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A Review on Biomineralisation of Organophosphorus Pesticides by Microbes.

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ABSTRACT

Pesticides are indispensible in the field of agriculture in preventing the loss of food and grains to pest. Organophosphorus forms a major group of pesticides which are broad spectrum insecticide and are being used worldwide. They are synthetic in origin and are normally esters, amides or thiol derivatives of phosphonic, phosphorothioic, phosphoric or phosphonothioic acids. There over 100 different organo phosphorus compounds in commercial use. Their mode of action is on nervous system by inhibiting acetyl cholinesterase leading to accumulation of acetylcholine causing neurotoxicity. They have adverse effect on the soil, soil organisms, plants, animals and human beings and thus affecting the food chain. Among various methods being employed for remediation of the environment, bioremediation has well established as eco friendly, efficient and cheaper than physical or chemical methods. Exploring the role of microorganisms in the metabolism of pesticides will give us a better understanding of bioremediation of the pesticide from soil. This review deals with most frequently used organophosphorus and the microbes which are able to mineralize it efficiently.

Keywords: Pesticide Contamination, Biomineralisation, Hydrolysis, Metabolites

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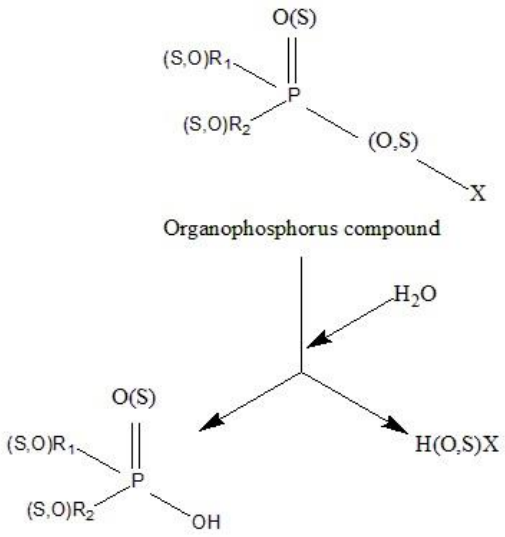
INTRODUCTION

Organophosphorus (OP) pesticides are also known as phosphate esters which are a group of pesticides used in large quantities in agriculture and health sectors. They act as acetyl cholinesterase (AchE) inhibitors, resulting in neurotoxicity and death. Medical treatment is difficult, with case fatality of 20% [1]. The organophosphorus pesticides were developed to replace organochlorine pesticides which were extensively used in 1940's (benzene hexa chloride, methoxy chlor, kepone, mirex, metalachlor, chlordane, Dichlorodiphenyltrichloroethane (DDT), etc.). Organochlorine pesticides are long persistent, accumulate in the environment, they possess the ability to drift to non-target area through runoff or groundwater or air and affect non target organisms. Hence, they were replaced by relatively less persistent and yet effective OP compounds. OP pesticides are relatively easier to degrade via microbial or environmental processes. Unlike organohalide pesticides, the OP pesticides accumulation is less pronounced due to their rapid breakdown in the environment and thus preferred over organohalides. But they are still highly neurotoxic to humans upon exposure and in some cases their degradation products are more toxic than the parent compound. OP compounds are efficiently absorbed by inhalation, ingestion, and skin penetration. They are strong inhibitors of cholinesterase enzymes that function as neurotransmitters, including acetyl cholinesterase, butyl cholinesterase, and pseudo cholinesterase. Each year poisons thousands of humans across the world. In 1995 in Tokyo subway, AumShinrikyo a religious sect, used sarin to poison people. In 2005, 15 victims were poisoned after accidentally ingesting ethion-contaminated food in a social ceremony in Magrawa, India resulting in mass poisoning. Exposure to OP has been attributed to frequent use of the pesticide in agricultural lands resulting as residues in fruits, vegetables, livestock, poultry products and municipal aquifers. For example, when sprayed on crops during the time of growing season, only a small amount of the pesticide applied reaches the target organisms, and most of the pesticide reach non target areas, the typical pesticide concentration that flow into aqueous waste range from 10,000 to 1 ppm [2,3]. Commonly used organophosphates in agriculture are parathion, methyl parathion, chlorpyrifos, mono crotophos, malathion, acephate, cyanophos, phospamidon, dichlorvos, phosmet, fenitrothion, tetra chlorvinphos, azamethiphos, profenofo sand azinphos-methyl. In order to avoid undesirable presence of pesticides in food products, it is vital to develop safe, convenient and economically feasible methods for pesticide detoxification [4]. General formula of organophosphorus compounds and major pathway of degradation is provided in Figure 1. This review deals with some of the most frequently used OP and the micro organisms which possess the potential to degrade OP.

