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Biodegradation of Organophosphorous Pesticide: Chlorpyrifos

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Abstract

At the present time extensive varieties of pesticides are being used but the demand for organophosphorus pesticide is increasing globally to control insect. Chlorpyrifos is a broad spectrum, moderately toxic, chlorinated organophosphate insecticide that is synthetic in origin and is normally ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acids. The mode of action involves inhibiting acetyl cholinesterase leading to accumulation of acetylcholine causing neurotoxicity. It is being transported by circulation far away from site of application leading to pollution of environment. Due to its persistent in nature, it is not only severely detrimental to the target pests, but also causes toxicity in non-target organisms including humans. It is thus critically important to develop methods to eradicate these pollutants from the environment. Lately, research activities in this area have demonstrated that microorganisms are potential tool in decaying chlorpyrifos into less harmful and non-toxic metabolites through a process known as bioremediation. This article therefore aims at giving an overview of the present status of research and future prospects in bioremediation of chlorpyrifos.

Keywords: Pesticide; Acetyl cholinesterase; Neurotoxicity; Persistent; Pollutant; Pollution; Chlorpyrifos; Bioremediation; Microorganism; Environment.

1. Introduction

Enhanced agricultural activities throughout the world have been required large scale synthesis of xenobiotic compounds leading to considerable pollution of the environment. Pesticides as agrochemicals were introduced in agriculture system to meet the demand of increased food needs of growing population act by preventing, destroying, repelling, or mitigating any pest [1, 2]. Despite numerous merits, they are now considered a necessary evil for their multifaceted toxicity, persistence and recalcitrance nature. It is ironic that only 0.3% of applied pesticide reaches to the target pest, and the rest which are not utilized, eventually dissipated into the environment [3, 4]. Pesticides commonly used during agricultural activities include organochlorines, organophosphates, pyrethroids and carbamates [5, 6]. Among these pesticides, organophosphorus pesticide alone is account for about 38% of the total pesticides used globally. The increase use of organophosphorus pesticide is because of its high effectiveness against target pests and relatively low toxicity to non-target organisms than other groups of pesticides [7, 8].

Chlorpyrifos is one of the most widely used chlorinated organophosphorous pesticide having the chemical name O, O-diethyl O-3, 5, 6-trichloropyridyl phosphorothioate (Fig. 1) and the trade names Lorsban, Agromil, Dhanwan, Dorson, Omexan, Dursban, Suscon Green, Empire, Equity to name a few. It is effective against a broad spectrum of insect pests of economically important crops which has been extensively used globally since 65s as an insecticide to control crop pests, mosquito as well as soil dwelling grubs, borers, cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants lice and subterranean termites. It is available in a variety of formulations, such as granules, wettable powder, dustable powder and emulsifiable concentrate. Chlorpyrifos is registered for use in nearly 100 countries and is annually applied to approximately 8.5 million crop acres [9]. However, this continuous and excessive use in agriculture sector has resulted in the persistence of their residues and contamination of various environmental compartments, such as soil, water and air. The contamination has been found up to about 24 kilometers from the site of application [8]. For instance, residues of chlorpyrifos have been detected in fruits, vegetables, and also in water and soil samples, in marine, sediments, streams, sumps, sloughs, rivers, urban storm drains, freshwater lakes, groundwater, fog, rain and air in many parts of the world [10].

The half-life of chlorpyrifos generally ranges between 10 and 120 days in soil but can range from 2 weeks to over 1 year depending on abiotic factors such as soil type, climate, and other conditions [7, 11]. In environment chlorpyrifos is hydrolyzed to 3, 5, 6-trichloropyridinol (TCP), which has more water solubility and therefore more mobile and easily leaches into ground as well as surface water with greater efficiency than chlorpyrifos itself [12, 13]. Half-life of TCP ranges from 65 to 360 days in soil and is reported as persistent pollutant by US EPA [14]. Chlorpyrifos is acutely toxic to bees, birds, mammals, aquatic life, and certain species of algae. Poisoning from chlorpyrifos and TCP may affect the central nervous system, the cardiovascular system, endocrine system as well as the respiratory system of non-target organisms [15, 16]. Acute exposure can result in such symptoms as numbness, tingling sensation, incoordination, dizziness, vomiting, sweating, nausea, stomach cramps, headache, vision disturbances, muscle twitching, drowsiness, anxiety, slurred speech, depression, confusion and in extreme cases, respiratory arrest, unconsciousness, convulsions, and even death [17].

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Due to the environmental concern associated with the accumulation of organophosphorus compounds in food products and water supplies, here is urgent to develop safe, convenient, and economically feasible methods for its detoxification from the environment. Mechanisms for cleanup of chlorpyrifos using conventional methods such as chemical treatment, volatilization and incineration have met public opposition because of the use of large volume of acids and alkalis and accumulation of recalcitrant residuals [18]. Generally, physical and chemical cleanup technologies are expensive and sometimes not much effective [19]. Biodegradation processes are increasingly attracting scientists' interest as novel technological methods to detoxify contaminated natural matrices and it is a cost-effective, reliable as well as easy to use technique which does not pose threat of secondary pollution to the environment [20, 21]. It exploits the use of microorganisms whether bacteria or fungi that show the capability to degrade chlorpyrifos through specific pathways by using them as carbon and energy sources rendering the contaminants harmless or less toxic products in most cases [22].

