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Review

Microbial degradation of pesticide: A review

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Excessive use of pesticides has been known to be hazardous to the environment, affect soil fertility as well may impart toxicity in living beings. Presently there have been physical, chemical, biological and enzymatic approaches implicated to reduce pesticides. Although aimed to eradicate, physical and chemical methods are inefficient. Curiously, microbial pesticide remediation has been cost effective and thermodynamically more affordable, which may use any physical mater soiled with pesticide. Under favourable conditions microbes have been reported to use pesticides as source of carbon, sulphur and electron donor. Microbes; bacteria, actinomycetes and fungi have been found to help remove or detoxify chlorinated pesticides; polychlorinated diphenyl, polycyclic aromatic hydrocarbons, organophosphorus. Major bacterial genera includes; *Bacillus, Pseudomonas, Flavobacterium, Moraxalla, Acinetobacter, Arthrobacter, Paracoccus, Aerobacter, Alkaligens, Burkholderia* and *Sphingomonas*. Fungi with pesticide degradation potential includes;*Fusarium, Aspergilus niger, Penicillium, Lentinulaedodes, Lecanicillium, Oxysporum*. Among the Actinomycetes the*Streptomycetes* have been found to successfully detoxify pesticides. Persistent organic pollutants in the form of pesticides have also been reported to be taken care by the microbial enzymes *viz-a-viz*; dehydrogenase, ligninase, oxygenase, peroxidises, phosphotriesterase, hydrolases, dehalogenase, laccase and organophosphorus acid anhydrolase. Microbial strategies and tools; enzymes and genes involved in pesticide catabolism are reviewed.

Key words: Chlorinated pesticides, organophosphors pesticides, bacteria, fungi, enzyme.

INTRODUCTION

Pesticides are organic chemical purposely presumably intended for increasing agricultural yield, soil productivity, products quality, minimizing losses of agricultural products caused by crop peet and to control the insect vectors for prevention of the outbreak of human and animal epidemics. Increased use of the pesticide and herbicides in agriculture has also been implemented for food storage. Recently, over 500 compounds are registered and used worldwide as pesticides or metabolites of pesticides. After World War II the use of pesticide in agriculture field has progressively increased leading to increased world food production. Amongst the South Asian countries of the total pesticide consumption, India is largest of pesticide consumer country which

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volatilization, but not the destruction of organic compounds. The formed organic compounds in the contaminated gas stream are then treated by either passing through an after burner where the contaminants are completely destroyed or condensed. This process would convert the gas into a liquid phase for further treatment or they are captured by carbon adsorption beds which make their immobilization, but do not destroy the contaminants.

Incineration

Another proven and most frequently used technology to remediate pesticide contaminated sites is incineration. Unlike desorption approach incineration causes complete destruction of contaminant. The soil, sludge or sediments with organic contaminants are best been removed bt incinerator. The organic compounds from the contaminated media are subsequently oxidized by applying heat and oxygen. The first stage of incineration involves heating of the contaminated media at temperature between 1,000 and 1,800°F that result in partial oxidation and the volatilization of the organics. The second stage results in the complete destruction of organics which operates at temperatures between 1,600 and 2,200°F. The resulting ash can then be disposed off in a landfill, if it meets safety regulations.

Phytoremediation

The cost-effective and aesthetically-pleasing method of remediating contaminated sites is an innovative technology is the phytoremediation (U.S. EPA, 1999, 2000). In natural ecosystems, substances generated by nature are metabolized by plants, which act as filters. Phytoremediation is new emerging technology that uses plants to remove contaminants from soil and water (U.S. EPA, 1999, 2000; Raskin et al., 2000). Many studies have been carried out to determine the effectiveness of remediating persistent pollutants with various plant species and more results are frequently being reported. Pesticide biotransformation in plants with reference to similarities and dissimilarities has been documented by Hall et al. (2000). Phytoremediation of ethion, a phosphorus pesticide was studied with water hyacinth; *Eichhornia crassipes* (Huilong el al., 2006). The study reported that ethion disappearance rate constants in culture solutions for non-sterile planted were 0.01059, sterile planted were 0.00930, non-sterile unplanted were 0.00294, and sterile unplanted were 0.00201 h^{-1}. These rate constants were significantly divergent and indicated that plant uptake and phytodegradation contributed 69% and while microbial degradation contributed 12% to the removal of the applied ethion.

Capacity of *lemna minor*, *Elodea* Canadensis and *Cabomba aquatic* to uptake pesticides; copper suplphate, flazasulfuron and dimethomorth were studied by Olette et al. (2008). All of these three plants were able to conduct phytoremediation of pesticides successfully.

