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DDT remediation in contaminated soils: a review of recent studies

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Abstract Over the past few decades significant progress has been made in research on DDT degradation in the environment. This review is an update of some of the recent studies on the degradation and biodegradation pathways of DDT and its metabolites, particularly in soils. The latest reports on human toxicity shows that DDT intake is still occurring even in countries that banned its use decades ago. Ageing, sequestration and formation of toxic metabolites during the degradation processes pose environmental challenges and result in difficulties in bioremediation of DDT contaminated soils. Degradation enhancement strategies such as the addition of chelators, low molecular organic acids, co-solvent washing and the use of sodium and seaweeds as ameliorant have been studied to accelerate degradation. This review describes and discusses the recent challenges and degradation enhancement strategies for DDT degradation by potentially cost effective procedures based on bioremediation.

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Introduction

DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane], has been widely used as insecticide to control mosquito-borne malaria and typhus. DDT was the first synthetic insecticide and marketed all over the world. The use of DDT has recently been prohibited in most countries due its deleterious impact of wildlife and human health via food chain (Purnomo et al. 2011) DDT is ubiquitous in the environment of both developed and developing countries. Its persistence in the environment together with its toxicity has led to concerns amongst health and environmental regulators as well as the general public. DDT is amongst the 21 persistent organic pollutants (POPs) that require immediate phasing out, according to the 2010 Stockholm convention. The presence of chlorine atoms in DDT and its metabolites, in conjunction with their low solubility and tendency to partition preferentially into the lipophilic phase makes them highly toxic to higher organisms.

Past two decades, several physicochemical and biological remediation methods have been studied. A number of bioremediation technologies have been developed to remove DDT from soils. This review discusses the recent research on degradation of DDT and its metabolites, challenges due to sequestration

and the recent strategies to enhance bioavailability, and related degradation issues.

DDT degradation in soil

Technical grade DDT, the most common formulation used as insecticide, contains 85 % *p*,*p*′-DDT, 15 % *o*,*p*′-DDT and traces of *o*,*o*′-DDT and other compounds, including three isomeric tris(chlorophenyl)methanes that can be detected in the environment, together with their oxidation product, tris(chlorophenyl)methanol. Most reports indicate that DDT is reductively dechlorinated to DDD under reducing conditions (Gao et al. 2011). Reductive dechlorination (RD) is also the major mechanism for the microbial conversion of both the *o*,*p*′-DDT and *p*,*p*′-DDT isomers to DDD. Biodegradation of DDT and similar chlorinated compounds largely involves co-metabolism pathways carried out by a great number of facultative and obligate anaerobic microorganisms under suitable environmental conditions. However, studies conducted by DeWeerd et al. (1990) identified *Desulfomonile tiedjei*, a bacterium that is able to couple the reductive dechlorination of 3 chloro benzoate for energy conservation. This opened up a novel type of energy metabolism in anaerobic microorganisms leading to the degradation of more chlorinated compounds.

A critical step in the degradation of DDT is the cleavage of carbon–chlorine bond. Microbial degradation requires the presence of enzymes that cleave this bond under physiological conditions (Häggblom and Bossert 2003). Reductive displacement is the predominant reaction for the displacement of chlorine in many aromatic organochlorine compounds under anaerobic conditions.

DDT undergoes dechlorination under reducing conditions and forms DDD which is able to degrade further to more polar products such as DDOH when subjected to aerobic treatment (Mwangi et al. 2010). Therefore, a sequential reductive step followed by an oxidative process mineralizes DDT. When subjected to aerobic biotic degradation, abiotic dehydrochlorination and photochemical degradation, the decomposition of DDT results in the formation of DDE. DDE is also a recalcitrant resulting in slower metabolization. Environmental transformation is the major process in the degradation pathway and can be subdivided into abiotic and biotic transformation.

Biotic transformation

Transformations of an organic contaminant by microorganisms can occur when they are able to utilize as carbon sources, or during co-metabolism. Biotic transformation can also occur through secondary processes such as microbially induced changes of pH and redox conditions (Fang et al. 2010; Thomas and Gohil 2011). Microbial transformations can involve one, or a combination, of these mechanisms. The transformation could be carried out by a single microbial species or a combination of several microbial species.