The main objectives of this article, however are to give a chronological overview on the research efforts undertaken worldwide to develop sensitive methods for biodegradation of chlorpyrifos as well as critically review mode of action and recent biotechnological advancements in the development of bio-catalysts and genetically modified organisms for chlorpyrifos and their possible application in bioremediation of contaminated ecosystems.

2. Mechanism of Action of Chlorpyrifos and Its Effects

Chlorpyrifos works basically the same way both in insects and other animals including humans through impairment of nervous system. It enters into organisms by contact and ingestion in addition with absorbed through the skin, gut and pulmonary membranes [23]. Inside animals it is converted to their oxidized forms or oxon which is about 3000 times as potent against the nervous system as chlorpyrifos itself [24, 25]. The mechanism of action by which chlorpyrifos-oxon exerts its toxic effect is related to binding and irreversibly inhibiting acetylcholinesterase (AChE-ase), an enzyme located in the nervous system and in the neuromuscular junction [26-28]. AChE-ase involves in the breaks down of a neurotransmitter chemical acetylcholine (ACh) that involves in the transmission of impulses across neuromuscular junction. The stimulant effect of ACh is rapidly hydrolyzed to avoid over-stimulating or overwhelming the nervous system by AChE-ase activity. The inhibition of AChE-ase by clorpyrifos oxon results in the accumulation of ACh, with consequent serious disruption of nervous activity [29]. Reduction in acetylcholinesterase activity to 10-20% of normal activity in man results usually in severe muscle paralysis, convulsions and ultimately death [30]. The mechanism of action of chlorpyrifos is shown in Fig. 2.

The exposure of chlorpyrifos has also been shown to interact with macromolecule synthesis (DNA, RNA, and proteins), neurotransmitter receptors and signal transduction pathways. Besides, it impedes respiration in the livers of laboratory animals by interacting with the activity of ATPase and cholesterol ester hydrolase, enzymes associated with cellular respiration and normal reactions to stress respectively [31, 32].

3. Environment Fate and Pathway of Degradation of Chlorpyrifos

The fate of chlorpyrifos is affected not only by its own physicochemical properties, but also by characteristics of the soil, environmental conditions and management practices [33] (Table 1). Its persistence increases with decreased temperature, decreased pH, and decreased light. It has also been reported to have short to moderate persistence in the environment as a result of several non-biological methods including volatilization, photolysis, abiotic hydrolysis, and also by microbial degradation that might occur concurrently. The half-life may varies from 10 to 120 days that has been attributed to different environmental factors, the most important of which are soil pH, temperature, moisture content, organic carbon content, and pesticide formulation [34]. The dissipative half-life is significantly longer in organic soils than mineral soils. As a result of photolysis and oxidation in air and on foliar surfaces, chlorpyrifos is oxidized to its oxon form, chlorpyrifos-oxon. Volatilization governs dissipation from foliage in the initial 12h after application, but cannot works in the days after application as the formulation adsorbs strongly to foliage or penetrates more deeply into the soil matrix. The most significant step for the complete degradation of the molecule in soil involves hydrolysis of P-O-alkyl and P-O-aryl bonds either enzymatically (as a result of microbial activity) or spontaneously to yield 3, 5, 6-trichloropyridinol (TCP) and Diethylthiophosphoric acid (DETP) from either chlorpyrifos or chlorpyrifos-oxon [35]. However, hydrolysis is slower in water containing clay minerals, humate, dissolved organic matter, and suspended sediment [36].

The major intermediate hydrolysis product of chlorpyrifos is TCP with greater water solubility than chlorpyrifos and causes the widespread contamination in environment. Also, TCP retards the proliferation of chlorpyrifos degrading microorganisms owing to its antimicrobial properties that's why in some earlier studies chlorpyrifos was reported to be resistant to the phenomenon of enhanced biodegradation [20, 21]. The later studies revealed considerable capacity of microorganisms in the efficient degradation of chlorpyrifos into CO_2 and organic matter [37-39]. Fig. 3 illustrates the degradation pathway of chlorpyrifos.

Table-1. Physicochemical and environmental fate properties of Chlorpyrifos [40]

Property	Values for Chlorpyrifos
Empirical formula	$C_9H_{11}C_{l3}NO_3PS$
Molecular mass	350.6 g/mol
Melting point	42-44°C
Vapor pressure (25°C)	1.0×10^{-3} Pa
Water solubility	0.39 mg/L at 19°C

	0.941 mg/l at 20°C 0.588 mg/L at 20°C
Hydrolysis (t½)	pH 5.0 : 73days pH 7.0 : 72days pH 9.0 : 16days
Soil sorption coefficient, K_{oc}	652-30, 381 L/kg
Dissociation characteristics	Practically non dissociative by nature
Partition coefficient octanol or water	Log K_{ow} = 5.0 at 24.5°C Log K_{ow} = 4.7 at 20°C
Henry's Law Constant (determines solubility of gas in liquid)	1.10×10^{-5} atm m^{-3} mol^{-1}