Two fungicides; dimethomorph and pyrimethanil removal from water by five macrophytes; *L. minor, S. polyrhiza, C. aqautica, C. palustris* and *E. candensis* were studied by Dosnon-Olette et.al (2009). Experiments conducted in laboratory were able to remove 10 to 18% dimethomorph and 7-2% pyrimethanil during four days incubation window period. The maximum removal rate during the test period was found to be 48 mg/gm fresh weight for dimethomorph and 33 mg/g for pyrimethanil. Out of five, the *L. minor* and *S. polyrhiza* showed the highest removal efficiency towards these fungicides (Dosnon-Olette et al., 2009). Phytoremediation of Chlorpyrifos in greenhouse conditions with water lettuce; *Pistia stratiotes* L and dukeweed *L. minor* L were addressed by Prasertsup et al. (2011). Relative growth rate (RGR) studies at initial chlorpyrifos concentrations up to 0.5 mg/L did not affect plant growth however RGR was found to be affected at or above 1 mg/L chlorpyrifos concentration. RGR inhibition was found to be -0.036 mg/g/day for *P. stratiotes* while it was -0.023 mg/g/day for *L. minor*. The maximum removal of chlorpyrifos by *P. stratiotes* and *L. minor* was noticed when chlorpyrifos was at an initial culture concentration of 0.5 mg/L, was 82 and 87%, respectively. The bioconcentration factor (BCF) of *L.minor* was significantly greater than that for *P. stratiotes* and therefore, at least under this greenhouse-based conditions; *L. minor* was more efficient than *P. stratiotes* for the accelerated removal of chlorpyrifos from water. In their study Riaz et al. (2017) indicated phytoremediation of pyrethroids by macrophytes and algae in freshwater was superior over organochlorine. The *Saccharaum officinarum* (sugarcane) along with *Candida* was found to exhibit augmented removal of lindane in doped soil (Salam et al., 2017). Removal rate for atrazine from eutrophicated lakes in China with *Potamogeton crispus*-planted and *Myriophyllum spicatum*-planted sediments could reach over 90%. The half life of atrazine was significantly reduced from 14.3 to 8.6 days with *P. crispus* and 9.72 days with *M. spicatum* (Qu et al., 2017). In an alternative studies removal of atrazine was effectively achieved with prairie grass (Khrunyk et al., 2017) and shrub willows (Lafleur et al., 2016). Wang et al. (2016) under laboratory conditions assessed the potential of *Acorus calamus* to remove chlorpyrifos at low concentration from water. While phytoremediation of terbuthylazine from polluted solution was found be effective with *Festica arundinacea*. Studies reported that herbicide detoxification was due to Glutathion S-transferase and ascorbate peroxidase (Buono et al., 2016). Removal of terbuthylazine with Italian ryegrass *Lolium multiflorum* L (Mimmo et al., 2015) was studied in liquid solutions. Phytoremediation of imazalil and tebuconazole (Lv et al., 2016) from

however; some are recalcitrant in nature (Richins et al., 1997; Mulchandani et al., 1999). Additionally, some other aspects such as physiological, ecological, biochemical and molecular play important roles in the microbial transformation of pollutants (Iranzo et al., 2001; Vischetti et al., 2002).

BACTERIAL DEGRADATION OF PESTICIDES

There are various sources of microorganisms having the ability to degrade pesticides. Generally, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of pesticide contaminated sites. The soil is the medium that mostly gets these chemicals, when they are applied to agricultural crops; additionally, the pesticide industry's effluent, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of pesticides are also rich source of pesticide degrader. In different laboratories around the world, presently there are collections of microorganisms identified and characterized for their pesticides degradation ability. The isolation and characterization of pesticides degrading microorganisms that is able to give the possibility to count with new tools to restore polluted environments or to treat wastes before the final disposition. Upon complete biodegradation of the pesticide, the carbon dioxide and water are formed by the oxidation of the parent compound and this process provides the energy to the microbes for their metabolism. The intracellular or extracellular enzymes of the microbes play major role in the degradation of chemical compounds.

Organochlorine pesticide and their degradation

These are organic compound containing at least one covalently bonded chlorine atom and are insecticides containing primarily of carbon, hydrogen and chlorine. These include a chlorocarbon, chlorinated hydrocarbon, organochloride, organochlorine or chlorinated solvent. Organochloride pesticides are synthetic. In the 1970s, these were widely used, mainly in the United States. Because of their wide structural variety and divergent chemical properties it has broad range of applications. Because of the effects of these compounds on the environment use of many derivatives are controversial. Although their use has been banned in many countries, they are still used in developing countries. Many xenobiotic compounds, especially the organochloride pesticides are recalcitrant and resistant to biodegradation (Diaz, 2004; Dua et al., 2002; Chaudhry and Chapalamadugu, 1991). The rate of breakdown of most of them is slow and they can remain in the environment for long time after application and also in organisms long after its exposure.They act by hyper excitation in the nervous system of the animals, causing acute toxic effects and their death frequently occurs due to respiratory failure after the disruption of nervous system function. Organochloride pesticides are cumulative in the organisms and causes chronic health effects, like cancer and neurological and teratogenic effects (Vaccari et al., 2006).

In general, these highly toxic and carcinogenic compounds persist in the environment for many year. Some of the most widely used organochlorine pesticides include, dieldrin, aldrin, alpha-BHC, beta-BHC, delta-BHC, gamma-BHC (Lindane), heptachlor, endosulfan, methoxychlor, aroclor and dichloro diphenyl trichloro ethane (DDT) (Menone et al., 2001; Patnaik, 2003) (Figures 1 and 2).

