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Bioremediation of Pesticides by Microorganisms: General Aspects and Recent Advances

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With the advent of Green Revolution, there had been a quantum jump in the use of synthetic pesticides to protect crops from various pests and diseases and to enhance the productivity throughout the world, and have now become a part of modern agriculture. Although they play a significant role in augmenting food production, their large scale and indiscriminate use in India has resulted in highly unstable ecosystem, development of resistance by insects, elimination of parasites, predators and pollinators, resurgence of minor pests and destruction of useful insects. Thus, looking towards the environmental concerns and health hazards resulting due to the continuous use of these noxious xenobiotic pesticides. It is highly desirable to detoxify them. Traditional methods of pesticide detoxification have relied on landfills and incineration, which generate secondary contamination problems due to leaching of pesticides into surrounding soil and groundwater supplies and production of potentially toxic by-product emissions. By contrast, bioremediation may be viewed as a more effective and environmentally benign clean-up technology since it results in partial or complete bioconversion of the organic pollutants to microbial biomass and stable non-toxic end-products. Rhizosphere harbours a variety of beneficial microorganisms having the potential for degradation of these pollutants. These indigenous rhizosphere competent microbial strains are being exploited these days for the degradation of organic pollutants through bioremedial processes. Microbial interactions and innate microbial community dynamics greatly influence natural bioremedial processes occurring in soil. Microorganisms involve various biochemical mechanisms for degradation of pesticides such as oxidative transformations by synthesizing various enzymes, hydrolytic transformations, aromatic nitroreductive processes, carbon–phosphorus bond cleavage reactions, pesticide conjugation reactions and formation

humankind has practiced composting, sewage treatment and fermentation since the beginning of recorded history.

PESTICIDES—TOXIC XENOBIOTIC ENVIRONMENTAL POLLUTANTS

There has been an enormous increase in the use of various synthetic pesticides that contribute to the spectacular increase in the crop yield. Soil, groundwater and sediments are the ultimate sinks for these pollutants, where they are either broken down to simpler forms or remain persistent. Some of the pesticides are susceptible to biomagnification and cause more danger to the environment. The safe and economical disposal of excess pesticide waste is a problem of considerable magnitude. Pesticides are considered *Xenobiotic* (*xenos* means "foreign" in Greek) compounds. These are synthetic foreign (man-made) organic compounds produced chemically and have no obvious counterpart in the natural world. Most of them are *recalcitrant* and resist their biodegradation. Over 1000 pesticides have been marketed for chemical pest control purposes. Pesticides include herbicides, insecticides, bacteriocides and fungicides etc. (Shah and Dhaliwal, 2006). Chemically, pesticides are of a wide variety, including chlorinated compounds, aromatic rings, nitrogen- and phosphorous-containing compounds and others. Toxicity and bio-accumulation potential of chlorophenol increases with the degree of chlorination and lipophilicity. The constituents of many pesticides are generally derivatives of benzene. The major groups of pesticides, their target organisms and common examples are given below (Table 2.1). The aromatic ring has large negative resonance energy, therefore, benzene and its derivatives are stable group of compounds.

Table 2.1 Major groups of pesticides, their target organisms and common examples

Group of Pesticide	*Target organisms*	*Common examples*
Insecticides	Insects	Carbamyl, DDT, aldrin, endosulfan, HCH, malathione, Lindane
Fungicides	Fungi	Bordeaux mixture, captan, dinocap, sulphur, thiram, Pentachlorophenol (PCP)
Herbicides/weedicides	Weeds	Atrazine, 2,4D, dinoseb, isoproturon, maleichydrazide, MCPA, Simazine, 2,4,5-T, Decamba
Nematicides	Nematodes	DBCB, ECB, methyl bromide, methy isocyanate
Rodenticides	Rodents	Aluminium phosphide, methyl bromide, sodium fluoroacetate, zinc phosphide
Avicides	Birds	Endrin, methyl parathion, fenthion, thallium
Bacteriocides	Bacteria	Bordeaux mixture, cupric hydroxide, streptomycin, tetracycline
Molluscides	Molluscs	Carbonyl, metaldehyde, methicarb, PCP, phorate
Acaricides	Mites	Chlorenthol, chlorfenson, cyhexatin, dicofol, dinoseb, DNOC

Toxic effects of organophosphate and carbamate pesticides occur in the nervous system where chemicals disrupt the enzyme that regulates acetyl-cholinesterase, a neurotransmitter. The pyrethroids are functional toxins that produce adverse effects in a secondary way as a consequence of neuronal hyper excitability. World Health Organization (WHO) estimates that one million pesticide poisoning cases occur every year globally. Not only this, a long term professional exposure

to these pesticides also results in increased risk of several chronic and fatal diseases such as cancer. About 100 active ingredients in pesticides have been found to cause cancer in experimental animals or humans (Encyclopedia of Pest Management, 2002).

PERSISTENCE AND BIOMAGNIFICATION OF PESTICIDES

Most of the pesticides are *recalcitrant* in nature and resist their biodegradation, while some are metabolized incompletely, and as a result, accumulate in the environment (Table 2.2).

Table 2.2 Duration of environmental persistence of some common pesticides

	Biocides	*Time for 75-100% disappearance*
1.	**Chlorinated insecticides**	
	DDT (1,1,1-tricholro-2,2-bis-(p-cholorophenyl) ethane)	4 years
	Aldrin	3 years
	Chlordane	5 years
	Heptachlor	2 years
	Lindane (hexachloro-cyclohexane)	3 years
2.	**Organophosphate insecticides**	
	Diazinon	12 weeks
	Malathion	1 week
	Parathion	1 week
3.	**Herbicides**	
	2,4-D (2,4-dichlorophenoxyacetic acid)	4 weeks
	2,4,5-T(2,4,5-trichlorophenoxyacetic acid)	20 weeks
	Dalapon	8 weeks
	Atrazine	40 weeks
	Simazine	48 weeks
	Propazine	1.5 year

(Source: Madigan and Martinko, 2006)

Some common examples of biodegradable and recalcitrant pesticides are given in Fig. 2.1.

Hexachlorohexane (HCH), endosulfan, parathione and methyl parathione, which were banned or restricted in US and Europe several years ago, are still applied in India to control insect pests affecting pulses and wheat. Unfortunately, the above-mentioned insecticides are not only very toxic but also poorly biodegradable. In the past, organochlorine pesticides were commonly used, but due to their persistence in nature and adverse effects on human health and the environment, most have been banned or taken off the market (e.g., DDT and chlordane). The chlorinated pesticides are relatively resistant to microbial attack. In general, the more extensive the chlorine substitution, the more persistent the pesticide.