Figure 1: General formula of organophosphorus compounds and major pathway of degradation [54].



Parathion and methyl parathion:

Parathion is also referred to as parathion-ethyl or diethyl parathion, (C10H14NO5PS), O,O-Diethyl O-(4-nitrophenyl) phosphorothioate (Figure 2a.). It is a broad spectrum organophosphate pesticide used to control numerous types of insects and mites on majority of crops [5,6].Methyl parathion (C8H10NO5PS), O,O-

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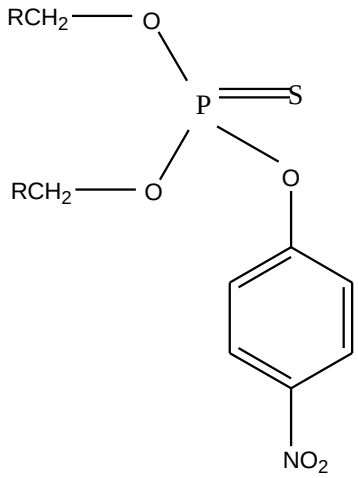
dimethyl O-(4-nitrophenyl) phosphorothioate is an insecticide and acaricide used to control boll weevils and many biting or sucking insect pests of agricultural crops. These chemicals act by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous systems of humans, animals and insects.

Figure 2a: Parathion and Methyl parathion Parathion: R=CH_3, Methyl parathion: R=H

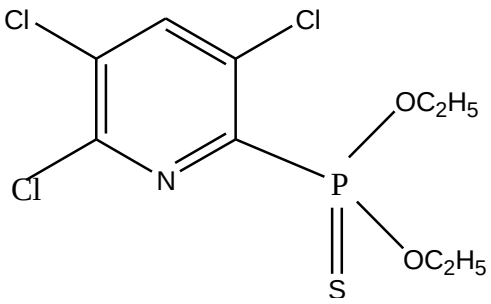


Siddramappa et al [7] isolated two bacterial strains Bacillus sp. and Pseudomonas sp., from parathion contaminated alluvial soils which were able to hydrolyze parathion effectively. Pseudomonas sp. hydrolyzed parathion and released nitrite from p-nitrophenol, Bacillus sp. could not degrade intact parathion but was capable of converting p-nitrophenol to nitrite. The study established bacterial degradation of parathion past the p-nitrophenol stage to the end product, nitrite. Choi et al [8] isolated bacterial strains which were able to utilize parathion as a sole source of carbon and energy, producing p-nitrophenol as the intermediate metabolite during the complete degradation of parathion. Analysis of 16S rRNA gene sequence indicated that the isolates were related to members of the genera, *Burkholderia*, *Arthrobacter*, *Pseudomonas*, *Variovorax*, and *Ensifer*. In another study by enrichment culture technique, a fungus *Penicilliumwaksmani* which had the efficiency to degrade parathion was isolated from an acid sulphate soil under flooded condition. The fungus tolerated parathion at concentrations as high as 1000 ppm. Initially, medium containing parathion supported less growth but at later stages the growth was equal to that of control treatment. Parathion was converted to aminoparathion by the fungus [9].

Chlorpyrifos:

Chlorpyrifos, **(**$C_9H_{11}Cl_3NO_3PS$)O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate is one of the organo phosphorus pesticide frequently used. It has the ability to suppress the acetyl cholinesterase AchE activity, exhibiting acute neurotoxicity (Figure 2b). The hydrolysis of chlorpyrifos, results in 3,5,6-trichloro-2-pyridinol (TCP) with greater water solubility than chlorpyrifos and causes widespread contamination in soils and aquatic environment. TCP is not only persistent in soil but possess antimicrobial activity which hinders biodegradation [10].