In 1939, the use of organochlorine pesticides started when Paul Hermann Müller realized that the DDT was an efficient insecticide (Matolcsy et al., 1988). During the World War II, for prevention of epidemics of typhus transmitted by lice, DDT powder was pulverized on the population's skin. In other countries, this insecticide was also used to control the malaria-bearing mosquitoes (Konradsen et al., 2004). Although most organochlorine were banned from some countries, organochlorine pesticides are still widely studied due to their recalcitrant nature, that is, even after years since the use has been banned, organochlorine contaminated sites are not rare. Both biotic and abiotic factors determine the fate of pesticides in the environment. The rate of different pesticides which are undergoes biodegradation varies widely.

Some pesticides like DDT and dieldrin are recalcitrant and they accumulate in the environment for a long time and enter into food chains and accumulate for decades after their application to the soil (Kannan et al., 1994). The *in situ* degradation of organochlorine pesticides by pure cultures has been proven. Among microorganisms, bacteria comprise the major group involved in organochlorine degradation, especially soil habitants belonging to genera *Bacillus, Pseudomonas, Arthrobacter and Micrococcus* (Langlois et al., 1970). Matsumura et al. (1968) reported that the evidence of the breakdown of dieldrin in the soil by a *Pseudomonas* sp. Studies with fungi have also evidenced the biodegradation of organochlorine pesticides. Ortega et al. (2011) evaluated marine fungi collected off the coast of São Sebastião, North of São Paulo State, Brazil for the biodegradation of organochlorine. The fungi strains were obtained from marine sponges. The fungi like *Penicillium miczynskii, Aspergillus sydowii, Trichoderma* sp., *Penicillium raistrickii,* and *Bionectria* sp. were previously tested in solid culture medium for their degradation capability. Table 1 presents some of the microorganisms that were able to degrade organochlorine pesticides. An extensive study on fungal degradation of heptachlor and heptachlor epoxide was carried out on the 18 species of white rot

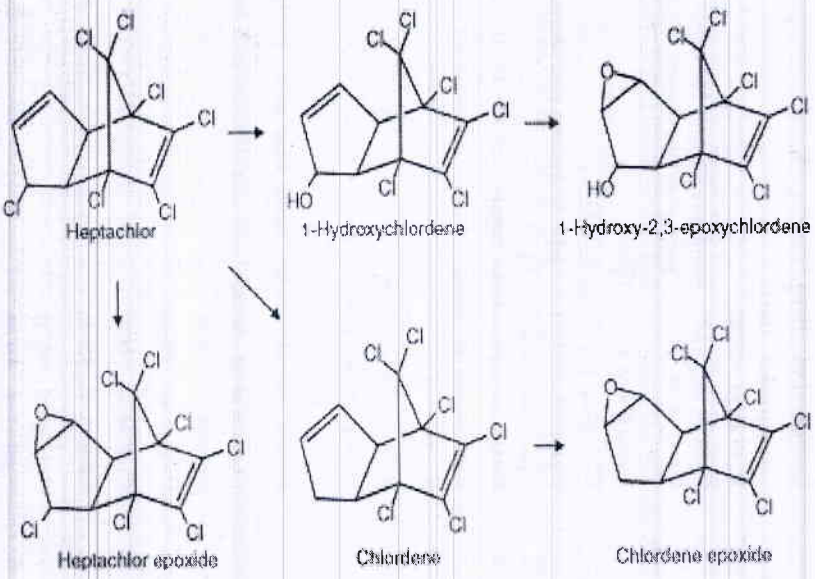

Figure 2. Organochlorine: Heptachlor degradation.