4. Bioremediation–A Potential Detoxification Technology

Bioremediation has now emerged as an innovative technology that is critically important for the clean-up of polluted sites. This technology is cost effective that does not pose the threat of secondary pollution to the environment. The contaminated soil, sediment as well as groundwater can be treated by bioremediation. The process can be carried out by using indigenous microorganisms or by adding an enriched culture of microorganisms. Degradation strategies exhibited by microbes include co-metabolism and catabolism. Incidental metabolism or co-metabolism is the biotransformation of a molecule coincidental to the normal metabolic functions of the microbe to partial intermediate with no net benefit. The later, catabolism or mineralization involves complete degradation and utilization of the degraded products as a nutritive or energy source. However, studies conducted in soil and water has led to the conclusion that the microbial activities are singularly most important mechanism in the degradation and detoxification of chlorpyrifos [41].

4.1. Biodegradation by Bacteria

Bacteria are considered as indispensable tools by the researchers now have proven to be potential candidate in the field of bioremediation of pesticide contaminated areas. Repeated application of pesticides for many years in any area has resulted in the evolution and adaptation of bacteria capable of degrading these anthropogenic compounds in that area [42]. Researchers have isolated and characterized bacteria showing potentiality towards degradation of chlorpyrifos. Some important chlorpyrifos degrading bacteria are listed in Table 2.

Table-2. Potential chlorpyrifos degrading bacteria

Bacteria	Rate of degradation (%)	Concentration (mg/L)	Duration (hours)	Reference
Enterobacter sp.	100	100	240	[38]
Alcaligenes faecalis	100	250	48	[43]
Sphingomonas sp.	100	100	48	[14]
Paracoccus sp.	100	50	96	[28]
Xanthomonas sp. 4R3-M1, *Pseudomonas* sp. 4H1-M3, and *Rhizobium* sp. 4H1-M1	-	10	-	[17]
Stenotrophomonas sp. YC-1	100	100	24	[44]
Gordonia sp. JAAS1	100	110	72	[19]
Cellulomonas fimi	100	50	72	[45]
Acinetobacter sp. MemCl4	98	-	144	[20]

Bacteria capable of degrading chlorpyrifos were also reported to promote several kinds of plants. Akbar and Sultan [35] isolated and characterized two bacteria *Achromobacter xylosoxidans* JCp4 and *Ochrobactrum* FCp1 able to degrade 93%-100% of input concentration (200mg/kg) of chlorpyrifos within 42days in sterilized as well as non-sterilized soils. These strains showed substantial plant growth promoting traits such as phosphate solubilization and production of ammonia and indole acetic acid. Feng, *et al.* [46] studied some endophytic bacteria with dual functions stimulating plant growth and degrading pollutants. They isolated five endophytic bacteria from chlorpyrifos treated rice plants showing more than 90% degradation of chlorpyrifos in 24h when the initial concentration was lower than 530 mg/L and identified as *Pseudomonas aeruginosa* RRA, *Bacillus megaterium* RRB, *Sphingobacterium siyangensis* RSA, *Stenotrophomonas pavanii* RSB and *Curtobacterium plantarum* RSC. All of these species shown to possess some plant growth promotional traits, including production of indole acetic acid and siderophore, secretion of phosphate solubilizing chemicals and 1-aminocyclopropane-1-carboxylate deaminase.

A bacterial strain *Bacillus subtilis* Y242 was isolated from agricultural wastewater and the efficiency of degrading chlorpyrifos was examined under different culture conditions. The bacterium was able to utilize chlorpyrifos as a sole carbon and energy source and showed 95.12% chlorpyrifos decomposition within 48h on a medium containing concentration up to 150 mg/L [47]. Fulekar and Geetha [48] reported the adaptation of

Pseudomonas aeruginosa NCIM 2074 to chlorpyrifos up to a concentration of 75 mg/L in a mineral salt medium. However, higher concentrations were detrimental to the growth and survival of the bacterium. Chishti and Arshad [49] reported growth linked biodegradation of chlorpyrifos (100 mg/L) by *Agrobacterium* sp. and *Enterobacter* sp. under shaking conditions. Up to 92% of the spiked chlorpyrifos was degraded within 18days at a neutral pH 7.0 and incubation temperature of 30°C. Eissa, *et al.* [18] isolated *Bacillus* sp. SMF5 capable of degrading chlorpyrifos and utilizing it as a sole source of carbon and phosphorus. The degradation was found to be optimum at 30°C and pH 7.0.

