

## Biodegradation of Xenobiotics

Biodegradation or biological degradation is the phenomenon of biological transformation of organic compounds by living organisms, particularly the microorganisms.

Biodegradation basically involves the conversion of complex organic molecules to simpler (and mostly non-toxic) ones. The term biotransformation is used for incomplete biodegradation of organic compounds involving one or a few reactions. Biotransformation is employed for the synthesis of commercially important products by microorganisms.

Bioremediation refers to the process of using microorganisms to remove the environmental pollutants i.e. the toxic wastes found in soil, water, air etc. The microbes serve as scavengers in bioremediation. The removal of organic wastes by microbes for environmental clean-up is the essence of bioremediation. The other names used (by some authors) for bioremediation are bio-treatment, bio-reclamation and bio-restoration.

It is rather difficult to show any distinction between biodegradation and bioremediation. Further, in biotechnology, most of the reactions of biodegradation/bioremediation involve xenobiotic.

### Xenobiotic:

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Xenobiotic (xenos-foreign) broadly refer to the unnatural, foreign and synthetic chemicals such as pesticides, herbicides, refrigerants, solvents and other organic compounds. Microbial degradation of xenobiotic assumes significance, since it provides an effective and economic means of disposing of toxic chemicals, particularly the environmental pollutants.

#### **Pseudomonas — The Predominant Microorganism For Bioremediation:**

Members of the genus *Pseudomonas* (a soil microorganism) are the most predominant microorganisms that degrade xenobiotic. Different strains of *Pseudomonas*, that are capable of detoxifying more than 100 organic compounds,

have been identified. The examples of organic compounds are several hydrocarbons, phenols, organophosphates, polychlorinated biphenyls (PCBs) and polycyclic aromatics and naphthalene.

About 40-50 microbial strains of microorganisms, capable of degrading xenobiotics have been isolated. Besides *Pseudomonas*, other good examples are *Mycobacterium*, *Alcaligenes*, and *Nocardia*. A selected list of microorganisms and the xenobiotics degraded is given in Table 59.1.

**TABLE 59.1 A selected list of microorganisms and the pollutants (xenobiotics) that are degraded by bioremediation**

<i>Microorganism</i>	<i>Pollutant chemicals</i>
<i>Pseudomonas</i> sp	Aliphatic and aromatic hydrocarbons—alkylaminoxides, alkylammonium benzene, naphthalene, anthracene xylene, toluene, polychlorinated biphenyls (PCBs), malathion, parathion, organophosphates.
<i>Mycobacterium</i> sp	Benzene, branched hydrocarbons, cycloparaffins
<i>Alcaligenes</i> sp	Polychlorinated biphenyls, alkyl benzene, halogenated hydrocarbons.
<i>Nocardia</i> sp	Naphthalene, alkylbenzenes, phenoxyacetate.
<i>Arthrobacter</i> sp	Benzene, polycyclic aromatics, phenoxyacetate, pentachlorophenol.
<i>Corynebacterium</i> sp	Halogenated hydrocarbons, phenoxyacetate.
<i>Bacillus</i> sp	Long chain alkanes, phenylurea.
<i>Candida</i> sp	Polychlorinated biphenyls
<i>Aspergillus</i> sp	Phenols
<i>Xanthomonas</i> sp	Polycyclic hydrocarbons
<i>Streptomyces</i> sp	Halogenated hydrocarbons, phenoxyacetate.
<i>Fusarium</i> sp	Propanil
<i>Cunninghamella</i> sp	Polycyclic aromatics, polychlorinated biphenyls.

### **Consortia of microorganisms for biodegradation:**

A particular strain of microorganism may degrade one or more compounds. Sometimes, for the degradation of a single compound, the synergetic action of a few microorganisms (i.e. a consortium or cocktail of microbes) may be more efficient. For

instance, the insecticide parathion is more efficiently degraded by the combined action of *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*.

### **Co-metabolism in biodegradation:**

In general, the metabolism (breakdown) of xenobiotics is not associated with any advantage to the microorganism. That is the pollutant chemical cannot serve as a source of carbon or energy for the organism. The term co-metabolism is often used to indicate the non-beneficial (to the microorganism) biochemical pathways concerned with the biodegradation of xenobiotics. However, co-metabolism depends on the presence of a suitable substrate for the microorganism. Such compounds are referred to co-substrates.