pesticide by the *lin* genes present in these three species (Pal et. al., 2005). Latter on Liu et al. (2007) demonstrated complete removal of hexachlorocyclohexane chlorinated pesticide from the soil with *S. japonicum*, with an efficacy of 2 mg/L/h. Studies by Jayashree and Vasudevan (2007) conducted studies on edosulfan remediation from the soil. In their experimental design they used synthetic surfactant Tween 80 and efficacy of *P. aeruginosa* to degrade endosulfan at neutral pH and at 8.5 pH was tested. In their studies they could demonstrate that surfactant could positively influence bacterial degradation of endosulfan. Bacterium could remove as high as 94% endosulfan at pH 8.5 producing endodiol and endosulfan sulphate, those were less toxic to soild (Jayshree and Vasudevan, 2007). When mixed ..tures of *Stenotrophomonas maltophilia* and *Rhodococcus erythropolis* were used for degradation of endosulfan (Kumar et al., 2008), studies revealed that 73 and 81% of α and β endosulfan were removed, respectively When tested individually the *S. maltophilia* had better efficacy over *R. erythropolis*. The metabolites produced by mixed culture degradation were found to be close to non-toxic to that of endosulfan when toxicity was tested against human polymorph nuclear cell detected by micronucleus assay (Kumar et al., 2008). Bacterium *Achromobacter xylosoxidans* CS5 was found to be able to utilize edosulfan as the sole carbon, sulphur and energy source from the activated sludge of Jiangsu, China (Li et al., 2009). Culture analysis with reference to pH, cell growth and residual endosulfan demonstrated CS5 strain could degrade endosulfan producing endosulfan diol and endosulfan ether as the major metabolites. Studies Tas et al. (2011) investigated role for *Dehalococcides* sp. in degradation of hexachlorobenzene dechlorination in river sediment. Curiously they could identify organism was able to perform activity anaerobically at various range of temeperature throughout the year and the *cbrA* gene product; trichlorobenzene reductive dehalogenase was found to be more important enzyme. Chaussonnerie et al. (2015) isolated and characterized two closely related species of *Citrobacter amalonaticus* those were able to transform recalcitrant chlordecone from pesticide contaminated soil. Endosulfan degradation by *P. aeruginosa* G1 (88.5%), *Stenotrophomonas maltophilia* G2 (85.5%), *B. atrophaeus* G3 (64.4%), *Citrobacter amolonaticus* G4 (56.7%) and *Acinetobacter lowffii* G5 (80.2%) was reported (Ozdal et al., 2016). Effect of biochar on the water soluble arsenic concentration and the extend of organochlorine degradation in a co-contaminated historic sheep-dip soil during 180d glasshouse incubation experiment was studied (Gregory et al., 2015). In this study microbial degradation activity was found to be enhanced after 60 day incubation. The biochar amended soil contained *Chryseobacterium, Flavobacterium, Dadobacterium,* and *Pseudomonadaceae* members. Increased microbiological dehydrogenase activity due to biochar amendment was responsible for enhanced degradation of organochlorine that was otherwise attenuated due to arsenic contamination. Abundance and distribution of potential microbes and functional genes associated with pentachlorphenol anaerobic mineralization in a continuous flow reactor was studied by Li et al. (2016). Microbes with potential reductive dechlorinators; *Dehalobacter, Sulfospirillum, Desifitobacterium and Desulfovibrio* spp. and phenol degrader *Cryptanerobacter* and *Syntrophus spp.* and putative functional genes; chlorphenol reductive dehalogenase *cprA*, benzoyl-CoA reductase *bam A* and seven types of putative nitrogenase reductase genes were determined (Table 1) (Li et al., 2016).

(Don and Pemberton, 1981) *Staphylococcus* (Sonkong et al., 2008), and *Pseudomonas* (Barragan-Huerta et al., 2007) carry out the reaction. The ability to degrade organochlorine has been documented in different genera of fungi. Among these, basidiomycetes show more resistance toward these compounds (Gomes Machado et al., 2005; Rigas et al., 2005). Recently it is reported that a strain of *Trichoderma harzianum* has capability to degrade organochlorines through an oxidative system (Katayama and Matsumura, 2009).

Another best example of organochlorine degradation is endosulfan degradation. Microorganisms play a key role in removal of such xenobiotic from the contaminated sites. The dynamic, complex and complicated enzymatic systems of the microbe degrade these chemicals by eliminating their functional groups of the parent compound. The endosulfan can undergo either oxidation or hydrolysis reactions. Several intensive studies on the degradation of endosulfan have been carried out which show, formation of endosulfan sulphate and endosulfan diol as primary metabolites.The *Mycobacterium tuberculosis* ESD enzyme degrades beta-endosulfan to the monoaldehyde and hydroxyether, but transforms alpha-endosulfan to the more toxic endosulfan sulphate. However, oxidation of endosulfan or endosulfan sulphate by the monooxygenase encoded by ese in *Arthrobacter* sp. KW yields endosulfan monoalcohol (Weir et al., 2006) alternatively, in somebacteria like *Pseudomonas aeruginosa* and *Burkholderia cepaeia*hydrolysis of endosulfan occur which yields the less toxic metabolite endosulfan diol (Kumar et al., 2007).

Bioremediation of organophosphorous

The first OP compounds degrading bacterium was isolated from a soil sample from the Philippines in 1973, and was identified as *Flavobacterium* sp. ATCC 27551 (Sethunathan and Yoshida, 1973). The biodegradation of organophosphorus compounds with their enzyme systems involved in the biodegradation has been extensively studied (Singh, 2008). Many of the pesticide degrading genes harbored on the plasmid DNA are reported (Chung and Ka, 1998; Laemmli et al., 2000). The plasmid carrying genetic system for the degradation of a complex compound is referred to as the catabolite plasmid. In the literature till date, the catabolite plasmids bearing several bacterial species viz, *Klebsiella, Moraxella, Rhodococcus, Pseudomonas, Flavobacterium, Alcaligenes, Acinitobacter and Arthrobacter* are reported (Sayler et al., 1990). Recently, the co-metabolic degradation of organo-phosphorus compound dimethoate is reported by bacterium *Raoultella sp.* X1 (Liang et al., 2009). Similary, by using *Paracoccus sp.* Lgjj-3, the biodegradation of dimethoate with detailed biochemical pathway is also reported (Li et al., 2010). Till date number of bacterial species has been reported for dichlorovos degradation. These include *Proteus vulgaris, Vibrio* sp., *Serratia* sp. and *Acinetobacter sp.* (Agarry et al., 2013).