TCP, the intermediate metabolite of chlorpyrifos, acts as a buffer in the soil and sediment that prevents the proliferation of chlorpyrifos degrading microbes. There have been no reports of mineralization of chlorpyrifos since its first use in 1965 [21]. Recently researchers have reported extensive mineralization of TCP to carbon dioxide to avoid its accumulation in the environment. *Bacillus firmus* [50], *Ralstonia* sp. T6 [51], *Bacillus pumillus* C2A1 [52], *Enterobacter* sp. B-14 [38], *Sphingobacterium* sp. JAS3 [19], *Cupriavidus* sp. DT-1 [53], *Alcaligenes* sp. JAS1 [54], *Paracoccus* sp. TRP [28], *Xanthomonas* sp. 4R3-M1 and *Pseudomonas* sp. 4H1M3 [17] are few bacteria that can mineralize the chlorpyrifos effectively. Feng, *et al.* [55] for the first time isolated a pure culture of bacteria capable of mineralizing TCP under aerobic conditions by a reductive de-chlorination pathway to yield CO_2, chloride and some unidentified polar metabolites. The bacterium was identified as a *Pseudomonas* sp. ATCC 700113. Li, *et al.* [14] demonstrated that the concentration of the chlorpyrifos had apparently no effect on the degradation rate, but when the concentration was higher than 200 mg/L, the bacterium stopped degrading its intermediate TCP and grew very slowly. The growth experiments by Rani, *et al.* [56] showed that *Providencia stuartii* MS09 was able to grow in the presence of high concentrations (50-700mg/L) of chlorpyrifos and utilized it as a source of carbon. However, the optimum concentration that supported bacterial growth over 24h was found to be 50-200mg/L chlorpyrifos, whereas at higher concentrations (300-700mg/L) an increased lag phase was observed, without inhibiting growth of the pesticide-utilizing bacterium. An effective chlorpyrifos-degrading bacterial strain, *Mesorhizobium* sp. HN3, was isolated and characterized by Jabeen, *et al.* [57]. This strain was capable of converting chlorpyrifos and TCP to diethylthiophosphate and of 3,5,6trichloro-2-methoxypyridine, respectively up to 400mg/L initial concentration at wide range of temperatures (30–40°C) and pH (6.0–8.0) while optimal degradation was achieved at 37°C and neutral pH (7.0) at an initial inoculum density 2×10^7 colony forming unit/mL of culture medium. Xu, *et al.* [58] reported complete mineralization of 100 mg/L chlorpyrifos by co-culturing of *Serratia* sp. a potential chlorpyrifos degrader and *Trichosporon* sp. a TCP mineralizing strain within 24h of incubation period. The results obtained from previous study clearly indicated that bacteria play a crucial role in detoxifying chlorpyrifos contaminated area.

4.2. Biodegradation by Fungi

Besides bacteria, many fungal isolates have been reported as potential degraders of chlorpyrifos as fungi account for up to 75% of soil microbial biomass [59]. Bioremediation in which fungi are involved is referred to as mycoremediation [39]. Only a few studies indicated that fungi degrade chlorpyrifos in liquid media. Ivashina [60] studied chlorpyrifos degradation by several microbial cultures maintained in liquid media containing 10ppm chlorpyrifos. Dissipation was more rapid in a sucrose-supplemented media containing *Trichoderma* sp. and glucose supplemented media containing *Bacillus* sp. than in control media containing no microorganisms. Chlorpyrifos disappeared from the microbial cultures in a linear fashion over a 2-week period. Jones and Hastings [61] isolated several forest fungi namely *Trichoderma harzianum*, *Penicillium vermiculatum*, and *Mucor* sp. capable of metabolizing 50ppm chlorpyrifos. After 28days, chlorpyrifos and its metabolite TCP were present in all cultures at levels of 2-5% and 1-14%, respectively. Fang, *et al.* [62] reported pH and temperature dependent biodegradation of chlorpyrifos by the *Verticillium* sp. DSP and concluded that the maximum degradation of 1mg/L chlorpyrifos was achieved at pH 7.0 and 35°C. Some degree degradation of chlorpyrifos by the yeast *Saccharomyces cervisiae* was reported by Lal and Lal [63]; where half the initial chlorpyrifos was recovered 12h after the cultures were inoculated with 1-10ppm. Bumpus, *et al.* [64] reported the ability of *Phanerochaete chrysosporium* to degrade 27.5% of chlorpyrifos during the 18days incubation in nitrogen-limited stationary cultures. Chen, *et al.* [65] isolated and characterized a new fungal strain *Cladosporium cladosporoides* strain Hu-01 which has high chlorpyrifos degradation activity. It utilized 50 mg/ml of chlorpyrifos as the sole carbon source and was able to tolerate upto 500 mg/ml of chlorpyrifos concentration. The optimum conditions for degradation were found to be 26.8°C and pH 6.5. It was able to metabolize chlorpyrifos completely under these conditions and no accumulation of TCP was observed.