### **Factors Affecting Biodegradation:**

Several factors influence biodegradation. These include the chemical nature of the xenobiotic, the capability of the individual microorganism, nutrient and O<sub>2</sub> supply, temperature, pH and redox potential. Among these, the chemical nature of the substrate that has to be degraded is very important.

### **Some of the relevant features are given hereunder:**

- i. In general, aliphatic compounds are more easily degraded than aromatic ones.
- ii. Presence of cyclic ring structures and length chains or branches decrease the efficiency of biodegradation.
- iii. Water soluble compounds are more easily degraded.
- iv. Molecular orientation of aromatic compounds influences biodegradation i.e. ortho > para > meta.
- v. The presence of halogens (in aromatic compounds) inhibits biodegradation.

Besides the factors listed above, there are two recent developments to enhance the biodegradation by microorganisms.

### **Bio-stimulation:**

This is a process by which the microbial activity can be enhanced by increased supply of nutrients or by addition of certain stimulating agents (electron acceptors, surfactants).

### **Bio-augmentation:**

It is possible to increase biodegradation through manipulation of genes. More details on this genetic manipulation i.e. genetically engineered microorganisms (GEMs), are described later. Bio-augmentation can also be achieved by employing a consortium of microorganisms.

**Enzyme Systems for Biodegradation:**

Several enzyme systems (with independent enzymes that work together) are in existence in the microorganisms for the degradation of xenobiotics. The genes coding for the enzymes of bio-degradative pathways may be present in the chromosomal DNA or more frequently on the plasmids. In certain microorganisms, the genes of both chromosome and plasmid contribute for the enzymes of biodegradation. The microorganism *Pseudomonas* occupies a special place in biodegradation.

A selected list of xenobiotics and the plasmids containing the genes for their degradation is given in Table 59.2.

**TABLE 59.2 A selected list of xenobiotics and the plasmids containing genes (in *Pseudomonas*) for biodegradation**

<i>Xenobiotic</i>	<i>Name of plasmid in Pseudomonas</i>
Naphthalene	NAH
Xylene	XYL
Xylene and toluene	TOL, pWWO, XYL-K
Salicylate	SAL
Camphor	CAM
3-Chlorobenzene	pAC25

**Recalcitrant Xenobiotics:**

There are certain compounds that do not easily undergo biodegradation and therefore persist in the environment for a long period (sometimes in years). They are labeled as recalcitrant.

**There may be several reasons for the resistance of xenobiotics to microbial degradation:**

- i. They may chemically and biologically inert (highly stable).
- ii. Lack of enzyme system in the microorganisms for biodegradation.
- iii. They cannot enter the microorganisms being large molecules or lack of transport systems.

iv. The compounds may be highly toxic or result in the formation highly toxic products that kill microorganisms.

There are a large number of recalcitrant xenobiotic compounds e.g. chloroform, freons, insecticides (DDT, lindane), herbicides (dalapon) and synthetic polymers (plastics e.g. polystyrene, polyethylene, polyvinyl chloride).

It takes about 4-5 years for the degradation of DDT (75-100%) in the soil. A group of microorganisms (*Aspergillus flavus*, *Mucor atenans*, *Fusarium oxysporum* and *Trichoderma viride*) are associated with the slow biodegradation of DDT.

### **Bio-magnification:**

The phenomenon of progressive increase in the concentration of a xenobiotic compound, as the substance is passed through the food chain is referred to as bio-magnification or bioaccumulation. For instance, the insecticide DDT is absorbed repeatedly by plants and microorganism.

When they are eaten by fish and birds, this pesticide being recalcitrant, accumulates, and enters the food chain. Thus, DDT may find its entry into various animals, including man. DDT affects the nervous systems, and it has been banned in some countries.

### **Types of Bioremediation:**

The most important aspect of environmental biotechnology is the effective management of hazardous and toxic pollutants (xenobiotics) by bioremediation. The environmental clean-up process through bioremediation can be achieved in two ways—in situ and ex situ bioremediation.

#### ***In Situ Bioremediation:***

In situ bioremediation involves a direct approach for the microbial degradation of xenobiotics at the sites of pollution (soil, ground water). Addition of adequate quantities of nutrients at the sites promotes microbial growth. When these microorganisms are exposed to xenobiotics (pollutants), they develop metabolic ability to degrade them.