The gas chromatographic analysis of chlorpyrifos degradation with *P. putida* strain MAS-1 revealed degradation was rapid. When growing in minimal salt medium containing 2 mg.ml Chlorpyrifos as the solitary carbon source it was demonstrated that as high as 90% chlorpyrifos was utilized by MAS-1 just in 24 h (Munazza et al., 2012). In a parallel study, Sasikala et al. (2012) isolated nine different bacteria from chlorpyrifos contaminated soil of which four were further used in preparation of consortium for the degradation of pesticide. In another study, it has been reported that 4 strains of *Pseudomonas* isolated from a water waste irrigated agri-soil in India, were able to utilize chlorpyrifos as an exclusive carbon source (Bhagobaty and Malik 2008). Rani et al. (2008) isolated chlorpyrifos degradting *Providencia stuartii* from agricultural soil experience with chlorpyrifos for 10 years in a row. While degradation of Chlorpyrifos by *Spingomonas* sp. Dsp-1 stain from contaminated water was addressed by Li et al. (2007). In an independent study, *B. cereus* mediated chlorpyrifos degradation from Chinese soil was demonstrated by Liu et al. (2012). Actinomycetes *Streptomycetes* strains have been known for their profound industrial application. Strains degrading chlorpyrifos were isolated (Briceno et al., 2012), ability to degrade pesticide was established in agar medium, interestingly degradation was found to be modulated by the changes in the pH of the cultivation medium (Briceno et al., 2012). Yet another pesticide Profenofos degrading bacteria were isolated by enrichment technique. These bacteria predominantly belonged to *Pseudomonas* genus and were able to remediate proficiently 90% pesticide within 90 h (Malghani et al., 2009). Under laboratory conditions Rayu et al. (2017) could demonstrate that *Xanthomonas* sp. 4R3-M3 and *Pseudomonas* sp. 4H1-M3 were able to use both chlorpyriphos and 3,5,6-trichloro-2-pyridinol as a sole carbon and nitrogen source. Degradation of cyhalothrin and other pyrethroides by *B. thuringiensis* strain was reported (Chen et al., 2015).

Studies demonstrated production of 3-phenoxyphenyl acetonitrile and *N-(2-isoproxy-phenyl)-4-phenoxy benzamide* as metabolites in the degradation pathway of pyrethroids. Cypermethrin degrading *Bacillus* sp. strain G2 was isolated from pesticides contaminate soil. The isolate was found to utilize a novel pathway producing intermediates as 4-propylbenzoate, 4-propylbenzaldehy, phenol M-tert-butyl and 1-dodecanol (Pankaj et al., 2016). Parte et al. (2013) tested ability of *Pseudomonas demolyticum* NCIM2112 strain to degrade sufonated aromatic amine, used as precursor in much OP synthesis. Under controlled laboratory condition NCIM2112 strain of *P. demolyticum* was found to successfully remediate sulfonated aromatic amine. In an independent study strain SMK of *Pseudomonas stutzeri* was obtained from the soil that received dichlorovos treatment for consecutive three years. Strain was tested