Mirex ($C_{10}Cl_{12}$) and Kepone ($C_{10}Cl_{10}O$) are extensively chlorinated insecticidal compounds (Fig. 2.2).

The extensive application of pesticides in Indian agriculture results in residual concentrations in almost every environment, drinking water and food. The term *biodegradation* has been used to describe transformations of every type, including those that yield products more complex than the

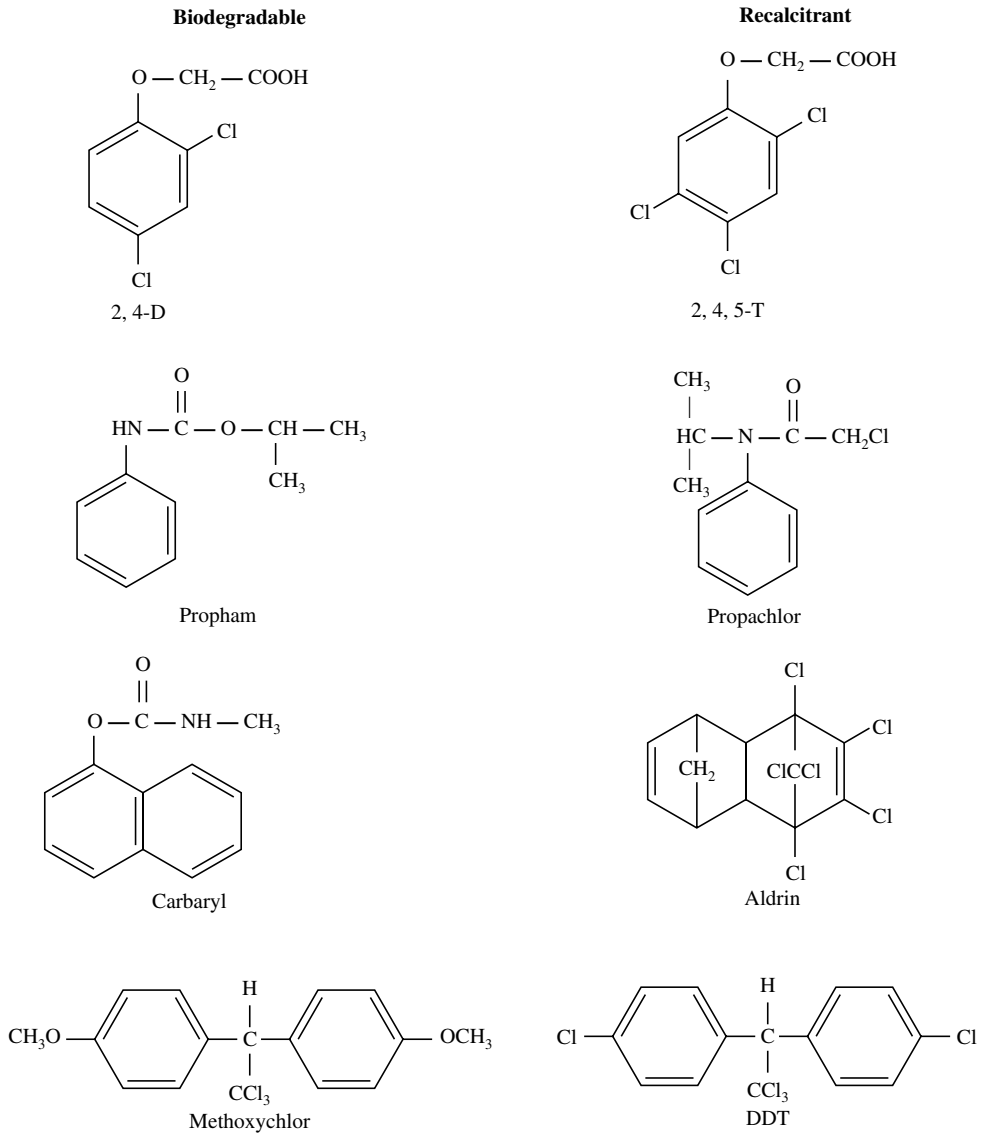


Fig. 2.1 Some examples of biodegradable and recalcitrant pesticides *(Source: Atlas & Bartha ,*1998).

starting material as well as those responsible for the complete oxidation of organic compounds to CO_2, H_2O, NO_3^- and other inorganic components. Occasionally, microbial transformations result in residues that are more stable than the parent compound, yet this phenomenon is sometimes called *degradation* because the parent compound disappears, while some call it *mineralization.*

What happens if a persistent pesticide is introduced into the ecosphere? It accumulates in the environment, not only at the place where it is applied but also at far distant places. The chlorinated hydrocarbon insecticides have been detected in remote and even arctic regions, thousands of miles away from the application site. It is important, therefore, to keep in mind that assessing

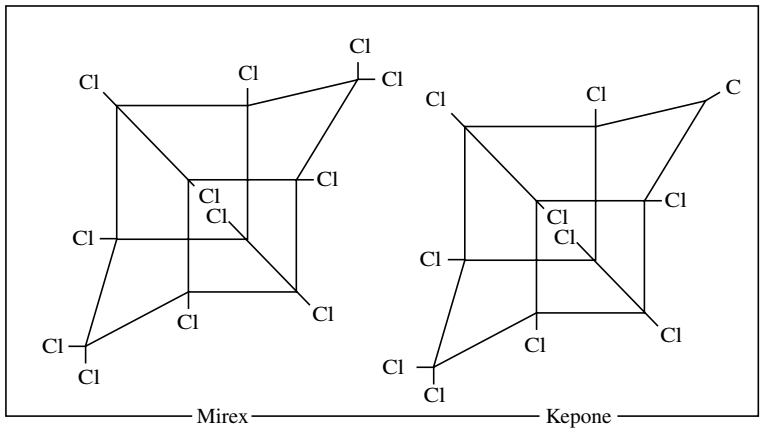


Fig. 2.2 Structural formulae of the extensively chlorinated insecticides, Mirex and Kepone (*Source:* Atlas and Bartha, 1998).

the effect of a persistent organic chemical only on the environment to which it is directly applied is insufficient. Distribution tends to dilute, and in the environments only indirectly exposed, they are generally present in the low parts per billion (ppb) ranges such as organochlorines. Then, how these low concentrations make so harmful effects? It is only because of a phenomenon called *biological magnification* or *biomagnification*. However, for a pollutant to be biomagnified, it must be both persistent and lipophilic. Because of their lipophilic character, minute dissolved amounts of these substances are partitioned from the surrounding water into the lipids of both prokaryotic and eukaryotic microorganisms. Their concentrations in the cells, compared to the surrounding medium, may increase by one to three orders of magnitude. Members of the next higher trophic level then ingest the microorganisms. Only 10-15% of the biomass is transferred to the higher trophic level, but the persistent lipophilic pollutant is neither degraded nor excreted to a significant extent and is preserved practically without loss in smaller biomass of the second trophic level, and consequently, its concentration increases. The same thing occurs at successively higher trophic levels. The top trophic level, composed of birds, mammalian carnivores and large predatory fish, may carry a body burden of the environmental pollutant that exceeds the environmental concentration by a factor of $10^4 - 10^6$. If the pollutant is a biologically active substance, such as a chlorinated hydrocarbon insecticide, DDT, at such levels it may cause death or serious damage to the affected organism (Fig. 2.3).