The growth of the microorganisms and their ability to bring out biodegradation are dependent on the supply of essential nutrients (nitrogen, phosphorus etc.). In situ bioremediation has been successfully applied for clean-up of oil spillages, beaches etc. There are two types of in situ bioremediation-intrinsic and engineered.

**Intrinsic bioremediation:**

The inherent metabolic ability of the microorganisms to degrade certain pollutants is the intrinsic bioremediation. In fact, the microorganisms can be tested in the laboratory for their natural capability of biodegradation and appropriately utilized.

**Engineered in situ bioremediation:**

The inherent ability of the microorganisms for bioremediation is generally slow and limited. However, by using suitable physicochemical means (good nutrient and O<sub>2</sub> supply, addition of electron acceptors, optimal temperature), the bioremediation process can be engineered for more efficient degradation of pollutants.

**Advantages of in situ bioremediation:**

1. Cost-effective, with minimal exposure to public or site personnel.
2. Sites of bioremediation remain minimally disrupted.

**Disadvantages of in situ bioremediation:**

1. Very time consuming process.
2. Sites are directly exposed to environmental factors (temperature, O<sub>2</sub> supply etc.).
3. Microbial degrading ability varies seasonally.

***Ex Situ Bioremediation:***

The waste or toxic materials can be collected from the polluted sites and the bioremediation with the requisite microorganisms (frequently a consortium of organisms) can be carried out at designed places. This process is certainly an improvement over in situ bioremediation, and has been successfully used at some places.

**Advantages of ex situ bioremediation:**

1. Better controlled and more efficient process.
2. Process can be improved by enrichment with desired microorganisms.
3. Time required in short.

**Disadvantages of ex situ bioremediation:**

1. Very costly process.
2. Sites of pollution are highly disturbed.

3. There may be disposal problem after the process is complete.

### **Metabolic Effects of Microorganisms on Xenobiotics:**

Although it is the intention of the biotechnologist to degrade the xenobiotics by microorganisms to the advantage of environment and ecosystem, it is not always possible. This is evident from the different types of metabolic effects as shown below.

#### **Detoxification:**

This process involves the microbial conversion of toxic compound to a nontoxic one. Biodegradation involving detoxification is highly advantageous to the environment and population.

#### **Activation:**

Certain xenobiotics which are not toxic or less toxic may be converted to toxic or more toxic products. This is dangerous.

#### **Degradation:**

The complex compounds are degraded to simpler products which are generally harmless.

#### **Conjugation:**

The process of conjugation may involve the conversion of xenobiotics to more complex compounds. This is however, not very common.

### **Types of Reactions in Bioremediation:**

Microbial degradation of organic compounds primarily involves aerobic, anaerobic and sequential degradation.

#### ***Aerobic bioremediation:***

Aerobic biodegradation involves the utilization of O<sub>2</sub> for the oxidation of organic compounds. These compounds may serve as substrates for the supply of carbon and energy to the microorganisms. Two types of enzymes namely mono-oxygenases and di-oxygenases are involved in aerobic biodegradation. Mono-oxygenases can act on both aliphatic and aromatic compounds while di-oxygenases oxidize aliphatic compounds.

#### ***Anaerobic bioremediation:***

Anaerobic biodegradation does not require O<sub>2</sub> supply. The growth of anaerobic microorganisms (mostly found in solids and sediments), and consequently the degradation processes are slow. However, anaerobic biodegradation is cost-

effective, since the need for continuous O<sub>2</sub> supply is not there. Some of the important anaerobic reactions and examples of organic compounds degraded are listed below. Hydrogenation and dehydrogenation — benzoate, phenol, catechol.

Dehalogenation — Polychlorinated biphenyls (PCBs), chlorinated ethylene's. The term de-chlorination is frequently used for dehalogenation of chlorinated compounds.

Carboxylation and decarboxylation — toluene, cresol and benzoate.

### ***Sequential Bioremediation:***

In the degradation of several xenobiotics, both aerobic and anaerobic processes are involved. This is often an effective way of reducing the toxicity of a pollutant. For instance, tetra chloromethane and tetrachloroethane undergo sequential degradation.