Anaerobic degradation by reductive dechlorination

RD is an important route for the biodegradation of organochlorine pesticides such as DDT. RD of many compounds is known to occur only among mutualistic anaerobic microbial communities and some (Mohn and Tiedje 1992). It results in the replacement of chlorine with hydrogen, with a net input of one proton and two electrons. The two electron transfer reactions occur in two sequential steps. The first electron transfer results in the dissociation of a chloride anion and the formation of a *p*,*p*-dichloro-diphenyl-dichloroethyl radical. The second electron transfer leads to protonation of the radical to form DDD (Bylaska et al. 2004). This process is termed dehalogenation and results in the removal of chlorine and the addition of hydrogen ions to the compound, increasing the susceptibility to oxidation and the formation of less toxic products. Literature suggests that DDT degradation usually follows this pathway. Corona-Cruz et al. (1999) studied anaerobic coupled with aerobic biodegradation of DDT. Ten kg of soil taken from an agricultural land treated with mixed anaerobic culture and aerobic culture using a fermentation reactor. The mixed anaerobic culture was from a soft drink company wastewater plant. For the aerobic treatment two types of cultures were used, a mixed culture containing five species of *Pseudomonas*, one of *Klebsiella*, four of *Rhodococci* and two strains of fungi and *Phanerochaete chrysoporium* was added. The effect treatments on degradation were compared to that containing only water or nutrient. An aerobic pure culture system using *P. chrysoporium* was also studied. This study observed a maximum DDT degradation of 84.4 % with coupled anaerobic

(with a mixed culture)–aerobic (with *P chrysoporium*) fermentation. The rate of degradation was mainly dependent on the rapid onset of reducing conditions in soil which in turn is dependent on the organic matter content (OM) (Gevao et al. 2002).

Microbial dechlorination can be divided into two types—co-metabolic and metabolic (Smidt and de Vos 2004). The co-metabolic conversion is catalysed mostly by metal ions containing heat stable tetrapyrroles, or enzymes such as coenzyme F430 (nickel) and vitamin B12. Vitamin B12 is biosynthesized by anaerobic bacteria presented as cofactors (Smidt and de Vos 2004). On the other hand, metabolic conversion is carried out by halo-respiring bacteria that utilize the energy generated during the dechlorination process for the synthesis of ATP.

A pure system study of Ahuja et al. (2001) with a mineral medium with 1 g/L of glucose showed accelerated dechlorination of DDT to DDD by up to 54 % by *Alcaligenes denitrificans* compared to a medium devoid of the bacteria. About 35 % of the DDT was transformed into DDD during the 2-week incubation period. However, no further transformations were observed after 2 weeks. A DDT metabolic fate study in human intestinal gut by Yim et al. (2008) demonstrated that *Eubacterium limosum* (ATCC 8486)—a strict anaerobe isolated from the human intestine can also transform DDT into DDD. The isolated bacteria was grown in a brain heart infusion medium containing DDT in anaerobic serum bottles, which was transformed completely into DDD in 16 days. However, the metabolites were not degraded any further. It was assumed was that *E. limosum* used organic electron donors to reductively dechlorinate DDT to DDD.

The formation of DDE during the breakdown of DDT was considered to be an end point without further degradation. Megharaj et al. (1997) investigated the bacteria capable of degrading compounds with a structure similar to DDE, such as the chlorobiphenyl and dichloroethylene groups, in order to determine whether bacteria possessed enzyme systems with sufficiently relaxed specificities to carry out parallel degradation of DDE. Four cultures (*Methylosinus trichosporium, Mycobacterium vaccae, Acetobacterium woodii* and *Clostridium butyricum*) were evaluated for their ability to co-metabolize DDE while growing on primary carbon substrates. However, DDE was not transformed during the 30-day incubation period, suggesting that these bacteria were unable to degrade these compounds even when they were provided with co-substrates that induce chlorobiphenyl and dichloroethylene group transforming enzymes.

Quensen et al. (2001) demonstrated the transformation of DDE to DDMU in Palos Verdes marine sediment microcosms. Sediment slurries spiked with 200 μg of ^{14}C labelled DDD or DDE were mixed with anaerobic marine media and kept statically at 22–25 °C. At the end of 32 weeks of incubation, DDE was dechlorinated to DDMU in both methanogenic and sulfidogenic microcosms. They also reported that DDD dehydrochlorination into DDMU was three orders of magnitude slower than DDE transformation. Eganhouse and Pontolillo (2007) predicted the long term fate of *p*,*p*′-DDE in box cores collected between 1992 and 2003 from Palos Verdes, using a multistep reaction model. Between 1992 and 2003, *p*,*p*′-DDE decreased by 43 % whereas that of *p*,*p*′ DDMU increased by 34 %. According to the multistep reaction model, the inventories of *p*,*p*′-DDE and *p*,*p*′ DDMU will continue to decline, whereas that of *p*,*p*′-DDNU will reach a maximum around the year 2014.

Quensen et al. (2001) later studied factors such as carbon (electron donors) and sulfate addition, methanogenesis and temperature in controlling the rate of DDE dechlorination. The study demonstrated that the addition of carbon in the form of glycerol stimulated DDE dechlorination indirectly by early onset of sulfate-limiting conditions by sulfidogenic bacteria, and occurrence of methane in the microcosm. Microbes under such conditions began metabolizing the remaining carbon substrates using DDE as the terminal electron acceptor to execute dechlorination. The study also noted higher temperatures shortened the lag time of DDMU formation as 16, 24 and 32 weeks of incubation at 22, 15 and 10 °C, respectively.