MICROBIAL POTENTIAL FOR DEGRADATION OF PESTICIDES

Microbial degradation of pesticides has been long recognized. Recent research has revealed a number of microbial systems capable of biodegradation of organic compounds. The natural capacity of microorga-nisms to degrade a large variety of synthetic herbicides and pesticides is the essence of microbial method for the degradation of soil contaminants (bioremediation) —the basis for green technologies. Another very interesting feature of microorganisms is that they can degrade some of the organic substances that are produced only synthetically. Although microbial degradation of pesticides does not always lead to detoxification, in many cases the products are much less hazardous and/or become susceptible to further degradation. The microbes having the potential

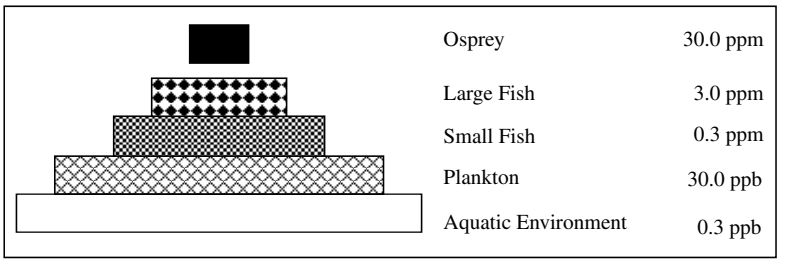


Fig. 2.3 Biomagnification of chlorinated hydrocarbon insecticide, DDT, to different trophic levels. (*Source:* Atlas and Bartha, 1998).

for pesticide degradation are mainly bacteria, especially actinomycetes and cyanobacteria, algae and fungi. Twenty-eight of the bacterial genera that can utilize aliphatic hydrocarbons have been isolated (Encyclopedia of Pest Management, 2002), and most common among them are the species of *Pseudomonas, Alcaligenes, Bacillus, Arthrobacter, Brevibacterium, Flavobacterium, Klebsiella, Methylococcus, etc.* Several fungi having pesticide degrading potential have also been identified, such as the species of *Aspergillus, Candida, Fusarium, Penicillium, Trichoderma, Rhodotorula, Pleurotus, Phaenerochaete,* etc. (Table 2.3). A relatively enormous catalytic power, large surface-volume area and rapid rate of reproduction of microorganisms contribute to the major role they play in the chemical transformation. Some organisms require an adaptation period for the constitutive synthesis of pesticide degrading enzymes before attacking it. Some, through random mutation, acquire this ability, while in others, the synthesis of degrading enzymes is induced in the presence of a particular pesticide.

The biochemical processes induced by microorganisms under aerobic and anaerobic conditions are mineralization, detoxification, cometabolism and activation. Unlike organochlorine pesticides, organophosphate, carbamate and pyrethroid pesticides are biodegradable. The main detoxification processes are hydrolysis and oxidation, with hydrolysis being the most efficient route for all three types of pesticides.

Table 2.3 Microorganisms capable of degrading organic compounds, pesticides or their metabolites

S.No.	*Degrading Microorganisms*	*Organic compound or pesticide*
I. Bacteria:		
1.	*Alcaligenes denitrificans*	Fluoranthene (PAH)
2.	*Alcaligenes faecalis*	Arylacetonitrils
3.	*Arthrobacter sp.*	Carbofuran, Parathion
4.	*Arthrobacter sp.*	EPTC, Pentachlorophenol, glyphosate
5.	*Bacillus sphaericus*	Urea herbicides, Parathion
6.	*Brevibacterium oxydans* DH35A	Cyclohexylamine
7.	*Burkholderia sp*. P514	1,2,4,5-Te CB
8.	*Clostridium*	Quinoline, Glyphosate
9.	*Corynebacterium nitrophilus*	Acetonitril, Carboxylic acid
10.	*Dehalococcoides ethanogenes*	Trichloroethylene (TCE)
11.	*Desulfovibrio sp*.	Nitroaromatic compounds
12.	*Flavobacterium sp.*	Pentachlorophenol, Parathion

Contd.

Contd.

13.	*Geobacter sp.*	Aromatic compounds
14.	*Klebsiella pneumoniae*	3&4 Hydrobenzoate
15.	*Methylococcus capsulatus*	Trichloroethylene
16.	*Nitrosomonas europaea*	1,1,1-Trichloroethane
17.	*Nocardia*	Quinoline
18.	*Pseudomonas stutzeri*	Parathion, Methyl parathion
19.	*Pseudomonas capaciea* *Pseudomonas sp.*	2,4,5-T Diazinon
20.	*Rhodococcus chlorophenolicus*	Pentachlorophenol (PCP)
II. Fungi and Yeast:		
1.	*Aspergillus flavus*	DDT
2.	*Aspergillus paraceticus*	DDT
3.	*Aspergillus niger*	2,4-D
4.	*Candida tropicalis*	Phenol
5.	*Chrysosporium lignorum*	3,4-Dichloroaniline
6.	*Fusarium oxysporum*	DDT
7.	*Phaenerochaete chrysosporium*	Lindane, DDT, Pentachlorophenol,
8.	*Pleurotus ostreatus*	DDT
9.	*Trichoderma viride*	DDT
III. **Ectomycorrhizal Fungi:**		
1.	*Paxillus involutus*	Mefluidide
2.	*Scillus luteus*	Mefluidide

(Source: Mateen *et al.,* 1994 *; Encyclopedia of Pest Management,* 2002*)*

BIOREMEDIATION: AN EMERGING TECHNOLOGY FOR ENVIRONMENT CLEAN-UP

The manufacturing and use of pesticides has been rising tremendously in India, and the waste generated by the pesticide industry has become a serious environmental problem due to the present insufficient and ineffective waste treatment technology involving physico-chemical and biological treatments. As a result, recalcitrant pesticide residues remain in surface soil, leading to the toxicity of soil-water environment. Considering the toxic effects of pesticides, it is essential to remove these chemo-pollutants from the environment. Bioremediation including phytoremediation and rhizoremediation is expected to be a useful clean-up method for soil contaminated with persistent organic pesticides including dieldrin and endrin (Matsumoto *et al*., 2009). Traditional methods of pesticide detoxification have relied on landfills and incineration, which generate secondary contamination problems due to leaching of pesticides into the surrounding soil and groundwater supplies and production of potentially toxic by-product emissions. Moreover, incineration, although approved by US EPA, is a very costly process requiring large amounts of energy (Chen and Mulchandani, 1998). From this point of view, biodegradation is a much safer and economical process for detoxifying pesticides. Many techniques of dispersal, collection, removal, landfill disposal and incineration simply dilute or sequester the contaminants or transfer them to another environment. By contrast, bioremediation may be viewed as a more effective and environmentally benign clean-up technology, since it results in the partial or complete bioconversion of the organic pollutants, such as pesticides, to microbial biomass and stable non-toxic end-products (Baker

and Herson, 1994). It is a relatively recent addition to the battery of clean-up strategies currently employed to restore and rehabilitate contaminated sites.