### ***Biodegradation of Hydrocarbons:***

Hydrocarbon are mainly the pollutants from oil refineries and oil spills. These pollutants can be degraded by a consortium or cocktail of microorganisms e.g. Pseudomonas, Corynebacterium, Arthrobacter, Mycobacterium and Nocardia.

### ***Biodegradation of Aliphatic Hydrocarbons:***

The uptake of aliphatic hydrocarbons is a slow process due to their low solubility in aqueous medium. Both aerobic and anaerobic processes are operative for the degradation of aliphatic hydrocarbons. For instance, unsaturated hydrocarbons are degraded in both anaerobic and aerobic environments, while saturated ones are degraded by aerobic process. Some aliphatic hydrocarbons which are recalcitrant to aerobic process are effectively degraded in anaerobic environment e.g. chlorinated aliphatic compounds (carbon tetrachloride, methyl chloride, vinyl chloride).

### ***Biodegradation of Aromatic Hydrocarbons:***

Microbial degradation of aromatic hydrocarbons occurs through aerobic and anaerobic processes. The most important microorganism that participates in these processes is Pseudomonas.

**The biodegradation of aromatic compounds basically involves the following sequence of reactions:**

1. Removal of the side chains.
2. Opening of the benzene ring.



Most of the non-halogenated aromatic compounds undergo a series of reactions to produce catechol or protocatechuate. The bioremediation of toluene, L-mandelate, benzoate, benzene, phenol, anthracene, naphthalene, phenanthrene and salicylate to produce catechol is shown in Fig. 59.1. Likewise, Fig. 59.2, depicts the bioremediation of quinate, p-hydroxymandelate, p-hydroxybenzoyl formate, p-toluate, benzoate and vanillate to produce protocatechuate.

Catechol and protocatechuate can undergo oxidative cleavage pathways. In ortho-cleavage pathway, catechol and protocatechuate form acetyl CoA (Fig. 59.3), while in meta-cleavage pathway (Fig. 59.4), they are converted to pyruvate and acetaldehyde. The degraded products of catechol and protocatechuate are readily metabolised by almost all the organisms.

### **Biodegradation of Pesticides and Herbicides:**

Pesticides and herbicides are regularly used to contain various plant diseases and improve the crop yield. In fact, they are a part of the modern agriculture, and have significantly contributed to green revolution. The common herbicides and pesticides are propanil (anilide), propham (carbamate), atrazine (triazine), picloram (pyridine), dichlorodiphenyl trichloroethane (DDT) monochloroacetate (MCA), monochloropropionate (MCPA) and glyphosate (organophosphate). Most of the pesticides and herbicides are toxic and are recalcitrant (resistant to biodegradation). Some of them are surfactants (active on the surface) and retained on the surface of leaves.

**Extra Material**

# BIOREMEDIATION

- Bioremediation refers to the **process of using microorganisms to remove the environmental pollutants or prevent pollution.**
- The removal of organic wastes by microbes for **environmental clean-up** is the essence of bioremediation.
- The other names used for bioremediation are **biotreatment, bioreclamation and biorestitution.**
- **Xenobiotics** broadly refer to the **unnatural, foreign and synthetic chemicals** such as pesticide, herbicide & other organic compounds.

## **TYPES OF BIOREMEDIATION :**

1. Biostimulation
2. Bioaugmentation
3. Intrinsic bioremediation

## Conclusion

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Xenobiotics (greek xenos = strange, foreign, foreigner) are chemically synthesized compounds that do not occur in nature and thus are 'foreign to the biosphere'. They have 'unnatural' structural features to which microorganisms have not been exposed to during evolution. Xenobiotics may resist biodegradation, or they undergo incomplete

biodegradation or just biotransformation. The definition of xenobiotics as compounds 'foreign to life' exhibiting 'unnatural' structural features does not necessarily imply that xenobiotics are toxic compounds, but many xenobiotics indeed are harmful to living organisms. Whereas xenobiotics may persist in the environment for months and years, most biogenic compounds are biodegraded rapidly. Exceptions are lignin, the structural polymer of woody plants, and, above all, the melanin polymers which are constituents of the cell wall of the spores of a number of fungi. Recalcitrance (i.e., the structureimmanent stability) of a xenobiotic molecule is mainly due to 'unphysiological' chemical bonds and/or substituents, which block the attack by

microbial catabolic enzymes. Type, number and position of bonds and substituents affect the xenobiotic character. However, it is not always easy to determine which structural moieties indeed are xenobiotic in the sense of 'foreign to life'. Some natural compounds show principally the same unusual structural features as xenobiotics, such as halogen substituents or nitro groups found in some antibiotics, or they contain stable chemical bonds like the ether and carbon-carbon bonds stabilizing lignin. Moreover, microorganisms throughout geological time have also been exposed to a variety of chemicals produced by abiotic natural processes:

### Bioremediation of Xenobiotics:

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Use of pesticides has benefited the modern society by improving the quantity and quality of the world's food production. Gradually, pesticide usage has become an integral part of modern agriculture system. Many of the artificially made complex compounds *i.e.* xenobiotics persist in environment and do not undergo biological transformation. Microorganisms play an important role in degradation of xenobiotics, and maintaining of steady state concentrations of chemicals in the environment. The complete degradation of a pesticide molecule to its inorganic components that can be eventually used in an oxidative cycle removes its potential toxicity from the environment. However, there are two objectives in relation to biodegradation of xenobiotics: *(i)* how biodegradation activity arises, evolves and transferred among the members of soil microflora, and *(ii)* to devise bioremediation methods for removing or detoxifying high concentration of dangerous pesticide residues. The characters of pesticide degradation of microorganisms are located on plasmids and transposons, and are grouped in clusters on chromosome. Understanding of the characters provides clues to the evolution of degradative pathways and makes the task of gene manipulation easier to construct the genetically engineered microbes capable of degrading the pollutants.

### Microbial Degradation of Xenobiotics

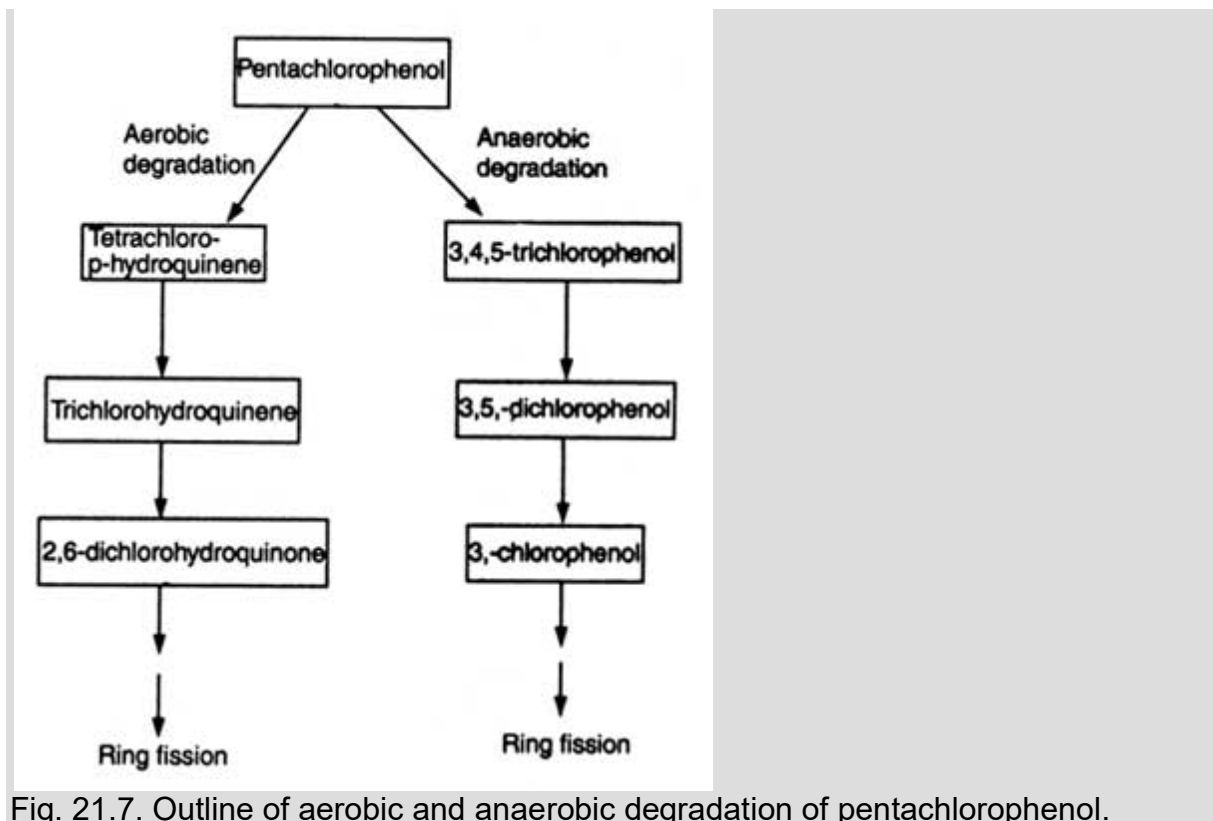


Fig. 21.7. Outline of aerobic and anaerobic degradation of pentachlorophenol.

Biodegradation of pesticides occurs by aerobic soil microbes. Pesticides are of wide varieties of chemicals e.g. chlorophenoxyalkyl carboxylic acid, substituted ureas, nitrophenols, tri-azines, phenyl carbamates, organo-chlorines, organophosphates, etc. Duration of persistence of herbicides and insecticides in soil is given in Table Otganophos-phates (e.g. diazion, methyl par-athion and parathion) are perhaps the most extensively used insecticides under many agricultural systems. Biodegradation through hydrolysis of p-o-aryl bonds by *Pseudomonas diminuta* and *Flavobacterium* are considered as the most significant steps in the detoxification of organophosphorus compounds. Organomercurials (e.g. Semesan, Panodrench, Panogen) have been practiced in agriculture since the birth of fungicides. Several species of *Aspergillus*, *Penicillium* and *Trichoderma* have been isolated from Semesan-treated soil. Moreover, they have shown ability to grow over 100 ppm of fungicide *in vitro*. The major fungicides used in agriculture are water soluble derivatives such as Ziram, Ferbam, Thiram, etc. All these are degraded by microorganisms.