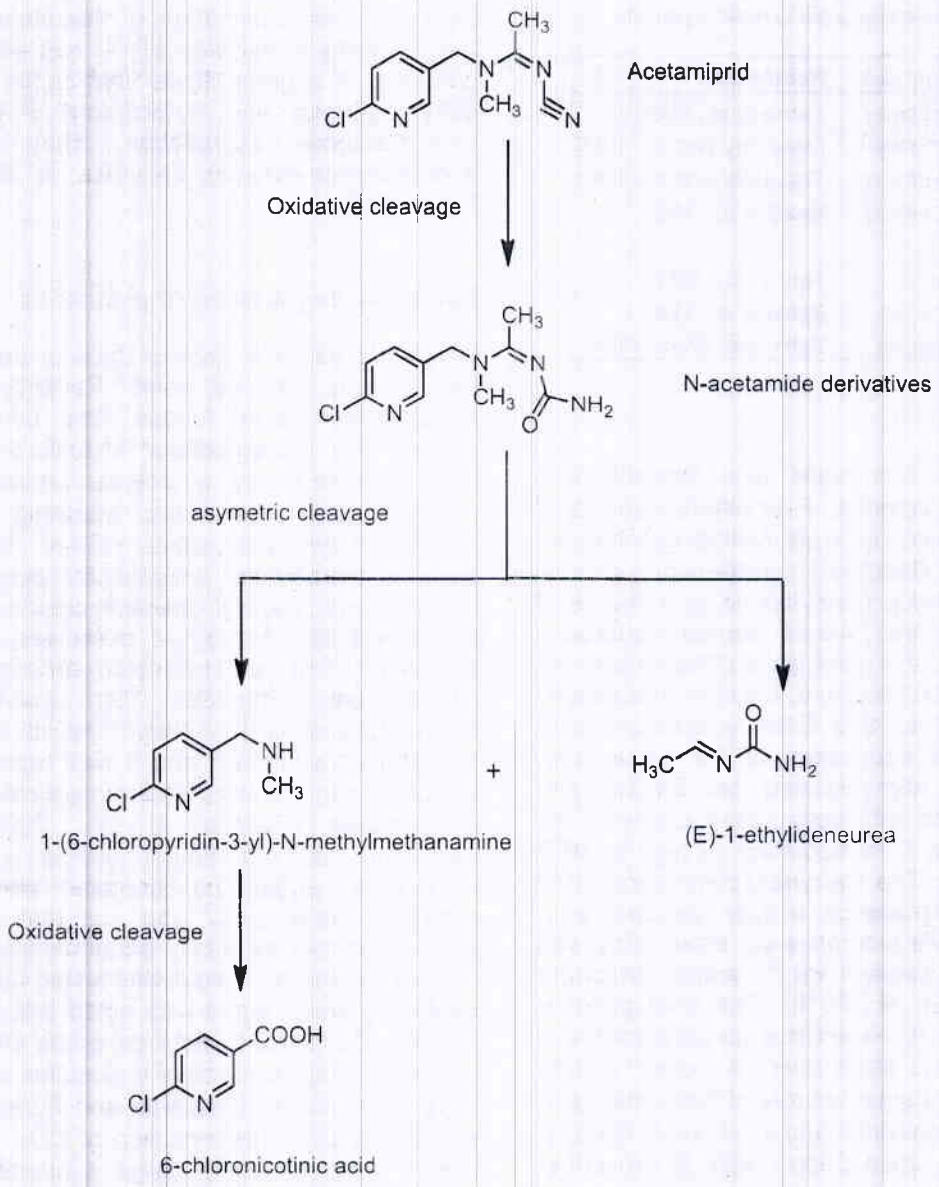

Figure 3. Proposed pathway for Acetamiprid degradation.

called carbofuran hydrolase, codified by the mcd gene, which was located on a plasmid is first described in *Achromobacter* sp. (Tomasek and Karns, 1989). Further studies showed that a wide variety of bacteria could degrade carbamates using carbofuran hydrolase. Among other genera *Pseudomonas, Mesorhizobium, Ralstonia, Rhodococcus, Ochrobactrum,* and *Bacillus* are the most notorious (Desaint et al., 2000). Fungal degradation of carbamates has also been reported. Of special interest is the report a novel hydrolase from *Aspergillus niger* capable of hydrolysing several N-methylcarbamate insecticides (Qing et al., 2006). Several Actinomycetes that metabolize carbamate pesticides were isolated. In most cases, this is initiated by hydrolysis of the carbamate at the ester linkage.

The bacteria like *Sphingomonas* sp. (Kim et al., 2004) and *Arthrobacter* sp. (De Schrijver and De Mot, 1999) degrade Carbofuran first into carbofuran phenol which was further degraded to 2-hydroxy-3-(3-methylpropan-2-ol) phenol. It has been reported that the fungicidecarbendazim was degraded by a microbial consortium obtained from several soil samples in Japanese paddy fields with continuous culture enrichment. Afterwards, the carbamate carbendazin was converted to 2-aminobenzimidazole by *Pseudomoans* isolates (De Schrijver and De Mot, 1999). Following table how the example of carbamate degrader. Shin et al (2012) isolated 37 carbofuran degrading bacteria representing 15 different 16 s RNA types. These included *Rhodococcus, Spingomonas, Spingobium* as known while

yeast extract and environmental factors improved the efficiency of the biodegradation process (Urlacher et al., 2004; Alzahrani, 2009]. Fungus uses chlorpyrifos as a sole energy and carbon source and causes its rapid degradation. Another fungus, basidiomycetes, degrades chlorpyrifos very effectively. The fungal strains such as *Fusarium oxysporum, Lentinulaedodes, Penicillium brevicompactum* and *Lecanicilliumsaksenae* has great potential for the biodegradation of the pesticides including difenoconazole, terbuthylazine and pendimethalin in batch liquid cultures and are investigated to be valuable as active microorganisms for pesticides degradation (Shi et al., 2012). The rot fungi isolates from contaminated soil degrades methomyl and diazinon pesticides. The temperature optima for maximum efficiency are 28°C. The higher rate of degradation is achieved by using mixture of fungal strains (Zhongli et al., 2001). By using mixed fungal strains, there is possibility of degrading mixed insecticides (DDT and chlorpyrifos). At low concentration of mixed insecticides there was the high efficiency of degradation is observed. It is reported in DDT and chlorpyrifos, that the efficiency of degradation was 26.94 and 24.94% respectively (Pieper et al., 2000). The phytopathogenic fungus easily grows up on organophosphonate herbicides and easily degrades herbicides (Shen et al., 2009). The pesticide like pirimicarb is degraded by *Trichoderma viride* and *T. harzianum* with high potential. Upon addition of activated charcoal the degradation capacity increases (Eapen et al., 2007). By enrichment culture method the strain, *Sphingomonas yanoikuyae*can degrade carbamate and pyrethrin (OPs) with high efficiency under harsh conditions, and analyzed by gas chromatography (Cases et al., 2005). The carbofuran is degraded by salt resistant actinomycete (Chougale and Deshmukh, 2007). Up to 95% degradation of carbafuran is carried out by the *S. alanosinicus*. It utilizes carbofuran as a sole source of carbon and is applicable to saline soils for its efficiency (Fu et al., 2004). More than 30 microorganisms have capablity of degrading the pesticides, of which *Gliocladium* genus has maximum activity for selective degradation of carbofuran (Fu et al., 2004).