Except for Quensen et al. (2001), whose study focused on a marine environmental sample, all the other studies were carried out in pure systems. Therefore the effect of these microorganisms in real contaminated sites needs to be evaluated. Furthermore the feasibility of obtaining similar results in complex heterogeneous environmental matrices such as soil should also be considered.

Aerobic degradation pathway

Degradation studies of DDT by oxidation reactions are often difficult because of the lack of a defined

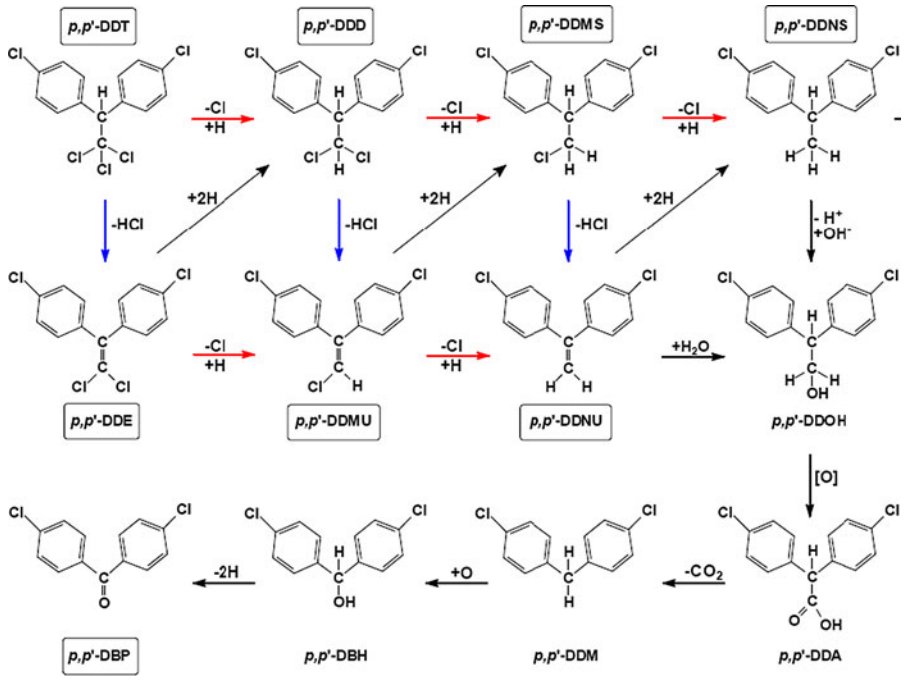

Fig. 1 Chart showing potential pathways for microbial degradation of DDT. *Blue arrows* represent dehydrochlorination reactions; *red arrows* represent reductive dechlorination reactions. (Eganhouse and Pontolillo 2007). (Color figure online)

mixed-function oxidase system in microorganisms. Therefore, analogue enrichment has been adopted in which a structural analogue is substituted for DDT. Diphenylmethane was used as a structural analogue of DDT in several studies (Megharaj et al. 1997). However, recent studies have identified microorganisms that can metabolise DDT aerobically without the need for a structural analogue. Considering the bioremediation options, recently several bacteria and fungi have been shown to enhance degradation processes in soil, using both pure and mixed cultures (Purnomo et al. 2011; Thomas and Gohil 2011; Fang et al. 2010; Xie et al.2011).

The first aerobic bacterial degradation of DDT was reported by Nadeau et al. (1994) using *Alcaligenes eutrophus* A5 in a minimal salts medium supplemented with 0.005 % yeast extract. The study showed *A. eutrophus* A5 biotransformed both *o,p′*-and *p,p′*-DDT with the formation of a yellow product after 30 days of incubation, which further degraded to 4-chlorobenzoic acid (4-CBA). The proposed catabolic pathway involved the initial oxidation of DDT at the *ortho* and *meta* position in the presence of dioxygenase. This resulted in the formation of 2, 3-dihydrodiol-DDT which was further degraded to form 4-CBA. A similar study conducted by Kamanavalli and Ninnekar (2004) reported that *Pseudomonas* sp. grown on biphenyl supplemented with 0.05 % wt/vol DDT accumulated metabolites after 5 days of incubation. Degradation was carried out via a meta-cleavage pathway to form 2, 3-dihydroxy DDT (Figs. 1, 2). This product further degraded to 4-CBA. However, there was no further microbial degradation of 4-CBA.

