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RESEARCH ARTICLE

REVIEW ARTICLE: CARBOFURAN-PERSISTENCE IN SOIL AND MICROBIAL DEGRADATION

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ABSTRACT

The use of pesticides in agricultural soil is very critical in protecting the crops of farmers' investment. The pesticide provides sure cover from the damage of the pests and insects. But the excessive use of the pesticides is also harmful for the environment due to its long term persistency in soil and slow or less degradation. This review is focused on the persistency of carbofuran in soil and the degradation of carbofuran-7-phenol by the action various micro organism on it, which are naturally present in the soil. Various fungi and bacterial species are found in soil which has a powerful impact on the degradation of carbofuran at ring structure. Such microbial strain degrades carbofuran by the hydrolysis at the furanyl ring.

Key words: Carbofuran, Bioremediation, 2-hydroxy-3-(3-methylpropan-2-ol)-phenol, Hydrolases, Phosphotriesterases.

INTRODUCTION

Agriculture has been the backbone of the Indian economy and it will continue to remain so for a long time. It has to support almost 17 per cent of world population from 2.3 per cent of world geographical area and 4.2 per cent of world's water resources. The economic reforms, initiated in the country during the early 1990s, have put the economy on a higher growth trajectory. Annual growth rate in GDP has accelerated from below 6 percent during the initial years of reforms to more than 8 percent in recent years. This happened mainly due to rapid growth in non-agriculture sector. The workforce engaged in agriculture between 1980-81 and 2006-07 witnessed a very small decline; from 60.5 percent to 52 percent (Pandey, 2009). The present cropping intensity of 137 per cent has registered an increase of only 26 per cent since 1950-51. The net sown area is 142 Mha. The net irrigated area was 58.87 Mha in 2004-05. Presently, the total net irrigated area covers 45.5 per cent of the net sown area, the remaining 54.5 per cent is rainfed. The degradation of land and surface as well as ground water resources results in fast deterioration of soil health. Losses due to biotic (insect-pests, diseases, weeds) and abiotic (drought, salinity, heat, cold, etc.) stresses account for about one-fourth of the value of agricultural produce (Pandey, 2009). The storage, transportation, processing, value addition and marketing of farm produce need to be improved to enhance household food, nutrition and livelihood security Indian agriculture is characterized by agro-ecological diversities in soil, rainfall, temperature, and cropping system. Besides favorable solar energy, the country receives about 3 trillion m^3, of rainwater, 14 major, 44 medium and 55 minor rivers share about 83 per cent of the drainage basin. About 210 billion m^3 water is estimated to be available as ground water. Irrigation water is becoming a scarce commodity. Thus proper harvesting and efficient utilization of water is of great importance (Pandey, 2009).

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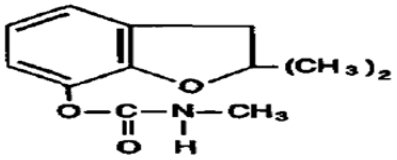
Environmental Impact of Modern Agriculture

Xenobiotic compounds are widely distributed in the environment as a result of their widespread use as pesticides, solvents, fire retardants, pharmaceuticals, and lubricants. Several of these chemicals cause considerable environmental pollution and human health problems due to their persistence and toxicity (Rossberg, 1986). Agricultural modernization due to increased market demand for agricultural products has greatly facilitated the industrial production and use of pesticides for pest management and vector control thus the diverse environmental contamination with pesticides (Ngowi, 2007). Carbamates, synthetic organic chemicals, are highly poisonous pesticides that have found wide usage in agricultural farms as insecticides, fungicides, herbicides, nematicide and acaricides (WHO, 1986). The non-target toxicity of carbamates extends from human beings to both aquatic and terrestrial organisms with high sensitivity in fish and earthworms (WHO, 1986) . Some of the carbamates used in horticultural farming include carbofuran (in form of Furadan), carbaryl, aldicarb and methomyl (Gibson, 2002). Many researchers have reported the biodegradation of various pesticides under different physiological conditions and isolated many bacterial species. It has thus become increasingly possible to isolate microorganisms that are capable of degrading xenobiotic and recalcitrant compounds from environments polluted with toxic chemicals (Gibson, 2002). This study focused on the isolation and characterization of methomyl and carbofuran degrading bacterial strains in selected agricultural soils (Kevin, 2012).