Bioremediation has been defined as a "*biological response to environmental abuse*". Thus by definition, *it is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms*. Bioremediation is a combination of two words — *bio* for biological, and *remediation* which means to remediate or eliminate. Depending upon the type of organism used in the process, bioremediation may be of different types:

Phytoremediation: The use of plants to clean-up the environment.

Mycoremediation: The use of fungi for the clean-up of contaminated environment. Bioremediation is often characterized as environment-friendly and cost-effective approach for the removal of contaminants from the environment. The application of fungal technology (mycoremediation) for the clean-up of contaminated soil holds promise since 1985 when the white-rot fungus *Phanerochaete chrysosporium* was found to be able to degrade a number of important environmental pollutants (Bumpus *et al.,*1985). Since then several strains of white-rot fungi have been demonstrated to attack many organopollutants.

There are many advantages of bioremediation technology:

(a) It harnesses natural biogeochemical processes.
(b) It is a cost-effective alternative.
(c) Toxic chemicals are destroyed or removed from the environment and not merely separated.
(d) Low capital expenditure.
(e) Less energy required when compared with other technologies.
(f) Less manual supervision requirement.

TYPES OF BIOREMEDIATION TECHNOLOGIES

Bioremediation technologies have been broadly divided into two categories based on whether biodegradation is stimulated *in situ* or carried out *ex situ* in compost heaps or bioreactors. Organic pollutants can be bioremediated using either *in situ* or *ex situ* systems (Fig. 2.4).

I. *In situ* Bioremediation Techniques

In situ bioremediation techniques involve enhancement of the biodegradation rate of organic contaminants within the affected area (soil, sediment and surface water or groundwater environments). In Latin, *in situ* means "in the origin place". It is the clean-up approach that directly involves the contact between microorganisms and the dissolved and sorbed contaminants for biotransformation (Alcalde *et al*., 2006). These types of technologies are less expensive, create less dust and cause less release of volatile contaminants. Potential advantages of *in situ* bioremediation methods include minimal site disruption, simultaneous treatment of contaminated soil and groundwater, minimal exposure of public and site personnel and low cost. But these techniques have some disadvantages also such as: time consuming method as compared to other remedial methods, seasonal variation of microbial activity under the influence of changing environmental conditions and problematic application of treatment additives (nutrients, surfactants and oxygen). When native microorganisms lack biodegradable capacity, genetically engineered microorganisms (GEMs) may be added to the surface during *in situ* bioremediation, although stimulation of

for the removal of by-products of raw materials and wastes. Bacteria are the most commonly used agents in these biodegradation processes.

3. **Bio-stimulation:** Bio-stimulation involves the introduction of nutrients or substrates such as fertilizers, to stimulate the growth and metabolism of the indigenous species performing biodegradation of pollutants.
4. **Biosparging:** Biosparging involves the injection of air under pressure below the water level to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by indigenous microorganisms. Biosparging increases the mixing in the saturated zone and thereby increases the contact between the soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

II. *Ex situ* Bioremediation Techniques

Ex situ bioremediation techniques are usually aerobic and involve the treatment of contaminated soil or sediments using solid or slurry phase systems. It actually involves the removal of waste material from polluted area and their collection at a place distant from the contaminated site to facilitate microbial degradation. There are certain limitations and disadvantages of this technology besides being a costly process. Based on the physical nature of the contaminant under treatment, this technology is classified into two types: (i) Slurry phase bioremediation systems involving treatment of solid liquid suspensions in bioreactors and (ii) Solid phase bioremediation system including land farming (soil treatment units) and soil piles, compost heaps and engineered bio piles. Composting techniques are used for the remediation of highly contaminated sites and have proved successful for military sites contaminated with explosives such as TNT, RDX and Tetryl.

(a) **Slurry phase bioremediation:** Slurry phase bioremediation is a controlled batch treatment technique in which excavated soil or sediments are mixed with water and treated in bioreactor vessels or contained ponds or lagoons. Processing of the soil involves separation of stones and rubbles from the contaminated soil to provide a low viscosity. Then the soil is mixed with a predetermined amount of water to form slurry. Thus, slurry phase treatment is a triphasic system involving three major components: water, air and suspended particulate matter including the desired microorganism. The concentration of water added depends on the concentration of pollutants, the rate of biodegradation, and the physical nature of the soil (USEPA, 2006). In addition to the provision of adequate aeration and mixing, nutrients are universally added, together with surfactants or dispersants as required. Optimum pH and temperature conditions are also provided in bioreactor vessels. Effective bioremediation has been obtained with slurry phase systems for soil and sediments contaminated with a wide range of organic compounds including pesticides, petroleum hydrocarbons, pentachlorophenol, polychlorinated biphenyls, (PCBs), etc.

Biologically, there are three types of slurry phase bioreactors: *Aerated lagoons, low-shear airlift reactor* and *fluidized-bed soil reactor*. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the rate of bioremediation of soil bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants. In general, the rate and extent

of biodegradation are greater in a bioreactor system than *in situ* or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of bioreactor systems, there are some disadvantages, such as the contaminated soil requires pre-treatment (e.g., excavation) or alternatively, the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor.