The potential of two 4,4′-dichlorobiphenyl (DCB)-degrading bacteria, *Rhodococcus globerulus* and *Pseudomonas fluorescens* to co-metabolize DDE was studied by Megharaj et al. (1997) during aerobic biphenyl degradation. However, after incubation for 30 days the bacterial cells pre-cultured on biphenyl did not result in DDE degradation, although biphenyl was completely degraded. In contrast, studies by Hay and Focht (1998) demonstrated that biphenyl-grown *Pseudomonas acidovorans* M3GY co-metabolically transforms DDE in a manner analogous to the initial transformation of biphenyl. After 50 days of incubation, 40 % of DDE transformed with the formation of nine metabolites suggesting a possible model for aerobic degradation. Later, Aislabie et al. (1997) isolated an aerobic gram positive bacterium, *Terrabacter* sp. strain DDE-1 from an agricultural soil. Metabolism of DDE to 4-chlorobenzoate was observed within 28 days of incubation when the bacterium was grown in a culture flask with minimal media and biphenyl. The pathway of degradation was oxidative

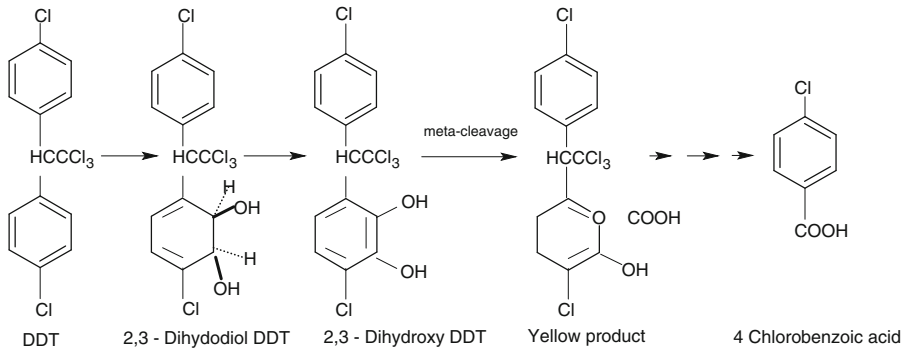

Fig. 2 The proposed pathway for the aerobic biodegradation of DDT (Nadeau et al. 1994; Kamanavalli and Ninnekar 2004)

displacement involving dioxygenase enzyme resulting in meta-ring cleavage. Ahuja et al. (2001) reported that *A. denitrificans* isolated from DDT contaminated soils was able to metabolize DDE by up to 24–26 % from its initial concentration, after 2 weeks of incubation. However they did not form metabolites such as DDMU and 4-CBA as reported in other studies. The study also reported that addition of glucose in the mineral medium accelerated the breakdown process.

Persistent of DDD in aerobic environments, can be a concern in contaminated sites. Hay and Focht (2000) showed that aerobic degradation of DDD by *Ralstonia eutropha* strain A5 occurred in mineral salts medium with biphenyl as a carbon and energy source. Metabolites were recovered for up to 25 days. In earlier reports, aerobic degradation of DDD was performed by either hydroxylation or monooxygenation. However, the pathway proposed by Hay and Focht (2000) was via dioxygenation and by *meta* fission, yielding *meta* and *ortho* substituted monohydroxy-DDD intermediates. Hay and Focht (2000) also reported that *R. eutropha* strain A5 could also aerobically transform DDT and DDE.

A DDT degrading consortium isolated from DDT contaminated fields was studied for their aerobic degradation abilities by Bidlan and Manonmani (2002). The consortium of four bacteria was acclimatised and maintained on minimal agar containing 5 mg/L DDT and 1/50 nutrient broth. This consortium was able to degrade 25 mg/L of DDT in 144 h. The study demonstrated that the bacterium *Serratia marcescens* degraded up to 25 mg/L of DDT in basal mineral medium. A pH of 7.5 and a temperature of 30 °C produced the maximum chloride release. The presence of additional carbon sources such as glycerol, peptone, yeast extract and tryptone soya broth also favoured the complete degradation of DDT. Recently a Gram-negative, strictly aerobic, *Pseudoxanthomonas jiangsuensis* sp. isolated from a long term DDT contaminated soil has shown to degrade DDT by using more polar lipids such as polar lipids diphosphatidylglycerol, phosphatidylethanolamine, and phosphatidylglycerol (Wang et al. 2011). Another, *Pseudomonas* bacterium designated as wax, which was capable of co-metabolizing DDT with other carbon sources, was isolated from a long-term DDT contaminated soil. In the presence of 100 mg/L glucose, the wax strain could degrade over 95 % of the total DDT, at a concentration of 20 mg/L, in 72 h, and could degrade over 60 % of the total DDT, at a concentration of 100 mg/L, in 144 h. Similarly Niu et al. (2012) reported that *Pseudomonas* strain 12-3 isolated from DDT contaminated shipyard in Guangzho, China could degrade up to 51 % DDT in 8 days under liquid culture conditions.