Pentachlorophenol (PCP) is a broad spectrum biocide which has been used as fungicide, insecticide, herbicide, algicide, disinfectant and antifouling agent. Bioreactors containing alginate immobilized + Polyurethane foam immobilized PCP

degrading *Flavobacterium* (ATCC39723) cells have been used to remove PCP from contaminated water. Absorption of PCP by Polyurethane immobilized matrix plays a role in reducing the toxicity of PCP. *Flavobacterium* removed and detoxified PCP (Zhong -Cheng, 1994). In other experiment *P. chrysosporium* enzyme (ligninase) has been found to dehalogenate PCP. Steps of PCP degradation has been shown in Fig.

**Table 1. Duration of persistence of insecticides and herbicides in soil.**

<b>Biocides</b>	<b>Time taken for 75-100% disappearance</b>
<b>A. Chlorinated insecticides</b>	
DDT (1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane)	4 years
Aldrin	3 years
Chlordane	5 years
Heptachlor	2 years
Lindane (hexachloro-cyclohexane)	3 years
<b>B. Organophosphate insecticides</b>	
Diazinon	12 years
Malathion	1 week
Parathion	1 week
<b>C. Herbicides</b>	
2,4-D (2,4-dichlorophenoxyacetic acid)	4 weeks
2,4,5-T	30 weeks
Atrazine	40 weeks

<b>Biocides</b>	<b>Time taken for 75-100% disappearance</b>
Simazine	48 weeks
Propazine	1.5 years

Source :

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Madigan *et al.* (1997). DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane) is an insecticide that persists in soil for four years. Degradation pathway of DDT involves an initial dechlorination of the trichloromethyl group to form 1,1-dichloro 2-ethane which then undergoes further dechlorination, oxidation and decarboxylation to form bis methane. Subsequent cleavage of one of the normal aromatic rings yields p-chlorophenyl acetic acid, which may also undergo ring cleavage. Microorganisms associated with DDT degradation are *Aspergillus flavus*, *Fusarium oxysporum*, *Mucor aterians*, *P. chrysosporium*, *Trichoderma viride*, etc. Environmental factors including pH, temperature, bioavailability, nutrient supply and oxygen availability affect biodegradation of pesticides.