(**b**) **Solid phase bioremediation:** Solid phase system includes organic wastes, manures, sewage sludge and municipal solid wastes. The traditional clean-up practices involve the informal processing of organic materials and production of compost, which may be used as soil amendment. In solid phase bioremediation, contaminated soil is excavated and placed into piles. Bacterial growth is stimulated through a network of pipes that are distributed throughout the piles. By pulling air through pipes, the necessary ventilation is provided for microbial respiration. Moisture is introduced by spraying the soil with water. Solid-phase systems require a large amount of space, and clean-ups require more time to complete than with slurry-phase processes. Some solid-phase treatment processes include land farming

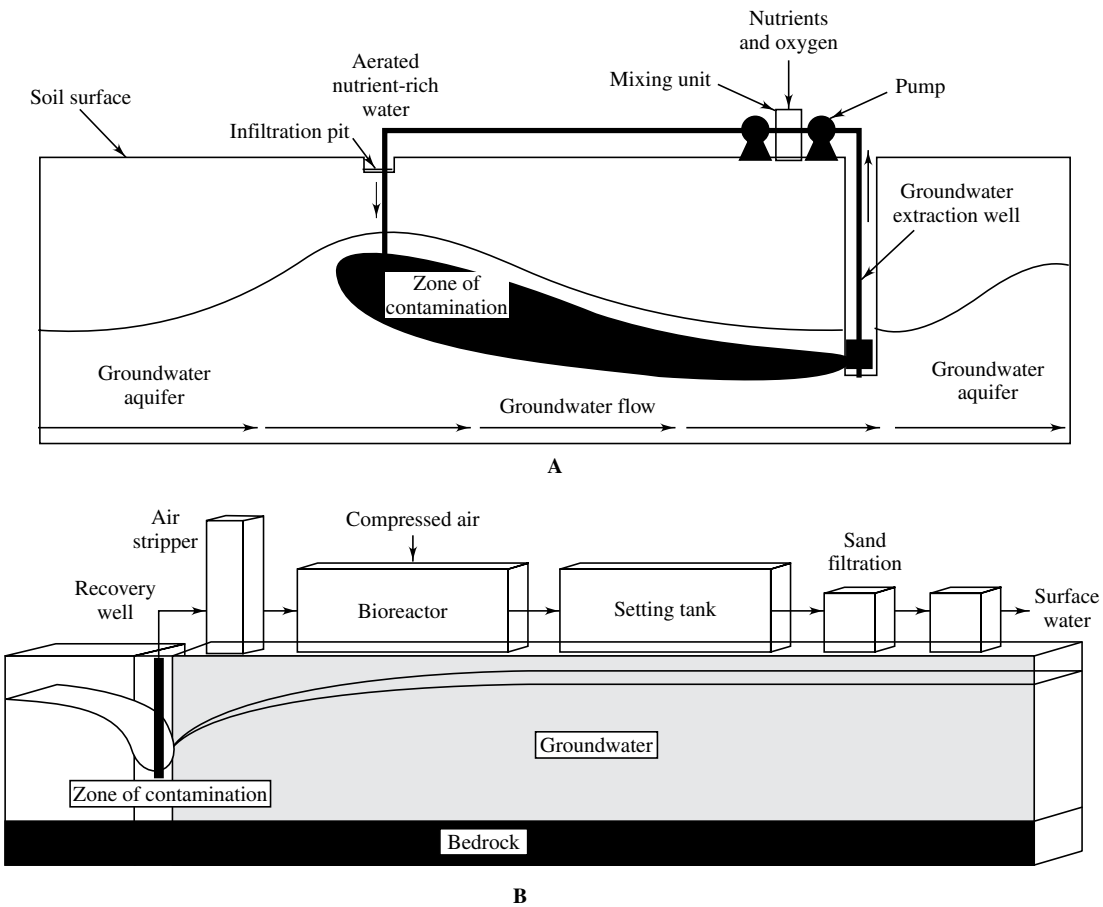


Fig. 2.4 Bioremediation of subsurface contaminated soil and groundwater by (A) *in situ* and (B) *ex situ* bioremediation technology (Source: Atlas and Bartha, 1998).

chemical groups on mineral surfaces, reactive organic compounds, and inorganic metals. The exact mechanism for microbial adaptation to pesticides is not well understood. Microorganisms may acquire genetic material to encode the biochemical mechanisms necessary to deal with a potential substrate. Microbial bioremediation can take place under *aerobic* as well as *anaerobic* conditions. In aerobic conditions, microorganisms use the available atmospheric oxygen for their metabolic functions in order to produce carbon dioxide and water through pesticide degradation. However, under anaerobic conditions, due to the absence of oxygen, microorganisms use these chemical compounds in the soil as substrate, breaking them down to obtain the energy they need. Field *et al.* (1995) reviewed the intrinsic chemical considerations that limit the biodegradability of aromatic pollutants in aerobic and anaerobic environments.

Pesticide-Bioremediation under Aerobic Conditions

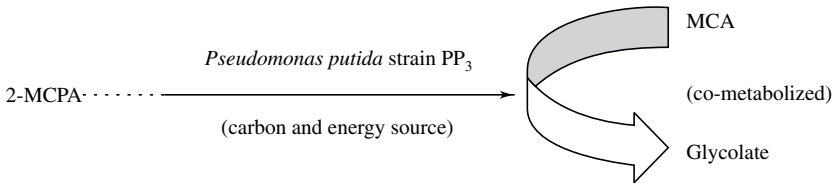
The use of *oxygenase* enzyme by aerobic species to initiate electrophilic attack on aromatic molecules is seriously hindered by the presence of numerous electron-withdrawing substituents, such as chloro, nitro and azo groups. Co-metabolism by the organisms allows the microbe to catalyze the hydrolysis reaction. An important factor in the bioremediation of pesticide contaminated soil is the availability of the contaminant to the microorganisms. This availability is a function of the affinity of adsorption of the organic compound to the soil. Moisture content also plays a significant role in microbial degradation of pesticides. Availability of the pesticide to the organism is dependent upon the solubility of the compound in water. A moisture content of 50% will give good degradation rates. The concentration of pesticide in soil also contributes to the rate of degradation. Often, when specific compounds such as pesticides are present in soil, significant populations of microbes that degrade these compounds are not present. Remediation of these soils is generally done with the aid of bio-augmentation, the development of specific seed cultures and inoculation of the soil or bioreactor. For some pesticides such as DDT, lindane and heptachlor, anaerobic degradation works better than aerobic degradation. (Fathepure *et al.*, 1995).