Unlike the process using bacteria, DDT degradation by fungi has received little attention. The bioremediation activity depends on the ability of fungal species colonized in the substrate to produce oxidative lignolytic enzymes and degradation rate has been reported to correlate with the lignolytic activity (Chung et al. 2009). It is also possible that fungal intracellular enzymes may be involved in DDT degradation. It has been reported that cytochrome P450 monooxygenage is involved in the degradation of DDT. DDT is metabolised under aerobic conditions by the P450 enzymes to DD, dicofol, FW-152, 2,2-bis(4-chlorophenyl acetic acid (DDA) and DBH (Purnomo et al. 2011; Szewczyk and Dlugonski 2009) Recently Purnomo et al. (2011) reported that DDT, DDD and DDE may also be directly transformed to DBP vial the fenton reaction by brown rot fungi (BRF). Unlike

white rot fungi, BRF does not have lignolytic enzymes and it generates hydroxyl radicals via extracellular fenton reaction (Purnomo et al. 2010a, b). Recent works on the addition of biological wastes such as different manure composts showed some degradation potential on DDT and its metabolites. Several fungi isolated from the mesophilic and maturation stages of cattle compost manure compost shown to degrade DDT (Szewczyk and Dlugonski 2009; Purnomo et al. 2011).

Most of the studies were carried out in pure systems and indicated the presence of metabolites that did not undergo further microbial degradation. Furthermore, pure system studies did not factor in ageing, bioaccessibility and bioavailability which can influence the degradation rates in environmental samples. Accessibility of the compound to the microorganism and favourable conditions in the local environment are essential for biodegradation (Alexander and Guerin 1999). Hence, degradation percentages reported from pure system studies can be considered as upper values when they are tested with complex and heterogeneous samples.

Abiotic transformations

The major abiotic transformation processes of DDT include hydrolysis, oxidation–reduction and photolysis.

Hydrolysis

Hydrolysis can represent a major transformation pathway in soil and water (Kookana et al. 1998). Hydrolytic degradation of pesticides in soil occurs in the soil pore water or on the surface of clay minerals. Hydrolysis can be biologically or chemically mediated and can occur in the presence of H_2O, H_3O^+ and OH^- and is termed neutral, acid and base hydrolysis respectively. Therefore, the reaction is strongly pH dependent. Temperature is an important factor governing the rate of hydrolysis in soil pore water. The rate of hydrolysis increases by a factor of 2 for every 10 °C rise in temperature (Gevao et al. 2002).

Oxidation–reduction reactions

Oxidation–reduction reaction involves the transfer of electrons from the reduced species to the oxidized species (Gavrilescu 2005). Organic compounds contain reduced forms of carbon favouring an oxidation reaction for degradation. However, organochlorines such as DDT are relatively oxidised because of the presence of chlorine moieties, which are highly electronegative and thus susceptible to reduction. Therefore, with increased chlorine or other halogen moieties, reduction is a more likely process than oxidation in natural environments.

Generating a reduced environment in soils, sediments and aquifers using abiotic methods has become a popular remediation strategy. An example of this technology is the use of zero valent iron (ZVI) as a reductant. ZVI serves as an electron donor under anaerobic condition stimulating biological dechlorination (Aulenta et al. 2006). ZVI has been successfully used to transform several halogenated organic compounds (Satapanajaru et al. 2003, 2006).

Photolysis

Sunlight induced photochemical transformation is one of the major abiotic pathways for degradation of organic pollutants on the surface layer of soil and water. Photochemical reactions may modify the physical and chemical properties of organic pollutants and significantly affect their environmental fate and behaviour in bulk soil (Senesi et al. 1998). Studies carried out by Zayed et al. (1994) showed that transformation of DDT to DDE occurred on exposure to sunlight. The study showed only 65 % of applied DDT remained in sunlight-exposed soil in comparison with 91 % of DDT in control which was not exposed to sunlight.

Factors affecting the bioremediation of DDT

Bioremediation aims for complete mineralization of contaminants to water and carbon dioxide without the build-up of intermediates. The fate and behaviour of organic pollutants in soil is governed by many factors including soil characteristics, compound properties and environmental factors such as temperature and precipitation (Reid et al. 2000; Shukla et al. 2010; Gao et al. 2011). The persistence of organic pollutants in soil is related to compounds' hydrophobicity (Cerniglia 1992).

Soil environmental factors

A number of factors control the rate of pesticide biodegradation in soil (Tables 1, 2). Contaminant adsorption is influenced by its physical and chemical properties (Fisher 1999; Zhao and Yi 2010). Soil clay and organic matter fractions play a major role in adsorption of pesticides to soil. Numerous researchers have demonstrated that natural organic matter enhances the apparent solubility or mobility of highly hydrophobic contaminants (Gavrilescu 2005). The hydrophilic/hydrophobic balance of both adsorbent and adsorbate strongly influence the adsorption of non-ionic pesticides on soil organic matter. Adsorption of organic compounds has a profound impact on their bioavailability to microorganisms. Organic compounds, when introduced into natural environments sorb to clay minerals, soil organic matter such as humic substances, or to other complex mineral/organic solids (Nam and Kim 2002).