Figure 2b: Chlorpyrifos



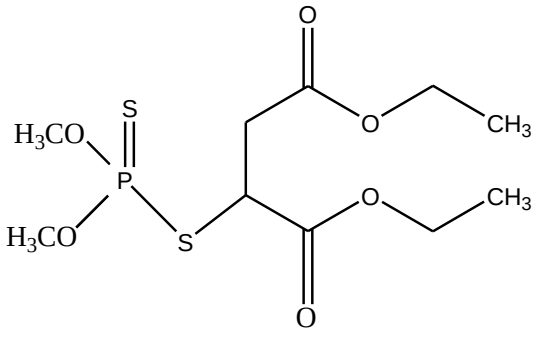
Chen et al. [11] isolated a new fungal strain Hu-01 with the capability of degrading chlorpyrifos and which was identified as *Cladosporium cladosporioides* based on 5.8S rDNA gene analysis. Strain Hu-01 had the ability to utilize 50 mg·L^{-1} of chlorpyrifos as the sole carbon source, and tolerated 500 mg $L^{-1}$of chlorpyrifos. In another study biodegradation of chlorpyrifosand its metabolite TCP was carried out with *Bacillus pumilus* C2A1. The strain C2A1 showed 90% degradation of TCP (300mg L^{-1}) within 8 days of incubation [10].In another investigation [12] the biodegradation of chlorpyrifos and TCP with bacterial consortium, which comprised of four different strains of *Pseudomonas* two isolates of *Agrobacterium* and *Bacillus* strain. The results confirmed the degradation of chlorpyrifos better than TCP.

Sasikala et al [13] conducted an investigation on chlorpyrifos degradation with nine morphologically different bacterial strains, one actinomycete and two fungal strains which were isolated from chlorpyrifos contaminated soil. Among the isolates *Pseudomonas putida* (NII 1117), *Klebsiella* sp., (NII 1118), *Pseudomonas stutzeri* (NII 1119), *Pseudomonas aeruginosa* (NII 1120) were found to degrade efficiently at neutral pH , temperature 37 °C with 500 mg L^{-1} of chlorpyrifos resulting in the presence of metabolites chlopyrifos-oxon and diethyl phosphorothioate. In another investigation *Alcaligenes* sp. JAS1 efficiently degraded 300 mg L^{-1} of chlorpyrifos within 12 h of incubation and TCP was degraded after 24 h in soil studies supplemented with nutrients and 48 h in the absence of nutrients [14]. In a similar study with *Aspergillus terreus* [15] and *Ochrobactrum* sp. JAS2 [16] the degradation pattern was found to follow a similar trend. The degradation characteristics of chlorpyrifos by a fungal strain *Verticillium* sp. DSP in pure cultures, soil, and on pakchoi (Brassica chinensisL.) was investigated resulting in efficient degradation of the pesticide [17].

Malathion:

Malathion ($C_{10}H_{19}O_6PS_2$), S-(1, 2-dicarbethoxyethyl)-O, O-dimethyl dithiophosphate, also known as carbophos, maldison and mercaptothion is a non systemic, wide-spectrum *organophosphorus* used to control the household and agricultural pests (Figure 2c). The most toxic metabolite of malathion is the oxidation product malaoxon which is formed in air possessing insecticidal activity of the parent compound. Malathion irreversibly inactivates acetylcholine esterase at various sites resulting in the accumulation of neurotransmitter acetylcholine at postsynaptic sites causing death. Malaoxon is an oxygen analogue of malathion and it can be found either as an impurity in malathion, or generated during the oxidation of malathion in air or soil [18]. Malaoxon breaks down more rapidly than malathion in alkaline and moist soil. Malathion is absorbed by practically all routes including the gastrointestinal tract, skin, mucous membranes, and lungs [19]. Toxic effects of malathion has shown to affect the central nervous system of invertebrates, immune system of higher vertebrates, reproductive functions of vertebrate, adrenal glands and tissues of fish [20].