Pesticide-Bioremediation under Anaerobic Conditions

Under anaerobic conditions, aromatic compound degradation is usually initiated by nucleophilic attack. Consequently, presence of chloro, nitro or azo ring substituents will favour initial reductive attack on aromatic pollutant by anaerobic bacteria. On the other hand, electron donating functional groups, such as amino groups, tend to reduce the susceptibility of aromatic compounds to anaerobic transformation, while increasing their susceptibility to aerobic degradation. Furthermore, the lack of any functional group has been associated with the observed recalcitrance of hydrocarbons in anaerobic environment. There are many degradation reactions that characterize the breakdown of pesticide anaerobically, such as: (i) addition of hydroxyl group replacing the hydrogen atom, (ii) oxidation of sulphur to SO_2. This is a common reaction that results in the formation of an epoxide group. The epoxide group may be resistant to microbial degradation and toxic to the cell material of the microorganisms, (iii) addition/removal of a methyl group. It is common in the methylation of arsenic pesticides, (iv) removal of chlorine. It is common among halogenated compounds. In this process a hydrogen atom or hydroxyl group replaces the chlorine atom, (v) chlorine migration. It is the movement of chlorine from one position to another position on the ring, (vi) reduction of a nitro (NO_2^-) group to amino group (NH_3), (vii) replacement of sulphur with oxygen. It may occur when an insecticide contains a sulphur-phosphorous double bond, (viii) cleavage of an ether

Natural microbial communities are complex assemblages in which various microorganisms are highly interdependent. The range of degradative capabilities represented in a complex community of many bacteria and fungi is far greater than the capabilities of any single organism alone. Further, the product of partial degradation of a xenobiotic by one organism may serve as a substrate for another organism. The concerted action of several different organisms may lead to complete mineralization of the xenobiotic. A microbial community is also likely to be more resistant than a solitary species to a toxic product of biodegradation, because one of its members may be able to detoxify it. Thus, composition of a microbial community responds to environmental conditions coming over time to exploit the nutrients in the most effective manner. The fate of organic compounds introduced into the soil is determined by a combination of physical, chemical and biological factors. A particular molecule may be removed by volatilization, leaching, adsorption, photochemical degradation or it may undergo abiotic oxidation or hydrolysis. Finally, the molecule may undergo biodegradation through the action of microorganisms. In some instances, the products of biological and nonbiological degradation may be identical. The laboratory studies of the fate of a single organic compound do not provide clear and accurate results for the persistence of xenobiotics in the environment, because in the natural habitats microbial communities are likely to be exposed to mixtures of several organic compounds and heavy metals. Many organisms are unable to grow in the presence of heavy metals. Thus, the bacteria that are able to degrade certain persistent organic pesticides such as polycyclic aromatic hydrocarbons or chlorinated organic compounds are likely to disappear from the environment when heavy metals are also present.

In this way microbial interactions in natural microbial communities greatly influence biodegradation or biotransformation of a particular pesticide. There are three key factors influencing microbial degradation of pesticides: (i) commensalism and mutualism, (ii) cometabolism and (iii) phenomenon of gratuitous biodegradation. *Commensalism* is an interactive association between two populations of different species that live together in which one population benefits from the association and the other is not affected. *Mutualism* is a symbiosis, an interaction in which two organisms of different species live in close physical association for their mutual benefits. *Gratuitous biodegradation* occurs when an enzyme is able to transform a compound other than its natural substrate. But transformation of unnatural substrate requires its binding to the active site of the enzyme without affecting its catalytic activity. There are several bacteria and fungi, which are of diverse metabolic potential and produce several enzymes able to act on a wide range of molecules. *Cometabolism* is the ability of an organism to transform a non-growth substrate as long as the growth substrate or other transformable compound is also present. A non-growth substrate is one that cannot serve as a sole source of carbon and energy for a pure culture of bacterium, and hence cannot support cell division. Some of the pesticides may appear as recalcitrants, but can be degraded by the process of cometabolism. A number of microbial cell bound and extracellular enzymes can catalyze the breakage of bonds in herbicide molecules, e.g., cometabolism of MCA (monochloroacetate) and the herbicide MCPA (monochloropropionate). *Pseudomonas putida* strain PP3 as such cannot metabolize MCA, but while metabolizing MCPA, the organism catalyzes dehalogenation of MCA (Chatterji, 2007).



BIOCHEMICAL MECHANISMS INVOLVED IN MICROBIAL DEGRADATION OF PESTICIDES

1. Oxidative Transformations

(a) Reactions by cytochrome P450

Oxygenation is the most frequent first step in the biotransformation of pesticides and other organic xenobiotics. Many of these reactions are mediated by oxidative enzymes, e.g., cytochrome P450s. These are the most extensively studied oxidative enzymes and are the most important enzymes in Phase I pesticide metabolism (Barrett, 2000). Cytochrome P450s are hemethiolate proteins that have been characterized in animals, plants, bacteria, and filamentous fungi. Cytochrome P450s often catalyze monooxygenase reactions, usually resulting in hydroxylation. Agrochemicals can influence cytochrome P450 systems by acting as effectors, thereby modifying pesticide metabolism, or by modulating overall metabolism of an organism. These effects can increase or decrease physiological activities, which may affect the growth and development.

(b) Transformation by peroxidases, phenoloxidases, and related oxidoreductases

In addition to P450s, microorganisms produce other oxidative enzymes (e.g., peroxidase, polyphenol-oxidase, laccase, and tyrosinase) that catalyze polymerization of various anilines and phenols (Dec and Bollag, 2001). White-rot fungi (*Phanerochaete chrysosporium*) offer high potential for xenobiotic transformation because they possess lignin peroxidase and manganese dependent peroxidases that can degrade a wide range of pollutants such as polychlorinated biphenyls (PCBs) and nitroaromatic explosives.

Some pesticides like propanil (herbicide) are partially biodegraded to form azo-compounds, which are carcinogenic and thus their biodegradation may lead to another problem. In most instances, polymerization products have reduced toxicity compared with the substrate, whereas polymerization of 3, 4-dichloroaniline (metabolite of propanil) by soil microorganisms results in the formation of carcinogenic tetrachloroazobenzene (Pothuluri *et al*., 1991) (Fig. 2.5).