4.3. Biodegradation by Co-culture of Micro-Organisms

Although researchers were successful in the isolation of many chlorpyrifos degrading microorganisms, however, there are very few reports of single microorganisms capable of efficiently degrading chlorpyrifos and its toxic metabolite TCP completely. Therefore, some approaches have been made towards co-culturing of microorganisms to ensure complete degradation of chlorpyrifos which was proven more efficient than single culture technique. John, *et al.* [66] assembled a novel bacterial consortium namely C5 consisting of *Staphylococcus warneri* CPI2, *Pseudomonas putida* CPI9 and *Stenotrophomonas maltophilia* CPI15 showing 90% degradation of chlorpyrifos (125 ppm) in 8days of incubation period at pH 7.0 and temperature 30ºC. The co-culture of a bacterial strain *Cellulomonas fimi*, that could transform chlorpyrifos to TCP and a fungal strain *Phanerochaete chrysosporium* that could utilize TCP showed complete mineralization of 50 mg/L CP within 16h at 33ºC and at pH 8.4 [45]. Sethunathan and Yoshida [67] reported degradation of CP by co-culture of *Flavobacterium* sp. and *Pseudomonas diminuta* in liquid media which were initially isolated from a diazinon treated field and by parathion enrichment, respectively. Xu, *et al.* [58] observed complete mineralization of 50 mg/ml of chlorpyrifos within 18h at 37°C by co-culturing *Serratia* sp. that could transform chlorpyrifos to TCP and *Trichosporon* sp. that further mineralized TCP.

Sasikala, *et al.* [68] reported efficient development of a consortium using four bacterial isolates namely *Pseudomonas putida* (NII 1117), *Klebsiella* sp., (NII 1118), *Pseudomonas stutzeri* (NII 1119), and *Pseudomonas aeruginosa* (NII 1120) capable of degrading chlorpyrifos to produce chlorpyrifos-oxon and Diethylphosphorothioate and concluded that the intracellular fractions of the consortium exhibited more organophosphorus hydrolase activity (0.171 ± 0.003 U/mL/min) while experiment was carried out at neutral pH and temperature 37°C with chlorpyrifos concentration 500 mg/L.

4.4. Enzymes Involved in Chlorpyrifos Biodegradation

Enzyme based bioremediation has now become emerging technology to decontaminate pesticide residues from agricultural soils and wastewater. Among various strategies used by the microorganism, the enzymes play a central role in the biodegradation of organophosphorous pesticide. Like most other organophosphorous pesticide, the most commonly studied enzymes for chlorpyrifos degradation are related to the phosphotriesterase (PTEs) or organophosphate hydrolase (OPH), which are capable of hydrolyzing organophosphorus pesticides at the central phosphorus atom containing P–O, P–F and P–S bonds. OPH activities in microbes is influence by various factors like varying incubation time period, pH, temperature, carbon sources, metal ions and various chemical compounds. Researchers had reported maximum OPH activity at 35°C and 37°C in *Pseudomonas stutzeri* S7B4 and *Pseudomonas aeruginosa* NL01, respectively [69]. Many researchers reported alkaline pH to be the optimum for maximum OPH activity [70]. Ningfeng, *et al.* [71] observed highest OPHC2 activity in *Pseudomonas pseudoalcaligenes* at pH 9.0. The OPH enzymes include Methyl Parathion Hydrolase (MPH), Mevalonate Pyrophosphate Decarboxylase (MPD) etc. The OPH was first isolated from *Pseudomonas diminuta* MG has the ability to hydrolyze a wide range of organophosphorus compounds [72]. Yang, *et al.* [73] discovered an opdA enzyme from *A. radiobacter* P230 that degraded a broad range of organophosphates. Singh, *et al.* [38] studied and reported a novel PTE enzyme system isolated from *Enterobacter* sp. B-14 encoding gene that had a different sequence from known organophosphate-degrading opd gene. Cui, *et al.* [74] isolated Methyl Parathion Hydrolase (MPH, E.C.3.1.8.1) differ from OPH from *Plesiomonas* sp. M6 that was proven to be crucial enzyme for hydrolyzing a broad spectrum of organophosphorus compounds. Khalid, *et al.* [75] identified *Pseudomonas putida* CP-1 that can grow efficiently on phosphorus free media while utilizing chlorpyrifos as a sole source of phosphorus. Cell free extract (CFE) obtained from *Pseudomonas putida* CP-1 further confirmed the triesterase activity as well as presence of phosphotriesterase as it hydrolyzes chlorpyrifos to yield TCP as a major metabolite. Most of the chlorpyrifos hydrolyzing enzymes are reported from bacterial species while there are few reports available in literature in case of fungal species. However, several fungi are reported to produce enzymes and therefore, have been suggested as potential candidates for bioremediation. Gao, *et al.* [11] purified and characterized a novel chlorpyrifos hydrolase from cell extract of the fungi *Cladosporium cladosporioides* Hu-01 and reported its involvement in the degradation of chlorpyrifos.