Temperature has a significant effect on the growth and physiological activity of microbes including uptake and enzymatic dehalogenation (Wiegel and Wu 2000). Jota and Hassett (1991) reported the influence of temperature is multifaceted, leading to changes in the adsorption and desorption kinetics of contaminants from soil particles. Samuel and Pillai (1989) reported DDT mineralization increased 10–14-fold as the temperature rose from 15 to 45 °C. Soil pH is also an important factor that can influence the degradation process. Andrea et al. (1994) attributed the persistence of DDT in a Brazilian field site to the low soil pH. Electron acceptors are a major factor for reductive dehalogenating organisms which play a major role in degradation under anaerobic conditions. The availability of electron acceptors affects the flow of electrons (i.e., availability of reductant usually in the form of readily decomposable organic matter) is required for reductive dehalogenation. Undefined substrates such as green manure, sludge supernatant and alfalfa have been found to stimulate dehalogenation of DDT in soils (Mohn and Tiedje 1992).

Constraints for microbial degradation of DDT

A large number of microorganisms have been identified as being able to bio-transform POPs (Table 3). The ability of soil microbial communities to degrade POPs, or hydrophobic organic contaminants (HOCs), is fundamental to soil health and fertility (Semple et al. 2003). Effective degradation occurs if microorganisms that possess catabolic ability to degrade the contaminants are present in sufficient numbers in soil. Catabolic ability is primarily due to co-evolution of soil microflora and naturally occurring organic compounds that are chemically analogous to POPs. However, the processes that control the evolution of catabolic activity in soils are not well understood (Macleod et al. 2001). Catabolic activity by bacteria can develop by adaptation, induction or depression of specific enzymes, or development of new metabolic capabilities through genetic changes such as plasmid transfer or mutation (Semple et al. 2003), and selective enrichment of organisms being able to transform contaminant(s). Microbial interaction with POPs

Table 1 Summary of soil factors involved in bioremediation (modified from Providenti et al. 1993)

Factors	Property	Effect of microorganisms	Effect on contaminants
Soil type	Texture (clay, sand and silt)	Diversity of microorganisms present	Bioavailability of contaminant depends on the texture of the soil.
	Organic matter	Microbial activity depends on the amount of carbon content.	Sorption of contaminants increases with increasing organic matter of soil.
Soil conditions	Soil moisture	Inadequate hydration depresses microbial metabolism and movement	Depends on the nature of contaminant. Reductive dechlorination is dependent on the moisture content of soil.
	Redox potential	For anaerobic metabolism presence of alternate acceptors are necessary	
	pH	Microbial activity dependent on pH	
	Temperature	Microbial metabolism varies with temperature	Temperature can affect contaminant solubility, sorption, viscosity and volatilization.

Table 2 Challenges encountered in remediating POP contaminated soils

Factors	Challenging conditions	Reasons
Soil	Ageing, sequestration, sorption and bioavailability	Organic compounds undergo time dependent sequestration in soils, resulting in a decline in bioavailability.
Contaminant	Recalcitrant metabolites	Breakdown of parent compound can sometimes lead to the formation of more persistent recalcitrant degradation products. DDE recalcitrance has been demonstrated in model ecosystems, anaerobic digesters, activated sludge plants and pasture soils. Formation of DDE from DDT breakdown is often considered a dead-end in the remediation process, although recent studies have showed the breakdown of DDE into other products
	Toxic metabolites	Cases of formation of toxic metabolites that affect microbial metabolism. For example significant levels of DDD as a result of DDT breakdown in soils can be antimicrobial for soil microorganism, which prevents further degradation of metabolite.
Microbial	Nutrients availability	Addition of organic and inorganic nutrients have been found to increase microbial metabolism of some target pollutants, decrease metabolism, shorten the adaptation period or have no apparent effect on metabolism. For example the addition of organic substrates such as methanol, glucose and acetone demonstrated significant dechlorination rates in PCBs contaminated anaerobic sediments.

requires a physical or chemical component involving the movement of the chemical in the physical environment (Bosma et al. 1997).

A compound's bioavailability is specific to an organism or a biological process that takes place. Megharaj et al. (2000) studied the effect of soil microorganisms (bacteria, fungi and algae), microbial biomass and dehydrogenase activity in long-term DDT contaminated soils. Viable counts of bacteria and algae declined with increasing DDT contamination in soil, while the fungal counts, microbial biomass and dehydrogenase activity increased in medium-level contaminated soil (27 mg DDT residues per kg of soil). At high levels of DDT contamination (34 mg/kg of soil) all the groups of microorganisms were affected. The study observed that species composition of algae and cyanobacteria was also altered in contaminated soils with sensitive species eliminated from medium and highly contaminated soils. The study also showed that cyanobacteria preferentially transformed DDT to DDD while the green algae converted DDT to DDE, which suggests the pattern of degradation was also species specific.