Figure 2c: Malathion



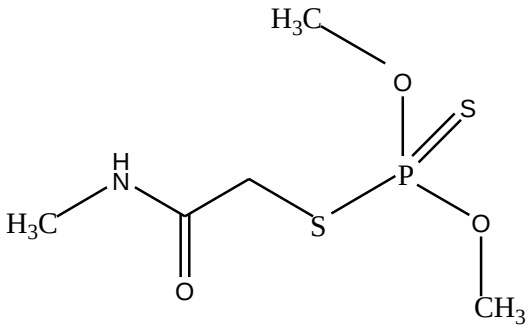
Degradation of malathion with bacterial strains, *Brevibacillus* sp. strain KB2 degraded 87.40% of malaoxon and 41.30% of malathion *Bacillus cereus* strain PU degraded 72.20% of malaoxon and 36.22% of malathion. Presence of metobolities such asmalmonocarboxylic acid and maldicarboxilic acid was confirmed by gas chromatography/mass spectrometry [21]. Malathion contaminated soil was bio remediated with *Bacillus thuringiensis* and *Bacillus cereus* by Singh et al [22]. with two systems, soil slurry system and soil box system. After four days of incubation by soil box system 65.87% of malaoxon and 30.93% of malathion was degraded and with soil slurry system 74.75% of malaoxon and 26.12% of malathion was degraded [23]. Similar works were carried out with successful degradation of malathion employing *Pseudomonas* [21]. *Bacillus*

thuringiensis from waste water in Egypt [24] *Pseudomonas* sp., *Pseudomonas putida*, *Micrococcus lylae*, *Pseudomonas aureofaciens* and *Acetobacter liquefaciens* [25]The bacterial strains were able to degrade malathion efficiently using it as a sole carbon source. *Fusarium oxysporum* was employed in degrading malathion [26,27]. There are reports on Malathion degradation by carboxyesterase enzyme and which is being detected in several fungi like *Aspergillus* sp., *Penicillum* sp., and *Rhizoctonia* sp. [28,29]. *Rhizopus oryzae* [30] Several *Aspergillus* sp. have showed immense potential in utilizing malathion as phosphorous and carbon source.

Dimethoate:

Dimethoate, ($C_5H_{12}NO_3PS_2$) O,O-dimethyl S-methyl carbamoyl methyl phosphorodithioate, is a systemic organophosphorus insecticide and acaricide (Figure 2d). Dimethoate is used extensively in many countries on a large number of crops for the control of a broad range of insects, such as aphids, red spider mites, pea midges, thrips, suckers and woolly aphids [31].Stable in aqueous media between pH 2 and 7, dimethoate is classified as a 'moderately hazardous' compound by WHO. Residues of dimethoate and its oxidized analogs have been detected in soil, fruits, vegetables and even cow's milk. Dimethoate can be decomposed in alkaline solution (half-life time (DT_{50}) is 12 d at pH 9) or at temperatures higher than 96°C [32, 33]. In the absence of biodegradation, the half-life of dimethoate in soil can be as long as 206 d at 25°C [34].

Figure 2d: Dimethoate



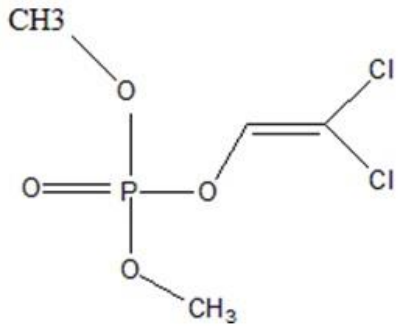
Biodegradation of dimethoate using *Paracoccus* sp. strain Lgjj-3 isolated from treatment wastewater was reportedby Lianget al. [35] Strain Lgjj-3 could utilize dimethoate as its sole carbon source for growth and degrade an initial concentration of 100 mg l^{-1} to non detectable levels within 6 h in liquid culture. During the degradation of dimethoate, seven metabolites, including dimethoate carboxylic acid, 2-(hydroxyl (methoxy) phosphorylthio) acetic acid, O,O,S-tri methyl thiophosphorothioate, O-methyl O,S-di hydrogen phosphorothioate, phosphorothioic O,O,S-acid, O,O,S-tri methyl phosphorothiate and O,O,O-trimethyl phosphoric ester, were successfully detected and identified based on GCMS analysis. Dimethoate is first hydrolyzed by the fission of the amide bond to form dimethoate carboxylic acid, which is subsequently decarboxylated into O,O,S-tri methyl thiophosphorothioate, which is further oxidized to form O,O,S-tri methyl phosphorothiate, and then to form O,O,O-trimethyl phosphoric ester. O,O,S-tri methyl phosphorothiate can also be hydrolyzed at the C-O bond to release a CH_3 group and cleaved at the S-C bond through a hydrolytic pathway to form O-methyl O,S-di hydrogen phosphorothioate. Dimethoate carboxylic acid can also be oxidated at the P=S bond to form the corresponding oxon derivative. Next, hydrolysis can occur at the C-O bond to form 2-(hydroxy (methoxy) phosphorylthio) acetic acid, which can be further hydrolyzed at the S-C bond to form O-methyl O,S-di hydrogen phosphorothioate. Phosphorothioic O,O,S-acid is finally formed by the hydrolysis of the C-O bond of O-methyl O,S-di hydrogen nphosphorothioate to lose another CH_3 group[35]. In another study *Raoultella* sp. X1, was found to degrade 75% of dimethoate[35,36].