4.5. Genes Involved in Chloryrifos Biodegradation

With rapid progression in the avenue of metagenomics and genome sequencing, the scope for investigating the novel genes possessing pesticide degradative efficiencies have opened up. In order to explore and successful implementation of these technologies in the field of remediation, it is crucial to identify microorganisms containing the efficient genes to degrade chlorpyrifos. Various catabolic genes including organophosphate-degrading genes (opd) and mpd from different microorganisms from different geographical regions have been characterized by many researchers. Most of these catabolic genes involved in degradation are extra-chromosomal or genomic while some other found in transposons [7, 76]. The first described opd gene was found in *Pseudomonas diminuta*, and was shown to be present on a plasmid [77]. A similar gene, opdA, is present in the chromosome of *Agrobacterium radiobacter* [78]. Guha, *et al.* [79] also reported the involvement of plasmids in degradation of chlorpyrifos by *Micrococcus* sp. isolated from soil. Singh and Walker [7] were successful in cloning opd gene capable of encoding OPH from geographically different regions and taxonomically different species. The opd genes isolated from *Bacillus diminuta* and *Flavobacterium* sp. ATCC 27551 were located on non-homologous plasmids that possess 100% similarity in DNA sequences. Very recently, a novel gene opdE (753 bp encoding a protein of 25 kDa) from *Enterobacter* sp. which showed no similarity to any previously isolated genes reported to degrade organophosphates was characterized by Chino-Flores, *et al.* [80]. Another gene, mpd, encoding an organophosphate degrading protein, was isolated from a parathion-methyl degrading *Plesiomonas* sp. that showed no homology to the known opd genes [14, 74] isolated *Sphingomonas* strain Dsp-2 having the ability to hydrolyze chlorpyrifos to TCP by the gene encoding the chlorpyrifos hydrolytic enzyme with its greater hydrolytic efficiency than the wildtype mpd from *Plesiomonas* sp. M6. mpd gene has also been reported to found in other genera of microbes. mpd gene located on the chromosome of *Stenotrophomonas* sp. YC-1 was reported by Yang, *et al.* [44].

5. Genetically Engineered Bacteria Involved in Chloryrifos Biodegradation

Along with genes and enzymes, genetically engineered microorganisms made with gene and enzyme engineering has become a rapidly growing and promising tool for degradation of various pollutants. Alteration in specificity of MPH in *Pseudomonas putida* JS444 enhances the rate of chlorpyrifos degradation. For the first time, MPH has been reported to display on the surface of microorganisms by using only N- and C-terminal domains of the Ice Nucleation Protein (INPNC) from *P. syringae* INA5 as an anchoring motif. A shuttle vector pINCM coding for INPNC–MPH was constructed and used to target MPH onto the surface of a *Pseudomonas putida* JS444 [81]. Cao,

et al. [82] cloned a novel 6012 bp gene cluster from TCP-degrading strain P2 responsible for dehalogenation of TCP. The gene cluster consisted of a monooxygenase gene (tcpA1), a flavin reductase gene (tcpB1), tcpR1, orf1 and orf2. TcpA1 and TcpB1 worked together to catalyze the dehalogenation of three chlorine of TCP, and generated a more readily biodegradable product of 3, 6-dihydroxypyridine-2, 5- dione. The cloning of mpd gene from chlorpyrifos degrading bacterial strains to *Escherichia coli* helps in developing its biodegradation capability. Wang, *et al.* [83] cloned *Escherichia coli* with opd gene that degrade chlorpyrifos co-metabolically. Yang, *et al.* [44] cloned the mpd gene from chlorpyrifos degrading bacterium *Stenotrophomonas* sp. isolated using chlorpyrifos as the sole source of carbon by enrichment method that degraded 100 mg/L of chlorpyrifos within 24h to DETP and TCP.

5.1. Immobilized Cells and Enzymes in Bioremediation

Various cells and enzymes are sensitive and unstable at extreme temperatures, in strong acids and alkaline solutions and also in organic solvents. Besides, they can lose their ability to degrade substrates under certain environmental stresses. Therefore, the immobilization of desired cells and enzymes on suitable materials as carriers is gaining much consideration to extend the process of pollutant degradation. However, the information regarding to the immobilization and characterization of enzymes that can degrade chlorpyrifos is limited [84]. Qiao, *et al.* [85] reported immobilized cells of recombinant *Escherichia coli* can detoxify an aqueous waste stream containing the insecticides, acetofenate and chlorpyrifos. An enhanced degradation of chlorpyrifos from 40.17% to 71.05% with the immobilization of cells in mixed culture of *Streptomyces* sp. was reported by Fuentes, *et al.* [86]. Vijayalakshmi and Usha [8] studied degradation of chlorpyrifos by free cells and calcium-alginate immobilized cells of *Pseudomonas putida* isolated from an agricultural soil. Ca-alginate immobilized cells of *Pseudomonas putida* had higher degradation efficiency of 96% for chlorpyrifos at 2% in comparison to 76% degradation by free cells. Wang, *et al.* [87] found 70 % degradation rate of chlorpyrifos in soil after 48h of treatment with immobilized fungal laccase on sodium alginate that was prepared by the method of embedding-crosslinking under different environmental conditions. Xie, *et al.* [84] studied the free enzyme from *Fusarium* sp. LK. ex Fx and reported it is stable at 40°C. However, after immobilization, the degrading enzyme was more stable at even higher temperatures of 50°C with only a slight loss in its initial activity, even after three repeated uses.

6. Conclusion

In accompanied with other pesticides, various concerns regarding environmental pollution and human health have been originated from the appliance of chlorpyrifos. Though a large number of microorganisms have been isolated and characterized that can degrade and mineralize this compound, extensive research is going on for the co-culturing of two or more microorganisms and development of efficient degradation pathways for chlorpyrifos. Further studies on the genes, enzymes and immobilization responsible for enhanced biodegradation can be beneficial in this regard. By integrating functional genomics, proteomics, transcriptomics and metabolomic data with bioremediation process, a more exact system of microbial bioremediation is expected. Additionally, more research on Omics-Based Approaches will enable us to detect even tiny amount of this anthropogenic compound.