Besides these, various fungal species including *Trametes* sp. and *Polyporus* sp. were able to degrade variety of chemicals including pesticides. Pesticide degradation ability was also reported using *A.fumigates, A. sydowii, A. terreus, A. flavus, Fusarium oxysporum* and *Penicillium chrysogenum* (Hasan, 1999).

MICROBIAL ENZYME SYSTEMS IN PESTICIDE BIODEGRADATION

Among various strategies used by the microorganism for degradation of xenobiotic compounds the enzymes play a key role in the biodegradation. The biochemical reactions for degradation are achieved through a number of different enzymes such as dehydrogenases (Bourquin, 1977; Singh and Singh, 2005), cytochrome p450 (Castro et al., 1985; Jauregui et al., 2003), dioxigenases (Nadeau et al., 1994; Van Eerd et al., 2003), ligninases (Pizzul et al., 2009). Several reports have documented the capability of different genera of fungi to degrade organochlorines. Bacterial enzymes have been found to achieve such detoxifying reactions (Singh et al., 1999; Yañez- Ocampo et al., 2009). The commonly used as a detoxification mechanism, especially in plants and insects, is conjugation with glutathione and this mechanism has also been reported in bacteria (Vuilleumier, 2001; Wei et al., 2001; Chaudhry et al., 2002). In these processes, bacteria and fungi are involved. They produces intracellular or extra cellular enzymes such as hydrolytic enzymes, oxygenases, peroxidases (Van Eerd et al., 2003).The basidiomycetes are more resistant to these compounds (Gomes Machado et al., 2005; Rigas et al., 2005). Recently a strain of *Trichoderma harzianum* is reported to degrade organochlorines through an oxidative system (Katayama and Matsumura, 2009).

It has been reported that a large group of bacterial genera are able to degrade organophosphates compounds. Hydrolysis is fundamental for the complete degradation of the molecule. The reported and studied enzymes are related to the phosphotriesterase, having capability to hydrolyzing organophosphate pesticides at the central atom of pesticides, phosphorus. Phosphotriesterase activity is the first and most important step in detoxification. A large number of bacterial genera have been reported for the carbamate degradation (Parekh et al., 1995). An enzyme called carbofuran hydrolase carry out the hydrolysis of the methylcarbamate linkage within carbamate (Figure 4).

Kim et al. (2004) studied the carbofuran degradation by bacteria like *Sphingomonas* sp. and *Arthrobacter* sp. by De Schrijver and De Mot (1999) and proposed the metabolic pathway. The Carbofuran first degraded into carbofuran phenol which was further degraded to 2-hydroxy-3-(3-methylpropan-2-ol) phenol. Slaoui et al. (2007) reported more than 30 microorganisms were capable of degrading the pesticides, out of which *Gliocladium* genus had maximum activity for selectively degrading carbofuran. Chougale and Deshmukh (2007) reported salt resistantactinomycete is capable of degrading carbofuran. One of seven actinomycetes, *S. alanosinicus*, is most effective and gives up to 95% degradation. It uses carbofuran as a carbon source and was found to be applicable to saline soils for its efficiency (Figure 5).

De Schrijver and De Mot (1999) reported that the fungicidecarbendazim was degraded by a microbial consortium obtained from several soil samples in Japanese paddy fields with continuous culture enrichment. Afterwards, the carbamate carbendazin was converted to 2-aminobenzimidazole by *Pseudomoans*

Table 4. Studies on pesticide degrading microorganism with their enzyme.