Carbofuran

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, molecular weight of 221.25 and a melting point of 150-152°C) and also known as Furadan, is a broad spectrum, systemic insecticide, acaricide and nematicide, currently registered for use on agricultural crops such as alfalfa (*Medicago sativa*), peanuts (*Arachis hypogaea*), rice (*Oryza sativa*), sugar cane (*Saccharum officinarum*), and especially corn (*Zea mays*) It is comparatively stable under neutral or acidic conditions, but degrades rapidly in alkaline media . This white, crystalline, solid of empirical formula $C_{12}H_{l5}NO_3$ is soluble at concentrations up to 700 ppm (mg/L) in water, but at < 30 ppm in various organic solvents. It degrades at >130° and supports combustion. It is used in a wide variety of crops against a large number of target species (Eisler, 1985).



Structure of Carbofuran

As a member of the carbamate class of pesticides, the action of carbofuran is largely based on its ability to inhibit acetylcholinesterase (AChE) in the nervous system and motor end plates of the target species. Carbofuran's toxicity in mammalian systems is also based on this property, though other mechanisms of toxicity may be operative. Carbofuran inhibits other cholinesterases (ChEs) besides nerve-localized AChE, including the plasma-localized butyryl ChE and the red blood cell-localized AchE (Rubin, 2006).

Persistence in Soil

Persistence of carbofuran in soils is a function of many factors, including pesticide formulation, rate and method of application, soil type, pH, rainfall, temperature, moisture content, and microbial populations (Ahmad, 1979; Deuel, 1979; Eisler, 1985; Fuhremann, 1980; Gorder, 1982). Soil pH is one of the more extensively documented variables affecting degradation; it may become increasingly important as acidic precipitation (acid rain) increases. Carbofuran decomposes rapidly at pH levels >7.0, but becomes increasingly stable as pH decreases. The hydrolysis half-life is about 16 years at a soil pH of 5.5; the half-lives are about 35, 6, and 0.25 days at pH levels of 7.0, 8.0, and 9.0, respectively (Fuhremann, 1980). Temperature and moisture content of soils were both positively related to degradation of carbofuran to 3-hydroxycarbofuran, 3-ketocarbofuran, carbofuran phenol, and 3-ketocarbofuran phenol (Eisler, 1985).

Pharmacokinetics

Hydroxylation (oxidation) and hydrolysis, along with polar conjugations, comprise the major metabolic transformations of carbofuran, creating esters or ester cleavage products. In the rat, data using carbonyl-14C-carbofuran indicate rapid absorption by the oral route, followed by carbamate hydrolysis and excretion, either through the lungs ($14CO_2$) or through the urine and feces. The data using ring-14C-carbofuran indicate rapid excretion, predominantly in urine. One bile cannulation study demonstrated carbofuran entry into the enterohepatic circulation. In this manner, appreciable cholinesterase inhibiting activity is maintained in the blood after the disappearance of the parent molecule (Rubin, 2006). In the most informative study to date, single doses of carbonyl-14C-carbofuran (0.4 mg/kg) or ring-14C-carbofuran (4 mg/kg) were administered orally to rats. By 24 hours, 43.4% of the administered carbonyl-14C-carbofuran dose had appeared as $14CO_2$, suggesting that hydrolysis of the carbamate ester bond was relatively rapid. At 32 hours, this proportion was 44.6%, remaining stable at that level through 120 hours. Urine accounted for 36.8% and 38.4% of the dose at 24 and 32 hours, respectively, while 1.9% and 2.4% of the dose appeared in feces. Thus by 24 hours, 82.1% of the administered carbonyl-14C-carbofuran had been excreted. By 32 hours excretion had risen to 85.6% and by 120 hours to 87.4%. The urinary and expired air data indicated that oral carbofuran was rapidly absorbed (Rubin, 2006).