References

[1] Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J. C., and García-Río, L., 2008. "The mobility and degradation of pesticides in soils and the pollution of groundwater resources." *Agricul, Ecosys. Environ.*, vol. 123, pp. 247-260.

[2] Javaid, M. K., Ashiq, M., and Tahir, M., 2016. "Potential of biological agents in decontamination of agricultural soil." *Scientifica*,

[3] Hossain, M. S., Chowdhury, M. A. Z., Pramanik, M. K., Rahman, M. A., Fakhruddin, A. N. M., and Alam, M. K., 2015. "Determination of selected pesticides in water samples adjacent to agricultural fields and removal of organophosphorus insecticide chlorpyrifos using soil bacterial isolates." *Appl. Wat. Sci.*, vol. 5, pp. 171-179.

[4] Van, d. W. H. M., 1996. "Assessing the impact of pesticides on the environment." *Agricul, Ecosyst. Environ.*, vol. 60, pp. 81-96.

[5] Chowdhury, M. A. Z., Banik, S., Uddin, B., Moniruzzaman, M., Karim, N., and Gan, S. H., 2012. "Organophosphorus and carbamate pesticide residues detected in water samples collected from paddy and vegetable fields of the Savar and Dhamrai Upazilas in Bangladesh." *Int. J. Environ. Res. Public Health*, vol. 9, pp. 3318-3329.

[6] Wilson, C. and Tisdell, C., 2001. "Why farmers continue to use pesticides despite environmental, Health and sustainability costs." *Ecol. Econ.*, vol. 39, pp. 449-462.

[7] Singh, B. K. and Walker, A., 2006. "Microbial degradation of organophosphorus compounds." *FEMS. Microbiol. Rev.*, vol. 30, pp. 428– 471.

[8] Vijayalakshmi, P. and Usha, M. S., 2012. "Optimization of chlorpyrifos degradation by Pseudomonas putida." *J. Chem. Pharm. Res.*, vol. 4, pp. 2532-2539.

[9] Mukti, S. S., Ahmed, G. U., Ahmed, Z. F., Sumon, K. A., and Fatema, M. K., 2018. "Histopathological study of female striped Gourami (Trichogaster fasciata, Bloch & Schneider, 1801) Gill exposed to Chlorpyrifos." *Int. J. Aquac.*, p. 8.

[10] Pengphol, S., Uthaibutra, J., Arquero, O. A., Nomura, N., and Whangchai, K., 2012. "Oxidative degradation and detoxification of chlorpyrifos by ultrasonic and ozone treatments." *J. Agricul. Sci.*, vol. 4, p. 164.

[11] Gao, Y., Chen, S., Hu, M., Hu, Q., Luo, J., and Li, Y., 2012. "Purification and characterization of a novel chlorpyrifos hydrolase from " *Cladosporium Cladosporioides Hu-01, PLoS One*, vol. 7, p. e38137.

[12] Li, B., Ricordel, I., Schopfer, L. M., Baud, F., Mégarbane, B., Masson, P., Lockridge, O., and Dichlorvos, 2010. "Chlorpyrifos oxon and Aldicarb adducts of butyrylcholinesterase, Detected by mass spectrometry in human plasma following deliberate overdose." *J. Appl. Toxicol.*, vol. 30, pp. 559-565.

[13] Manclús, J. J. and Montoya, A., 1995. "Development of immunoassays for the analysis of chlorpyrifos and its major metabolite 3, 5, 6-trichloro-2-pyridinol in the aquatic environment." *Anal. Chim. Acta.*, vol. 311, pp. 341-348.

[14] Li, X., He, J., and Li, S., 2007. "Isolation of a chlorpyrifos-degrading bacterium, Sphingomonas sp. strain Dsp-2, and cloning of the mpd gene." *Res. Microbiol.*, vol. 158, pp. 143-149.

[15] Kavitha, P. and Rao, J. V., 2008. "Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, Gambusia affinis." *Environ. Toxicol. Pharmacol.*, vol. 26, pp. 192–198.

[16] Shafiq-Ur-Rehman, R. S. and Waliullah, M. I. S., 2012. "Chlorpyrifos-induced neuro-oxidative damage in bee." *Toxicol. Environ. Health Sci.*, vol. 4, pp. 30-36.

[17] Rayu, S., Nielsen, U. N., Nazaries, L., and Singh, B. K., 2017. "Isolation and molecular characterization of novel chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol-degrading bacteria from sugarcane farm soils." *Front Microbiol.*, vol. 8, p. 518.

[18] Eissa, F. I., Mahmoud, H. A., Massoud, O. N., Ghanem, K. M., and Gomaa, I. M., 2014. "Biodegradation of chlorpyrifos by microbial strains isolated from agricultural wastewater." *J. Am. Sci.*, vol. 10, pp. 98-108.