Pesticides class	Microorganism	Enzymes	Reference.
Organochlorine	Bacteria *Klebsiella* sp., *Alcaligenes* sp. *Staphylococcus* sp. *Pseudomonas*. sp.	Dehalogenases	Franken et al., 1991; Sharma et al., 2006; Kwon et al., 2005; Don and Pemberton, 1981. Sonkong et al., 2008. Barragan-Huerta et al., 2007
Organophosphate	*Flavobacterium* and *Pseudomonas* sp. *Agrobacterium radiobacter* *Alteromonas* sp. *Plesiomonas* sp. *Achromobacter*, *Pseudaminobacter*, *Ochrobactrum*and *Brucella* *Alteromonas undina*, *Alteromonas haloplanktis* *B. diminuta* and *Flavobacterium* sp Fungi: *Pleurotus ostreatus*. *Aspergillus* sp. *Penicillium* sp.	Organophosphorus hydrolase(OPH), organophosphorus acid anhydrolase (OPAA), Laccase Aspergillus enzyme (A-OPH) Penicillium enzyme (P-OPH)	Singh and Walker, 2006; Serdar et al., 1982.Somara and Siddavattam, 1995; Horne et al., 2002; Cheng et al., 1996; Cheng et al., 1997; Zhongli et al., 2001; Zhang et al., 2005; Chen and Mulchandani (1998); Chen et al., 1999; Amitai et al., 1998; Liu et al., 2001; Liu et al., 2004; Liu et al., 2001
Carbamate	*Achromobacter* sp. *Pseudomonas*, *Mesorhizobium*, *Ralstonia*, *Rhodococcus*, *Ochrobactrum*, *and Bacillus*.	Carbofuran hydrolase	Tomasek and Karns, 1989; Desaint et al., 2000.
Pyrithroid	*Serratia*, *Pseudomonas*. *Fungi: Aspergillus niger*	Carboxyl esterase, phosphotriesterase. Pyrethroid hydrolase	Grant et al., 2002. Liang et al., 2005

insights into the molecular events that lead to the development of enhanced pesticide degradation phenomenon is achieved through advancement in the technique of isolation of pesticide degrading microorganisms and the characterization of genes encoding pesticide degradation enzymes. For optimization of the enzymes, metabolic pathways and organisms relevant for biodegradation in various genetic approaches have been developed and used (Pieper et al., 2000). New information on the metabolic pathways and bottlenecks of degradation is still gathering, requiring the need to reinforce the available molecular toolbox. Nevertheless, the incorporated genes or enzymes, even in a single modified organism, need to be introduced within the regulatory and metabolic network for their proper expression (Shimizu, 2002; Pieper et al., 2000; Cases et al., 2005). Firstly it was demonstrated that the detoxification of organophosphate pesticides by genetically engineered microorganisms and the genes encoding these hydrolases have been cloned and expressed in *Streptomyces lividans*, *Yarrowia lipolytica P. pseudoalcaligenes*, *E. coli*, *and Pichia pastoris* (Wu et al., 2004; Fu et al., 2004; Wang et al., 2012; Shen et al., 2009). The site-directed mutagenesis has been used to improve the substrate specificity and stereo selectivity of OPH (Casey et al., 2011; Van Dyk and Brett, 2011). To generate OPH variants with up to 25-fold improvements in hydrolysis of methyl parathion, directed evolution have recently been used (Cho et al., 2002). Up to 700-fold improvement was obtained, and the best variant hydrolyzes chlorpyrifos at a rate similar to that of the hydrolysis of paraoxon by wild-type OPH (Cho

Table 5. Studies on pesticide degrading microorganism with their catabolic gene.

Microorganism	Gene
Bacteria:	
Pseudomonas diminuta	*opd*
Alteromonas sp.	*opaA*
A. radiobacter	*opdA*
Nocardia sp.	*adpB*
Escherichia coli	*pepA*
Pseudomonas monteilli	*hocA*
Burkholderia caryophilli	*pehA*
Bacillus cereus	*phn*
Burkholderia sp. *JBA3*	*ophB*
Stenotrophomonas sp. *SMSP-1*	*ophC2*
	opdB
Lactobacillus brevis.	*imh*
Arthrobacter sp. *scl-2*	*mpd*
Ochrobactrum sp. *Yw28, Rhizobium radiobacter*	*oph*
Arthrobacter sp	*mph*
Arthrobacter sp. *L1.*	*mpdB*
Burkholderia cepacia	*opdE*
Enterobacter sp.	*lin*
Spingobium francense	*lin*
Spingobium indicum	*lin*
Spingobium japonicum	

et al., 2004). Linuron degrading bacterium *Variovorax sp* strain SRS16 contain the *libA* gene that encodes a hydrolase enzyme, which initiates degradation. An alternative hydrolase; HylA was found to exist in *Variovorax* sp. Strain WLD1. Both the *libA* and *hylA* could contribute linuron degradation in the same environment (Horemans et al., 2016). Aryloxyphenoxy Propanoate (AOPP) degrading strain *Rhodococcus ruber* JPL-2 was isolated by Hongming et al. (2015). A novel esterase gene *feh*, encoding phenoxaprop-p-ethyl hydrolyzing carboxyesterase (FeH) was cloned from *R. ruber* JPL2. The ability of *Streptomyces* strain alone or as mixed culture to remove pentachlorophenol and chlorpyrifos was studied (Fuentes et al., 2013). It was shown that without exhibiting any antagonism growth of mixed culture was superior to that of pure culture. Pure culture of *Streptomyces* sp. A5 and quadruple culture could remove pentachlorphenol efficiently whereas *Streptomyces* sp. M7 presented the best removal of chlorpyrifos. Immobilized cells were curiously able to remediate both pentachlorphenol and chlorpyrifos than that of free cells (Fuentes et al., 2013).

CONCLUSION

Biodegradation processes can be employed for the pesticide degradation using microbes. Microbial degradation processes applied for bioremediation involves use of variety of different microbes including bacteria, fungi and actinomycetes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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