapping within internal pores of, humic substances (Senesi & Miano 1995). Hydrophobic active sites of humic substances include aliphatic side chains or lipid portions, and aromatic lignin-derived moieties with high C content and a number of small polar groups. The association of DDT with the soluble humic fractions can result in an increase in the solubility, and hence mobility, of DDT (Ballard 1971), but the resultant effects on biodegradation rates are virtually unknown. Fujimura et al. (1994) determined the influence of dissolved humic substances on DDT biodegradation using pure cultures of *Bacillus* and an unidentified Gram variable rod B116. When grown in diluted nutrient broth in the presence of either humic or fulvic acids *Bacillus* sp. B75 was unaffected. Numbers of viable cells of strain B116 decreased, however, indicating that the presence of humic or fulvic acids had a possible bactericidal effect. Biotransformation of DDT by B116 was inhibited by both humic and fulvic acid. *Bacillus* sp. B75 was inhibited by high concentrations (500 mg/l) of humic acids. In contrast to fresh humic acid, the aged material did not inhibit the growth of B116 at 25 mg/l. It was proposed that the inhibitory activity of the humic and fulvic acids was because of the presence of free radicals in solution.

Once bound to soil organic matter, pesticide residues are only slowly released. Nevertheless, DDT residues could be released by addition of fresh soil (Hussain et al. 1994; Varca & Magallona 1994; Xu et al. 1994) which is indicative of a microbially mediated mechanism. Likewise, chemical pre-treatment of contaminated soils may enhance the release of bound DDTr. Keller & Rickabaugh (1992) and Parfitt et al. (1995) have investigated the treatment of soil with surfactants to release DDTr. Not only do surfactants significantly increase the aqueous solubility but they can effectively displace sorbed DDTr from organic matter surfaces and clay particles. Parfitt et al. (1995) were able to remove 25–45% of the DDTr by leaching DDT-contaminated Lismore silt loam from Canterbury with Triton-X and polypropylene glycolethyoxylate. As surfactant treatment enhances the water solubility of DDT it is feasible that it will also enhance DDTr biodegradation. You et al. (1995), for example, found that the nonionic surfactant Triton X-114 was effective in enhancing the biotransformation of DDT under reducing conditions in soil slurry reactors. DDT was transformed to DDD via DBP and after 30 days only 6–12% of the DDT remained. Very little effect was seen with DDE in these experiments. The residual DDT was assumed to be tightly bound to the soil. No removal of DDT was detected in sterile controls. However, the level of DDT contamination in the soil used is 1940 mg/kg which is many orders of magnitude above that in agricultural soils. It is uncertain whether surfactant treatment would have a similar effect on the biodegradation rates of DDT at levels of less than 10 mg/kg as are found in New Zealand soils.

CONCLUSIONS

Investigations into the microbial degradation of DDTr are useful for the development of methods for the remediation of contaminated soils.

Laboratory studies have shown that microbes have the ability to metabolise DDTr and different pathways for attack have been described. In soils DDT is converted to DDD and DDE. It is known that conversion of DDT to DDD is enhanced under anaerobic conditions, and that DDD can be converted to DDMU and DBP. However, little is known about the conditions which favour degradation of DDD. The same applies to the breakdown of DDE. Mineralisation of DDT, DDD, and DDE in soil occurs at a very slow rate. Residues are found in soils 20 years after application ceased. In situ biodegradation may be limited because a complex set of environmental conditions are required for the process including a mix of aerobic and anaerobic conditions and the presence of alternative growth substrates. To enhance degradation we may need to introduce microbes with DDTr metabolising abilities. In this respect, ligninolytic fungi or chlorobiphenyl degrading bacteria are promising candidates. In addition, contaminated soil may need to be treated in such a way that contact between the microbe and the substrate is promoted. To this end surfactant treatment may well prove useful. Although bioremediation of DDT-contaminated soils is difficult, all avenues for research have not been closed.

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