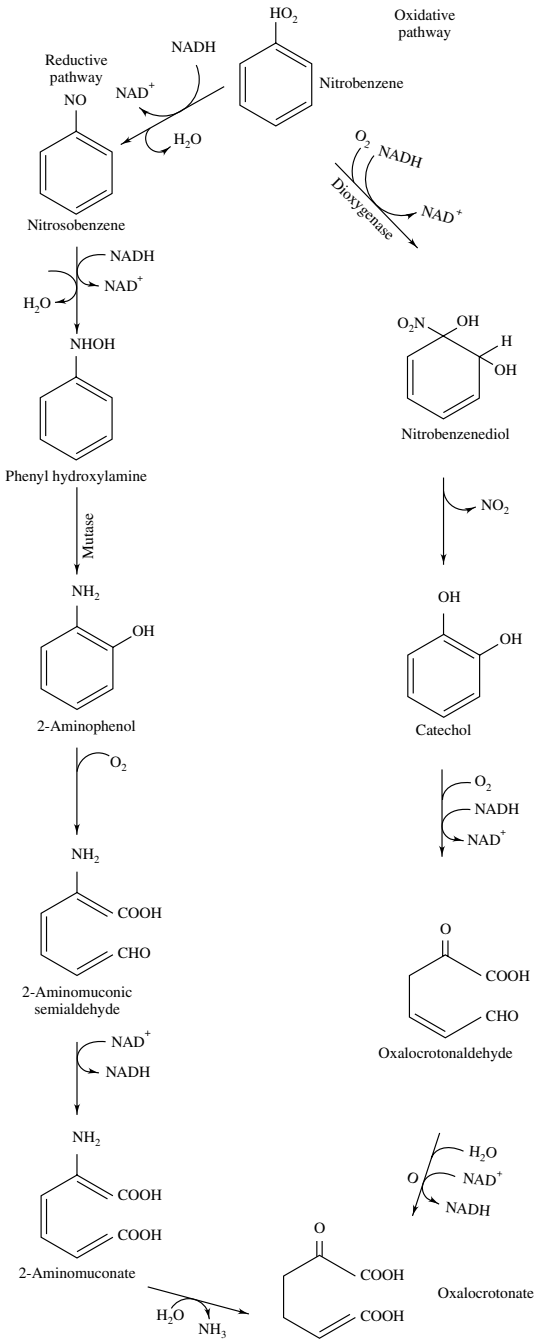


Fig. 2.6 The oxidative and reductive pathways of nitrobenzene biodegradation by *Comamonas* sp. and *Pseudomonas pseudoalcaligenes*. *(Source:* Atlas & Bartha, 1998*)*

nitro-substituted aromatics are more easily transformed under reductive than under oxidative conditions. The oxidative and reductive pathways of nitrobenzene biodegradation by *Comamonas* sp. and *Pseudomonas pseudoal caligenes* respectively, are shown in Fig. 2.6.

2. Hydrolytic Transformations

Hydrolytic enzymes cleave the bonds of a substrate by adding -H or -OH group from H_2O to each product. There are many hydrolytic enzymes that are capable of metabolizing a variety of substrates, particularly those containing amide, carbamate or ester functional groups. These enzymes may be compartmentalized or extracellular reactions can occur under aerobic and anaerobic conditions. Ester hydrolysis is commonly carried out by *esterase* and to a much lesser extent by *lipases* and *proteases*. Four types of esterases have been characterized in *Pseudomonas fluorescens*, each differing in protein structure, cellular localization, and substrate specificity. Atrazine was traditionally considered to be moderately persistent, but now bacteria are known that can completely mineralize atrazine. The gene regions encoding the first three enzymes of atrazine degradation have been isolated and characterized from *Pseudomonas* sp. strain ADP. This bacterium mineralizes high concentrations of atrazine under both growth and non-growth conditions, using the herbicide as the sole nitrogen source. The *atzA* gene encodes atrazine chlorohydrolase, which dechlorinates atrazine hydrolytically to the nonphytotoxic metabolite hydroxyatrazine (Fig. 2.7). The next step in the degradation pathway is hydrolytic removal of the aminoethyl group from hydroxyatrazine by *atzB* gene product, hydroxyatrazine ethyl amidohydrolase. Finally, the *atzC* gene encodes for another amidohydrolase that converts *N*-isopropylammelide to cyanuric acid. Wackett *et al.* (2002) have recently sequenced the complete catabolic plasmid pADP-1 from this strain and have identified three additional genes *atzD, atzE,* and *atzF* encoding for cyanuric acid amidohydrolase, biuret hydrolase, and

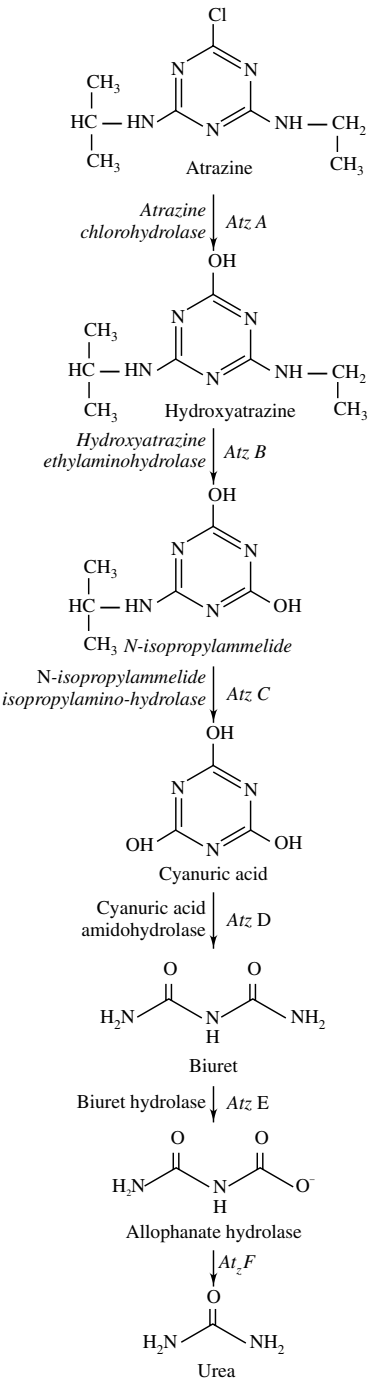


Fig. 2.7 Metabolic pathway for degradation of Atrazine by *Pseudomonas* sp. strain ADP *(Source:* Wackett *et al.*, 2002*;* Martinez *et al.,* 2001*)*.

nitroaromatic xenobiotics is mediated by *nitroreductase* enzymes found in aerobic and anaerobic bacteria, and several genera of fungi that have varying sensitivities to O_2 concentrations. There is a potential to develop transgenic crops that express a bacterial nitroreductase gene to metabolize diphenyl ether herbicides, thereby providing crop tolerance to these herbicides (Hoagland and Zablotowicz, 2001). Numerous bacteria are capable of partial nitroreduction, resulting in NH_3 release and subsequent ring cleavage.

4. Carbon-Phosphorus Bond Cleavage Reactions

Organophosphonates used as pesticides have a carbon-to-phosphorus(C–P) bond, which does not undergo photochemical, hydrolytic, thermal, or chemical degradation. However, many organophosphonate compounds do not persist in the environment because of microbial degradation. Degradation of C–P bonds has been extensively studied in bacteria. For instance, a gene cluster designated *phn,* consisting of 17 genes from *Escherichia coli*, is responsible for the degradation of a wide range of phosphonates and is likely to encode for a C–P lyase (Kim *et al.,* 1993). The enzyme(s) responsible for direct cleavage of organophosphonate C–P bonds is known by the general name *C–P lyase*.

5. Pesticide Conjugation Reactions

Pesticide conjugation has recently been defined as the "*metabolic process whereby an exogenous or endogenous natural compound is joined to a pesticide or its metabolite(s) facilitating detoxification, compartmentalization, sequestration, and/or mineralization.*" (Hall *et al.,* 2001). Conjugation of pesticides often involves utilization of the existing enzymatic machinery and is therefore called a *cometabolic process*. Uridine diphosphate-glucosyl (UDPG) transferase, an enzyme involved in cellulose biosynthesis, mediates pesticide-glucose conjugation and pesticide-glucose ester conjugation reactions. As mentioned above, glucose esters of pesticides are cleaved by esterases, often resulting in the release of the pesticide.