Bioavailability

In terms of biodegradation, bioavailability can be defined as the extent to which a contaminant is available for biological conversion, which in turn, is a function of the biological system, physico-chemical properties of the contaminant and environmental factors (Juhasz et al. 2000). Once the transfer across the membrane has occurred, storage, transformation, assimilation, or degradation can take place within the organism (Semple et al. 2004). Bioavailability is therefore not an inherent property of a compound or of a microorganism (Nam and Kukor 2003). Morrison et al. (2000) assessed the decline in bioavailability of DDT, DDE and DDE to earthworms (*Eisenia foetida*) in field soil samples treated 49 years earlier, and in laboratory-spiked soils which were aged for 190 days. About 30, 12, 34 and 20 % of DDT, DDE, DDD and the total of the three compounds, respectively, was bioavailable in the long term contaminated field soil samples, and was consistent with the laboratory soil sample aged for 190 days, which also showed reduced bioavailability. However, it may also be noted that in spite of 49 years of aging, earthworms were still able to assimilate significant amounts of DDT and its metabolites from soil.

Bioavailability is also affected by sequestration of contaminants in soil. Sequestration of contaminants occurs due to the interactions between the contaminant and solid fractions in the soil (Xing and Pignatello 1997; Schlebaum et al. 1998). As a consequence, compounds move from accessible soil compartments into less accessible, or inaccessible, compartments resulting in limited "bioavailability".

Table 3 Microorganisms capable of bio-transforming DDT and other POPs

POPs	Microorganism	Metabolite	Reference(s)
DDT	*Eubacterium limosum*, *Ralstonia eutropha* strain A5	DDD	Yim et al. (2008); Hay and Focht (2000)
	Trichoderma viride	DDD, DDE	Matsumura and Boush (1968)
	Pseudomonas acidovorans, *Terrabacter* sp.	DDE	Hay and Focht (1998); Aislabie et al. (1997)
Aldrin	*Pseudomonas* sp., *Micrococcus* sp., *Bacillus* sp.	*trans*-Aldrindiol	Patil et al. (1970)
	Aspergillus niger	Dieldrin	Korte and Porter (1970)
Dieldrin	*Bacillus* sp.	*trans*-Aldrindiol	Matsumura and Boush (1967)
	Arthobacter sp., *Bacillus* sp.	CO2	Jagnow and Halder (1972)
Endrin	*Pseudomonas* sp., *Micrococcus* sp., *Arthobacter*	Ketoendrin	Patil et al. (1970)
PCBs	*Desulfomonile tiedjei*, *Desulfitobacterium*, *Dehalobacter restrictus*	Mono and di-chlorobiphenyls	Borja et al. (2005)

Contaminant diffusion in organic matter is conceptualized as two regions. There is a "rubbery" region exhibiting a linear, faster and reversible character and a "glassy" region containing holes where slow, non-linear, reversible sorption and entrapment may also occur and account for desorption retardations (Wilson and Naidu 2008). A growing body of evidence supports the argument that the bioavailability of some organic compounds, as well as the ease of extractability of these organic compounds, diminishes as the residence time of the compound in complex environmental matrices such as soil, sediment and aquifers, increases (Nam and Kim 2002). This process is known as ageing, resulting in organic compounds becoming more strongly associated with soil solid phases over time.

The main mechanisms involved in ageing are sorption and diffusion which are interactions between the contaminant and the solid fractions within soil. Organic compounds are rapidly taken up by sorption from the bulk aqueous phase on to external sorption sites of the sorbent, and then they move slowly into internal sites, or sites that are less accessible with time for microbial uptake. Physical sequestration of organic compounds may occur by association with the polymeric structure of the matrix organic matter (a process that is referred to as intra-organic matter diffusion), or by diffusion into tortuous pores located within the matrix of a micro-porous particle (Nam and Kim 2002).

Bioavailability of DDT to microorganisms decreases with increasing contact time. Studies of Robertson and Alexander (1998) showed the ageing of DDT in soil significantly reduced its acute toxicity to house fly (*Musca domestica*), fruit fly (*Drosophila melanogaster*) and German cockroach (*Blattella germanica*). The study showed that after 270 days of ageing DDT was no longer toxic to house fly, while the mortality percentage decreased to 44 and 68 % in the case of fruit fly and cockroach, respectively. The study therefore also showed that the toxicity of aged DDT was also organism-specific.

Recent innovative strategies

The long term ageing of DDT in soils results in additional constraints to treating contaminated soils. Slow desorption is a major impediment to remediation of many contaminated sites (Yang et al. 2001). Therefore recent studies have tested chemical or biological additives that may increase bioavailability by enhancing desorption from long term DDT contaminated soils (Table 4).