Dichlorvos:

Dichlorvos, ($C_4H_7Cl_2O_4P$),2,2-Dichlorovinyl dimethyl phosphate is a commonly used organophosphate, abbreviated as DDVP, used against household insects, public health and stored products (Figure 2e). Dichlorvos was first synthesized in the late 1940's[37].It has been manufactured commercially and used throughout the world since 1961[38]. It was first found as a insecticidal impurity of trichlorfon, which is rapidly converted to dichlorvos at above pH 6[40].Dichlorvos is manufactured by the dehydrochlorination of trichlorfon in aqueous

alkali at 40-50 °C or by the reaction between chloral and trimethylphosphate [38]. Like all organophosphates, dichlorvos exerts its toxic effects, by inactivating the enzyme, acetylcholine esterase (AchE).

Figure 2e: Dichlorvos.

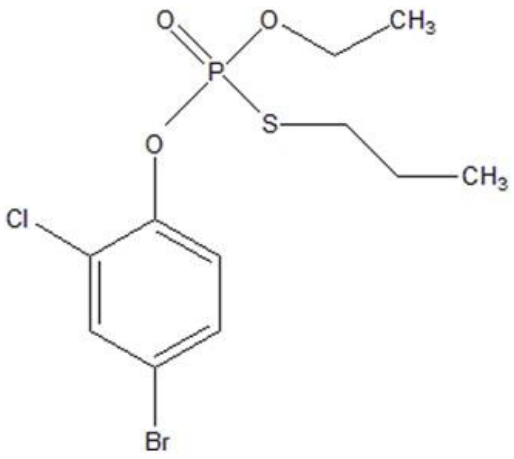


Jaya et al. [39] isolated bacterial strains *Bacillus* and *Pseudomonas* by selective enrichment technique. These isolates were tested for their ability to degrade the respective insecticides in mineral salts medium. Within 7 days of incubation, nearly 75% of chlorpyrifos and phorate and 50% of dichlorvos, methyl parathion and methomyl were degraded by cultures of soil bacteria. Jiying et al. [40] evaluated the rate of dichlorvos degradation by a natural microbial community on rape leaves and found that more dichlorvos was degraded on microbial-population-inhabited leaves than on surface-sterilized leaves. Six dichlorvos-degrading bacteria were isolated from the natural community and identified with 16S rRNA gene sequences are from the genera *Pseudomonas*, *Xanthomonas*, *Sphingomonas*, *Acidovorax*, *Agrobacterium* and *Chryseobacterium*. Upon microbial degradation dichlorvos forms desmethyldichlorvos which further breaks down into phosphate and water.

Profenofos:

Profenofos ($C_{11}H_{15}BrClO_3PS$), O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioateis one of the organophosphorothiolate insecticide used in agriculture (especially in cotton) for pest control (Figure 2f). Profenofos is extremely toxic to fishes and macro invertebrates, and its acute toxicity brings about the inhibition of the acetylcholinesterase activity, with toxic effects on humans. According to the reports from the US Environmental Protection Agency and previous research works, profenofos is a potential contaminant in a wide range of terrestrial and aquatic ecosystems, and its residues have been found in foods and vegetables [42,43].