Hazard identification

Acute toxicity, oral: The acute toxicity of carbofuran is thought to result largely from its ability to carbamylate, and thus inhibit, acetyl cholinesterase (AChE) at synapses and neuromuscular junctions. Consequent local accumulations of acetylcholine (ACh) generate a plethora of cholinergic signs and symptoms. Due to the reversibility of the carbamate-AChE bond, recovery is expected when exposures are low (Rubin, 2017).

Acute toxicity, inhalation: There was insufficient observational detail from the acute inhalation toxicity studies to establish a critical inhalation no-observed-effect-level (NOEL). Acute inhalation risk was thus gauged by the critical oral LED05 of 0.01 mg/kg[7].

Acute toxicity, dermal: One human dermal toxicity study was reviewed for this document. It was found to be inadequate for risk assessment purposes. Nonetheless, evidence from that study indicated that humans may be substantially more sensitive by the dermal route than rabbits, for which an acceptable 21-day repeat-dose dermal study was available. On this basis it was decided that the potential for dermal toxicity should be evaluated using the healthprotective rat critical oral LED05 of 0.01 mg/kg, which was lower than the lowest dose at which RBC cholinesterase inhibition was noted in the human study (0.5 mg/kg) or at which overt toxicity was noted (4 mg/kg)[7].

Oncogenicity: There was no indication from the chronic toxicity studies in laboratory animals that carbofuran induced tumors. However, one prospective epidemiologic study indicated a positive correlation between reported carbofuran exposure and incidence of lung cancer in pesticide applicators from Iowa and North Carolina (RR.3). Possible confounding factors and conflicting results using different referent populations suggested that caution be exercised in the interpretation of this study. It should nonetheless be recalled that, in view of evidence that *N*nitrosocarbamates are oncogenic, formation of *N*-nitrosocarbofuran has been demonstrated in the guinea pig stomach (Rubin, 2006).

Genotoxicity: *In vivo* tests indicated that carbofuran induced chromosome abnormalities and micronucleus formation in mice. Furthermore, sperm abnormalities were induced in mice upon intraperitoneal injection. The latter observation was

consistent with similar observations in the male reproductive systems of several species subjected to chronic, reproductive, and/or developmental toxicity tests. Data from four additional studies indicated that the *N*-nitroso derivatives of carbofuran were genotoxic in several *in vitro* tests (Rubin, 2006).

Neurotoxicity: To the extent that many of the acute oral and dermal effects noted above may be driven by inhibition of brain or peripheral neural AChEs, they are classifiable as neurotoxic. A rat 13-week neurotoxicity study provided evidence for gait impairments and reduced limb grip strength after dietary exposure to carbofuran. A separate rat developmental neurotoxicity study produced possibly CNS-related neural disruptions in pups exposed during gestation, though the doses were higher than those eliciting frank cholinergic signs or ChE inhibition in other studies (Rubin, 2006).

Bioremediation of Carbofuran

Bioremediation can be defined as any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Bioremediation may be employed to attack specific soil contaminants, such as degradation of chlorinated hydrocarbons by bacteria[12].Biotransformation of various pollutants is a sustainable way to clean up contaminated environments. These bioremediation and biotransformation methods harness the naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons, polychlorinated biphenyls, polyaromatic hydrocarbons and metals. Microbial degradation is an important step in the disappearance and, in most cases detoxification of pesticides (Racke, 1988). Degradation of pesticides is the breaking down of toxic chemicals into nontoxic compounds and, in some cases, back into their original elements.

There are some strictly chemical reactions which take place in soil that aid in degradation, however, the most common type of degradation occurs through the activity of microorganisms, especially the fungi and bacteria (Sukop, 1996). There is nothing mysterious about microbial degradation of pesticides. Microorganisms simply supply a medium and an energy source for rather simple chemical reactions to take place (Lal, 1982). They in return obtain food, essential elements, or energy to carry on their life functions (Khawaja, 2012). The pesticide or insecticide does not readily disappear from the environment; soil microorganism may be responsible for the removal of insecticide. Several factors are involved in insecticide degradation like transformation, photochemical mechanism, physical mechanism, chemical mechanism, microbial degradation and bioremediation. Bioremediation can be defined as any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Bioremediation may be employed to attack specific soil contaminants. Clean up Biotransformation of various pollutants is a sustainable way to contaminated environments. These bioremediation and biotransformation methods harness the naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons, polychlorinated polyaromatic hydrocarbons and metals (Kanne, 2016).