Desorption of HOCs using surfactants and co-solvents have been studied extensively (Smith et al. 2004). Numerous studies have been conducted using a range of amendments as ameliorants for desorption of contaminants from soil. Some ameliorants were used to change the properties of the soil whilst enhancing the rate of mass transfer of HOCs into the solution phase. Research conducted by Yang et al. (2001) showed that strong chelating agents such as oxalate, pyrophosphate, citrate and ethylene-diamine-tetra acetic acid can alter the association between soil organic matter (SOM) and the inorganic matrix which

Table 4 Strategies to increase bioavailability and remediation of contaminants

Amendment	Remediation process	Results	Reference
Organic substrate—acetone, methanol, glucose and acetate	Reductive dechlorination of PCB's	Up to 70 %-dechlorination of tetrachlorobiphenyls and 80 % with hexa and pentachlorobiphenyls	Nies and Vogel (1990)
Reducing agent + Surfactant (Triton X-114 and Brij 35)	Accelerated DDT transformation due to increased solubility of DDT	Increased transformation of DDT with reduced accumulation of DDD and formed other products such as DDOH and DBP	You et al. (1996)
p,*p*′ DDT, DDD and DDE to sediment slurries	Sediments are adapted to the compounds	Dechlorination of DDT and formation of DDD, DDMU and trace of DBP	Huang et al. (2001)
Low molecular organic acids	Partial dissolution of soil structure through the chelation of inorganic ions, potentially enhancing bioavailability	Citric and oxalic acid removed 2.1 and 1.9 % DDE compared to water treated soil.	White et al. (2003)
Cosolvent washing—50 % 1-propanol	Enhanced solubilisation of contaminants	Enhanced desorption of *p*,*p*′ DDT	Smith et al. (2004)
Oxalate	Disruption of organo-mineral linkages due to chelation resulting in solubilisation of soil organic matter and inorganic ions	Increased desorption of *p*,*p*′ DDT ranging between 11 and 54 %.	Luo et al. (2006)
Vitamin B12 with ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate (abiotic)	Electrolytic dechlorination of DDT and DDD	DDO, DDNU and DDMS formed in the range of 73–82 %	Jabbar et al. (2007)

affect the structure of the SOM itself. These studies showed that restructuring of soil particles due to chelation of metals resulted in SOM desorption, which enhanced the availability of HOCs to the aqueous phase. In a fairly recent study, Kantachote et al. (2004a) reported that adding 0.5 % (w/w) seaweed provided the optimum dissolved organic carbon (DOC) of about 227 mg/kg of soil and Na^+ for mobilising DDT from soil. This led to enhanced DDT transformation, resulting in 80 % biodegradation of DDT from soil in 6 weeks under anaerobic conditions. However, the study also showed that DOC above 300 mg/kg of soil exhibited decreased rates of degradation. The study reported this could be due to the transformation of seaweed derived carbon sources in preference to DDT and a reduction in bioavailability due to binding of these pollutants to the seaweed and DOC in soil solution. In another study, Kantachote et al. (2004b) attributed the enhanced degradation of DDT to increased dispersion of soil in the presence of Na^+, and the consequent release of DOC. The study showed that addition of 30 mg Na^+/kg of soil resulted in the transformation of 95 % of DDT in soil compared to transformation of 75 % of DDT in soil with no amendments. The study showed Na^+ addition increased the levels of DOC and anaerobic bacteria. Some of the amendments changed the properties of the geo-sorbent itself. This change can enhance the rate of mass transfer of hydrophobic organic compounds into the solution phase which could be utilized by the microbes for degradation.

Although, some ameliorants are promising, they are yet to be tested for wider range of soils. Furthermore, the presence of various factors both in terms of environment and the contaminant can affect the degradation rate and pattern of DDT and its degradation products. Hence new strategies using additives to soil are a significant step forward, but still needs further testing and validation to develop successful field technologies.

Conclusions

(i) Given the resilience and recalcitrant nature of DDT significant effort is being made to develop

effective and reliable techniques for remediating contaminated soils. Research conducted over the last 20 years demonstrates partial degradation of DDT. Research has shown DDT has been converted into DDE, DDD and 4-CBA. However, complete mineralization of DDT is yet to be reported, and moreover, most of the studies were carried out in pure systems that require further assessment in real environmental conditions.

(ii) Recent studies show that the persistence of DDT and its degradation products for several decades, due to ageing and sequestration and bioavailability, are challenges to developing remediation processes.

(iii) A decade ago knowledge was limited regarding various environmental factors that enhance bioremediation of DDT from contaminated sites. More recently, amendments such as carbon rich substrates and zero valent iron were found to improve the reductive dechlorination process. These can play a major role in bringing about a favourable environment for degradation.

(iv) Studies report that DDE is no longer a "dead end" in the degradation process in that it can be degraded in anaerobic conditions to DDMU and in aerobic conditions to 4-CBA. DDT degradation by aerobic bacteria has also opened up new areas of research.

(v) Although DDT is one of the most difficult contaminants to degrade, recent advances have created new avenues in remediating contaminated sites. However, much still needs to be done to mineralize DDT completely.

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