Microbial pesticide conjugation reactions include *xylosylation, alkylation, acylation*, and *nitrosation* and can occur intra- or extracellularly. During fungal degradation of lignin, carbohydrates are generated, but toxic phenols are also concomitantly released. These phenols are extracellularly conjugated to xylose as a detoxification mechanism. The biotransformed pesticide is released into the soil, where it is susceptible to further metabolism by bacteria. Both fungi and bacteria use methylation as a common conjugation reaction to detoxify xenobiotics. For example, formation of *O*-methylated pentachlorophenol by fungal cultures of *Trichoderma virgatum* results in a less toxic, but more recalcitrant, pentachloroanisole. *Phanerochaete chrysosporium* methylates chlorophenoxyacetic acid via a manganese-lignin peroxidase, which is an extracellular degrading enzyme system (Joshi and Gold, 1993). Phenols and anilines in soil are often acylated by fungi. For example, the herbicide metobromuron is metabolized by microbes to 4-bromoaniline, which is then acylated to form 4-bromoacetaniline (Fig. 2.9). Glutathione-*S*-transferases that function as reductive dehalogenases from *Sphingomonas* strains are involved in the dechlorination of pentachlorophenol and lindane.

6. Formation of Bound Pesticide Residues

Pesticides (mainly conjugated pesticides) are often bound to plant cell walls. Bound pesticide residues are generally considered as those that cannot be extracted with aqueous and organic

solvents, however, a more precise definition has been provided: *"a bound xenobiotic residue is a residue associated with one or more classes of endogenous macromolecules. It cannot be dissociated from the natural macromolecule using exhaustive extraction or digestion without significantly changing the nature of the associated endogenous macromolecules"* (Skidmore *et al.,* 1998). On the basis of reports in literature, it appears that xenobiotics are incorporated randomly into different cell wall components; however, little is known about the type of linkages involved in this binding. *Phanerochaete chrysosporium* has been shown to mineralize bound chloroaniline and 2, 4-dichlorophenol, indicating that these compounds may become bioavailable. However, the biological relevance of typically low concentrations of bound pesticide residues is not known. Presently, the U.S. Environmental Protection Agency requires no characterization of bound pesticide residues if concentrations are less than 0.05 ppm of the parent equivalents or 10% of the total pesticide residue. If concentrations exceed these levels, determination of the bioavailability is required.

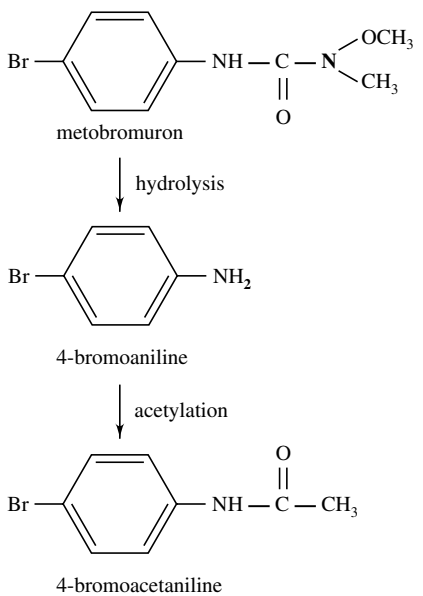


Fig. 2.9 Biotransformation of the herbicide metobromuron by microorganisms.

BIOREMEDIATION OF PESTICIDE IN THE RHIZOSPHERE

Chemicals released by plants may enhance xenobiotic degradation, and it may therefore be beneficial to use plants in the remediation of contaminated soils. There are three general mechanisms by which rhizosphere may act to enhance co-metabolism of anthropogenic contaminants:

(i) Rhizosphere may allow selective enrichment of degrader organisms that have densities too low to significantly degrade xenobiotics in root-free soil.

(ii) The rhizosphere may enhance growth-linked metabolism or stimulate microbial growth by providing a natural substrate when the concentration of xenobiotics is low or unavailable.

(iii) Rhizosphere is rich in natural compounds that may induce cometabolism of xenobiotics in certain microorganisms that carry degradative genes or plasmids. This may permit initial degradation of xenobiotics that would otherwise be unavailable as carbon sources.

inorganic solid supports by adsorption on solid surfaces such as glass, entrapment in polymeric gels, encapsulation, or intermolecular cross-linking. Although preparing supports can be time-consuming and expensive, the support can generally be reused. Enzymatic treatment holds great promise in bioremediation of contaminated soil and water. Reductive dehalogenation (RDE), is the only significant mechanism for the breakdown of halogenated aromatic, aliphatic, and heterocyclic compounds like PCBs, TCE, hexachlorobenzene, and halogenated pesticides such as heptachlor and aldrin. In theory, certain micro-sites within the rhizosphere are favourable (anaerobic conditions, low redox potentials, and available electron acceptors) for RDE, thus facilitating the transformation of halogenated compounds. Spatial and temporal heterogeneity in O_2 distribution in the rhizosphere environment usually (but not always) provides microbes with localized environments that are anaerobic and have low redox potential, thereby favouring RDE reactions (Barkovskii, 2001). Moreover, most of the terminal electron acceptors, such as nitrate, ferric iron, sulphate, and quinones are abundant in the rhizosphere. The bioavailability of hydrophobic contaminants determines the rate of xenobiotic transformation and mineralization.

CONCLUSION

Technologies using microorganisms with extensive biodegradative capacities have been developed for use in both pollution prevention and site remediation. Bioremediation, the technology employing living organisms, especially microorganisms, for the removal of pollutants from the environment is one of the several technologies that may be applied. For bioremediation to be effective, the pollutant must be subject to microbial attack or microbial transformation, the metabolic product must be safe and the process must not cause adverse ecological side effects. Environmental conditions must permit *in situ* growth of the microorganism capable of bioremediation or transformation of the pollutant so that it can be biodegraded *ex situ* in bioreactors. By selecting an effective indigenous strain or by developing a recombinant pesticide degrading microorganism together with optimized environmental conditions, bioremediation becomes a cost effective and more ecofriendly approach for restoring environmental quality. In many cases, especially for pesticides which are being used today extensively for enhancing crop productivity, bioremediation can biodegrade, detoxify or immobilize hazardous pollutants and is becoming a widely used technology for environmental clean-up. Moreover, the use of biological control agents replacing (partially or completely) synthetic chemical pesticides under integrated pest management system (IPM) appears to be more helpful in minimizing environmental pollution.

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