Microorganisms involved in the biodegradation of pesticides

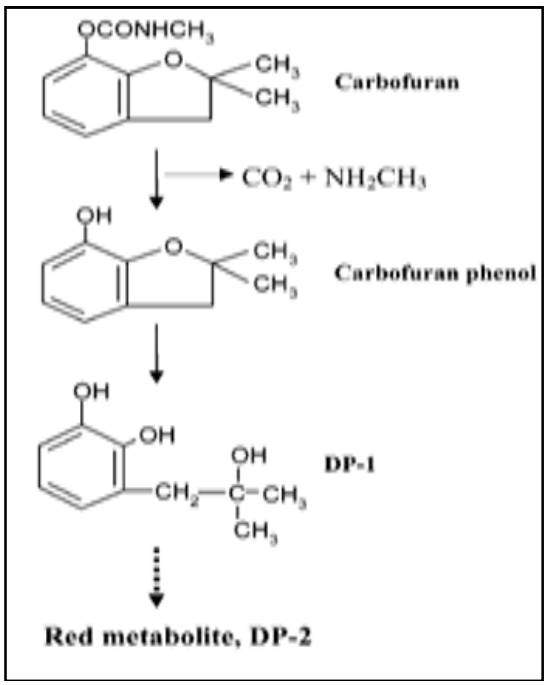
Fungal biodegradation of Carbofuran

The pesticide or insecticide does not readily disappear from the environment; soil microorganism may be responsible for the removal of insecticide. The isolated organisms can flourish in the carbofuran pesticide using farms by utilizing them as their source of energy when others source are limited or unavailable. Kanne et al isolated carbofuran degrading fungi from hill soils and different areas in Tamil Nadu . From the study it has been confirmed that the organisms are capable of degrading the pesticide. In this analysis, used the several species of fungi, used the identified species called *Aspergillus spp, Penicillium* spp and *Trichotherium spp*. With help fungi, soil fertility can be increased and will remove the pesticide contamination. Using HPLC, the ability of degradation capability of fungi was monitored and it can able to degrade carbofuran pesticide (Kanne, 2016).

Bacterial biodegradation of Carbofuran

A number of bacteria capable of degrading carbofuran, including strains such as *Pseudomonas*, *Flavobacterium*, *Achromobacterium*, *Sphingomonas*, and *Arthrobacter*, have been isolated and characterized in an effort to better understand the bacterial role to remove carbofuran from the environment (Chaudhry, 1988; Desaint, 2000; Karns; 1986; Parekh, 1995; Ramanand, 1991; Tomasek, 1989). Karpouzas *et al.,* isolated and characterized 23 carbofuran-degrading bacteria from two geographically distant soils from the Soakwaters Field. These isolates were classified on the basis of Restriction Fragment Length Polymorphism (RFLP) patterns of the 16S rRNA gene and partial 16S rRNA sequence analysis. Nine of them showed high similarity to *Pseudomonas* strains, seven showed similarity to the *Flexibacter/ Cytophaga/Bacteroides* group (Karpouzas, 2000). Onunga *et al.,* isolated two carbofuran degrading bacterial strains from the rice field with a history of carbofuran use and identified the isolated by 16SrRNA sequencing as *Bacillus cereus, Bacillus thurigiensis.* The degradation of carbofuran was followed by measuring its residues in liquid cultures using high performance liquid chromatography (HPLC) (Onunga, 2015).