Figure 2f: Profenofos

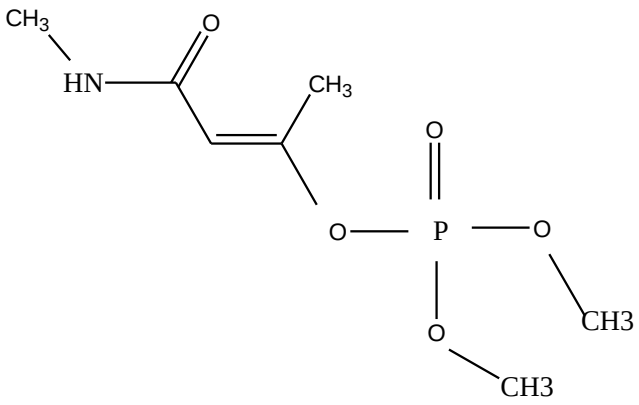


Profenofos degradation was carried out with Pseudomonas aeruginosa which was isolated from a long term profenofos exposed soil by using enrichment technique. Bioremediation of profenofos contaminated soil with 200 µg/g profenofos, resulted in a higher degradation rate than control soils. In a mineral salt medium, removal of profenofos was 86.81% within 48 h of incubation resulting in the final degradation of profenofosto 4 bromo-2-cholorophenol (BCP)[44]. In another study two pure bacterial cultures *Pseudomonas putida* and *Burkholderia gladioli* at pH 5.5-7.2 with temperature range from 28 °C to 36 °C were able to degrade 200 µg/g of profenofos[45].

Monocrotophos:

Monocrotophos, ($C_7H_{14}NO_5P$) dimethyl-(E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate, abbreviated as MCP is a non specific systemic, acaricide (Figure 2g.). MCP is generally used extensively against pests of cash crops such as tobacco, maize, cotton, sugarcane, groundnut, soybeans, rice, and vegetables and against insects, mites, ticks, spiders [46]. Bioremediation of wastewater containing MCP by Arthrobacter *Atrocyaneus*, *Bacillus megaterium*, and *Pseudomonas mendocina* was highest at pH 8.0, but maximum reduction in Chemical Oxygen Demand (COD) was at pH 7.0. Removal of MCP and reduction in COD by *B. megaterium* and *P. mendocina* were highest at 35°C, while with *A. atrocyaneus*, which was maximum at 30°C, under aerated culture condition and inoculum density of 108 cells/ml [47]. Monocrotophos is characterized by a P-O-C linkage and amide bond, and has been reported to be degraded as a sole carbon or phosphorus source in liquid media by *Pseudomonas aeruginosa* sp., *Clavibacter Michiganense* sp. [48]*Arthrobacter Atrocyaneus* sp., *Bacillus megaterium* sp. and *Pseudomonas mendocina* [47]. Similar works were conducted by Gajendiran and Abraham [49] using *Aspergillus oryzae* SJA1 isolated from Oryza sativa field with efficient degradation of MCP and in another investigation *Achromobacter* strain JAS10 followed similar strategy in degrading MCP[50]. Metabolic reactions, such as N-demethylation, O-demethylation, hydroxylation of N-methyl groups and cleavage of the phosphate-crotanamide linkage, occur during the metabolism of monocrotophos by microbial cultures in soil [51]. The formation of O-desmethylmonocrotophosmonomethyl phosphate, dimethyl phosphate, N-methylacetoacetamide and N-methylbutyramide has also been observed. *Aspergillus* sp. and *Pencillium* sp. was successfully in degrading 75% and 50% of 200 mg l^{-1} MCP in a period of 4 d [52,53].

Figure 2g: Monocrotophos



CONCLUSIONS

Pesticides cause adverse effects and are persistent in the environment, they are indispensible and the only way to safely alleviate the problems caused by pesticides is to detoxify their effects through bioremediation. Contrastingly, microbes are the major agents which can decrease the effects caused by pesticides considerably. Microbes posses the ability to degrade pesticides to nontoxic compounds. The isolation and evaluation of potential pesticide degrading microbial genes encoding the enzymes for degradation will yield unique insights into the molecular organization and sequence of events followed in degradation. The information can improve understanding the microbial diversity in bioremediation.

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