An initial step for the bacterial degradation of carbofuran has been generally shown to occur via hydrolysis at the moiety of *N*-methylcarbamate linkage, giving carbofuran-7-phenol (2,3-dihydro-2,2-dimethyl-7-benzofuranol) and methylamine as the resultant degradation products, which has been well-documented in bacteria capable of growth on carbofuran as a sole source of carbon and nitrogen(Topp, 1993). Many studies reported involvement of plasmids in mineralization of carbofuran, focusing mostly on only its methylcarbamate moiety (Feng, 1997). A preliminary study on plasmid-mediated catabolism of carbofuran at ring structure was first reported by Head *et al* (Head, 1992). A more detailed characterization of plasmids encoding mineralization of carbofuran at the ring structure has been described in *Sphingomonas* sp. strain CF06 (Kim, 2004). Bacteria that hydrolyze Carbofuran, have shown complete degradation of the aromatic ring structure Feng *et al* reported that strain CF06 of the genus *Sphingomona*s is capable of completely mineralizing the aromatic ring of Carbofuran without detection of the metabolites involved.



Proposed Degradation Pathway of Carbofuran by *Sphingomonas* sp. strain SB5 (Kim, 2004)

Kim *et al* identified strain SB5a of *Sphingomonas* sp., isolated from soil with a five year Carbofuran application history, which showed activity on the aromatic ring by hydrolysis of Carbofuran 7-phenol, producing an intermediary metabolite called 2-hydroxy-3-(3-methylpropan-2-ol)-phenol, which is then converted into red metabolites (Kim, 2004). Subsequently, Park *et al* characterized, by mass spectrometry and nuclear magnetic resonance analysis (NMR), one of these red metabolites as 5-(2-hydroxy-2-methylpropyl)- 2,2-dimethyl-2,3-dihydro-naphtho [2,3-6] furan-4,6,7,9-tetrone, further suggesting that this red metabolite comes from the condensation of some metabolites of the degradation of 2-hydroxy- 3-(3-methylpropan-2-ol)-phenol (Park, 2006). On the other hand, Castellanos *et al* isolated and characterized strain FND-3 of *Novosphingobium* sp., isolated from a waste water treatment system in a manufacturing company of pesticides in China, finding that this bacteria, in addition to degrading the Carbofuran aromatic ring with the hydrolytic pathway and producing the metabolite 2-hydroxy-3-(3-methylpropan-2-ol) phenol, had the ability to hydrolyze the ether bond of the Carbofuran furanyl ring, producing the metabolite 2-hydroxy-3-(3- methylpropan-2-ol) benzene-N-methyl carbamate and Carbofuran, generating the metabolite 5-hydroxy Carbofuran (Castellanos, 2013).

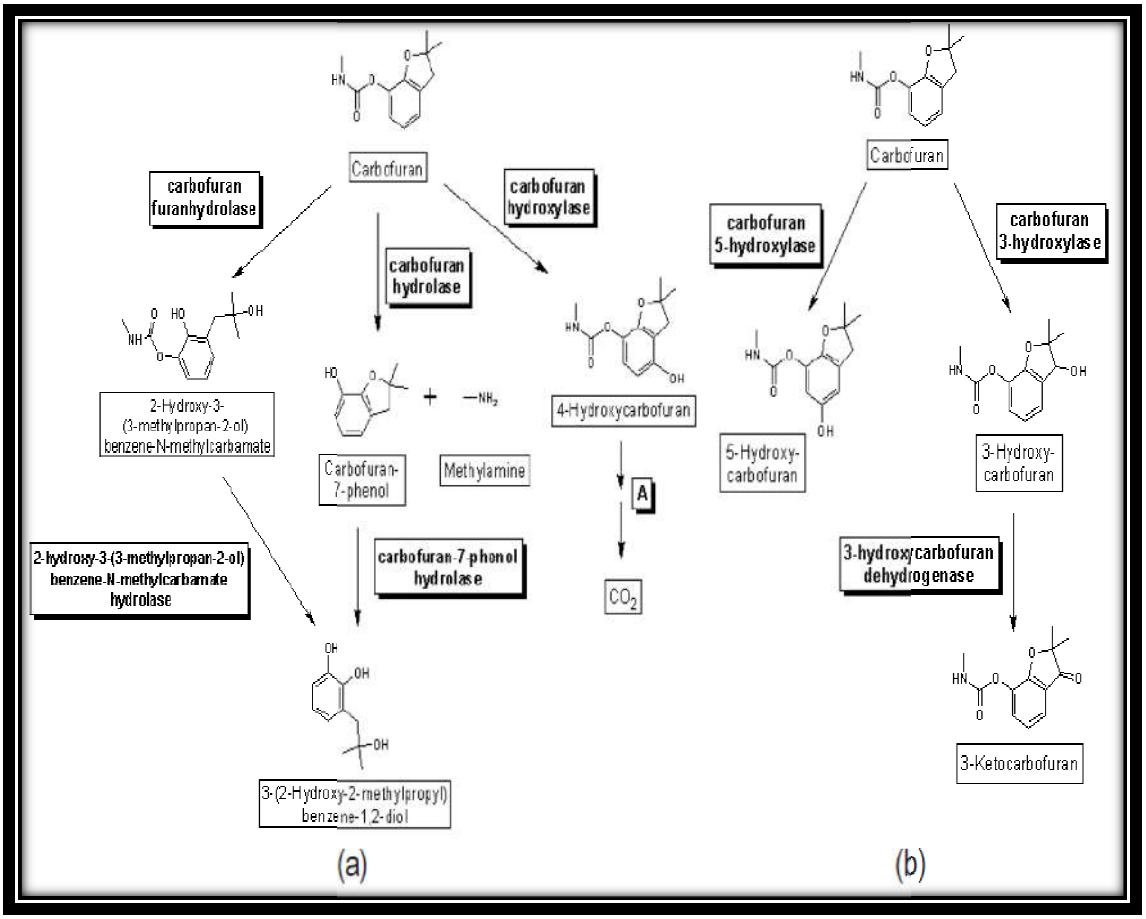
Mechanism of Carbofuran Biodegradation

Biodegradation that involves the capabilities of microorganisms in the removal of pollutants is the most promising, relatively efficient and cost-effective technology. Biodegradation is a process that involves the complete rupture of an organic compound in its inorganic constituents. The microbial transformation may be driven by energy needs, or a need to detoxify the pollutants, or may be fortuitous in nature (cometabolism) (Paul, 2005). Fungi and bacteria are considered as the extracellular enzyme-producing microorganisms for excellence. White rot fungi have been proposed as promising bioremediation agents, especially for compounds not readily degraded by bacteria. This ability arises from the production of extracellular enzymes that act on a broad array of organic compounds. Several bacterial that degrade pesticide have been isolated and the list is expanding rapidly (Bass, 2011). The three main enzyme families implicated in degradation are esterases, glutathione S-transferases (GSTs) and cytochrome P450 (Bass, 2011). Enzymes are central to the biology of many pesticides (Riya, 2012). Applying enzymes to transform or degrade pesticides is an innovative treatment technique for removal of these chemicals from polluted environments. Enzyme-catalyzed degradation of a pesticide may be more effective than existing chemical methods. Enzymes are central to the mode of action of many pesticides: some pesticides are activated *in situ* by enzymatic action, and many pesticides function by targeting particular enzymes with essential physiological roles.

Enzymes are also involved in the degradation of pesticide compounds, both in the target organism, through intrinsic detoxification mechanisms and evolved metabolic resistance (Scott, 2008). For pesticides degradation, three are mainly enzyme systems involved: hydrolases, esterases (also hydrolases), the mixed function oxidases (MFO), these systems in the first metabolism stage, and the glutathione S-transferases (GST) system, in the second phase (Li, 2007). Several enzymes catalyze metabolic reactions including hydrolysis, oxidation, addition of an oxygen to a double bound, oxidation of an amino group (NH_2) to a nitro group, addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2) to an amino group, replacement of a sulfur with an oxygen, metabolism of side chains, ring cleavage. The process of biodegradation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant molecule, which is dependent on both accessibility and bioavailability (Ramakrishnan, 2011). Metabolism of pesticides may involve a three-phase process. In Phase I metabolism, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar or amino acid, which increases the water solubility and reduces toxicity compared with the parent pesticide. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also non-toxic. In these processes fungi and bacteria are involved producing intracellular or extra cellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc (Van Eerd, 2003).

Hydrolases

Hydrolases are a broad group of enzymes involved in pesticide biodegradation. Hydrolases catalyze the hydrolysis of several major biochemical classes of pesticide (esters, peptide bonds, carbon-halide bonds, ureas, thioesters, etc.) and generally operate in the absence of redox cofactors, making them ideal candidates for all of the current bioremediation strategies[36] As an example of the catalytic activity of enzymes hydrolases, the degradation pathway of carbofuran, a pesticide the group of carbamates is presented. This pesticide can be transformed in the environment and different metabolites are generated and accumulated in potentially contaminated sites (soil, water and sediments, mainly). Different organisms isolated from contaminated sites that have been identified and characterized as transformers of carbofuran, resulting in different metabolites (Yan, 2007).



Pathway for Degradation of Carbofuran

Phosphotriesterases (PTEs)

Among the most studied pesticide degrading enzymes, the PTEs are one of the most important groups (Chino-Flores, 2012). These enzymes have been isolated from different microorganisms that hydrolyze and detoxify organophosphate pesticides (OPs). This reduces OP toxicity by decreasing the ability of OPs to inactivate AchE (Singh, 2006; Ghanem, 2005; Shen, 2010). The first isolated phosphotriesterase belongs to the *Pseudomonas diminuta* MG species. This enzyme shows a highly catalytic activity towards organophosphate pesticides. The phosphotriesterases are encoded by a gene called *opd* (organophosphate-degrading). *Flavobacterium* ATCC 27551 presents the *opd* gene encoding to a PTE[44].The gene was cloned and sequenced by Mulbry *et al* (Mulbry, 1989). These enzymes specifically hydrolyze phosphoester bonds, such as P–O, P–F, P–NC, and P–S, and the hydrolysis mechanism involves a water molecule at the phosphorus center (Ortiz-Hernández, 2003). Different microbial enzymes with the capacity to hydrolyze MP have been identified, such as organophosphorus hydrolase (OPH; encoded by the *opd* gene), methyl-parathion hydrolase (MPH; encoded by the *mpd* gene), and hydrolysis of coroxon (HOCA; encoded by the *hocA* gene), which were isolated from *Flavobacterium* sp. (Sethunathan, 1973), *Plesimonas* sp. strain M6[48] and *Pseudomonas moteilli (*Horne. 2002*)* respectively. The phosphotriesterase enzyme is a homo-dimeric protein with a monomeric molecular weight of 36 Kda. As a first step in the PTE organophosphorous pesticide hydrolysis mechanism, the enzymatic active site removes a proton from water, activating this molecule, them, the activated water directly attacks the central phosphorus of the pesticide molecule producing an inversion in its configuration The oxygen is polarized by the active site, with the participation of a zinc atom (Kapoor, 2011). This enzyme has potential use for the cleaning of organophosphorus pesticides contaminated environments (Ortiz-Hernández, 2003).

Conclusion and Future Perspectives

The indiscriminate use of pesticides, especially carbofuran has a problematic impact on the environment. Bioremediation is emerging beneficial phenomenon for the degradation of harmful pesticides in the soil. The isolation of carbofuran degrading bacteria from the soils with a history of carbofuran application indicates the presence of pesticide-degrading bacteria in the soil. Several Bacterial and fungal species having potential to degrade carbofuran have been identified by several researchers. These species of bacterial and fungal strains are enabling us to propose a tentative pathway for carbofuran degradation. In order to fully understand carbofuran degradation in these soils, a complete elucidation of degradation mechanisms involved should be carried out. This study is the report about the metabolism of carbofuran degradation pathway by micro organisms and by the enzymatic degradation. In future prospects the identified native bacterial strain can be developed and used for bioremediation of carbofuran-contaminated soil to protect its quality and fertility.

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