[19] Abraham, J., Shanker, A., and Silambarasan, S., 2013. "Role of Gordonia sp. JAAS1 in biodegradation of chlorpyrifos and its hydrolysing metabolite 3, 5, 6-trichloro-2-pyridinol." *Lett. Appl. Microbiol.*, vol. 57, pp. 510-516.

[20] Pailan, S., Sengupta, K., Ganguly, U., and Saha, P., 2016. "Evidence of biodegradation of chlorpyrifos by a newly isolated heavy metal-tolerant bacterium Acinetobacter sp. strain MemCl4." *Environ. Earth Sci.*, vol. 75, p. 1019.

[21] Racke, K. D., Laskowski, D. A., and Schultz, M. R., 1990. "Resistance of chlorpyrifos to enhanced biodegradation in soil." *J. Agric. Food Chem.*, vol. 38, pp. 1430-1436.

[22] Aislabie, J. and Lloyd-Jones, G., 1995. "A review of bacterial degradation of pesticides." *Aust. J. Soil Res.*, vol. 33, pp. 925-942.

[23] Simon, D., Helliwell, S., and Robards, K., 1998. "Analytical chemistry of chlorpyrifos and diuron in aquatic ecosystems." *Analytica. Chimica. Acta.*, vol. 360, pp. 1-16.

[24] Chambers, J. E. and Carr, R. L., 1993. "Inhibition patterns of brain acetylcholinesterase and hepatic and plasma aliesterases following exposures to three phosphorothionate insecticides and their oxons in rats." *Toxicol. Sci.*, vol. 21, pp. 111-119.

[25] Sultatos, L. G., 1991. "Metabolic activation of the organophosphorus insecticides chlorpyrifos and fenitrothion by perfused rat liver." *Toxicology*, vol. 68, pp. 1-9.

[26] Shen, Y. J., Lu, P., Mei, H., Yu, H. J., Hong, Q., and Li, S. P., 2010. "Isolation of a methyl parathion-degrading strain Stenotrophomonas sp. SMSP-1 and cloning of the ophc2 gene." *Biodegradation*, vol. 21, pp. 785-792.

[27] Thompson, C. M. and Richardson, R. J., 2004. *Anticholinesterase insecticides*. Pesticide Toxicology and International Regulation, pp. 89-127.

[28] Xu, G., Zheng, W., Li, Y., Wang, S., Zhang, J., and Yan, Y., 2008. "Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by a newly isolated Paracoccus sp. strain TRP." *Int. Biodeter. Biodegr.*, vol. 62, pp. 51-56.

[29] Eaton, D. L., Daroff, R. B., Autrup, H., Bridges, J., Buffler, P., Costa, L. G., Coyle, J., McKhann, G., Mobley, W. C., *et al.*, 2008. "Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment." *Crit. Rev. Toxicol.*, vol. 38, pp. 1-125.

[30] Zalat, O. A., Elsayed, M. A., Fayed, M. S., and Abd, E. M. M. K., 2014. "Validation of UV Spectrophotometric and HPLC Methods for Quantitative determination of chlorpyrifos." *Int. Lett. Chem. Phys. Astron.*, vol. 2, pp. 58-63.

[31] Civen, M., Brown, C. B., and Morin, R. J., 1977. "Effects of organophosphate insecticides on adrenal cholesteryl ester and steroid metabolism." *Biochem. Pharmacol.*, vol. 26, pp. 1901-1907.

[32] Sakai, R., 1990. "Toxicity of organic phosphates and chloronaphthalenes, Okayama Igakkai Zasshi." *Journal of Okayama Medical Association*, vol. 102, pp. 997-1005.

[33] Halima, M., Tan, Y. A., Ismail, B. S., and Nashriyah, M., 2010. "Downward movement of chlorpyrifos in the soil of an oil palm plantation in Sepang, Selangor Malaysia." *J. Oil Palm Res.*, vol. 22, pp. 721-728.

[34] Racke, K. D., Coats, J. R., and Titus, K. R., 1988. "Degradation of Chlorpyrifos and its hydrolysis products, 3, 5, 6-trichloro-2- pyridinol, in soil." *J. Environ. Sci. Health B.*, vol. 23, pp. 527-539.

[35] Akbar, S. and Sultan, S., 2016. "Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement." *Braz. J. Microbial.*, vol. 47, pp. 563-570.

[36] Gebremariam, S. Y., Beutel, M. W., Yonge, D. R., Flury, M., and Harsh, J. B., 2012. "Adsorption and desorption of chlorpyrifos to soils and sediments." *Rev. Environ. Contam. Toxicol.*, pp. 123-175.

[37] Kulshrestha, G. and Kumari, A., 2011. "Fungal degradation of chlorpyrifos by Acremonium sp. strain (GFRC-1) isolated from a laboratory-enriched red agricultural soil." *Biol. Fertil. Soils*, vol. 47, pp. 219-225.

[38] Singh, B. K., Walker, A., Morgan, J. A. W., and Wright, D. J., 2004. "Biodegradation of chlorpyrifos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils." *Appl. Env. Microbiol.,* vol. 70, pp. 4855-4863.

[39] Singh, H., 2006. *Mycoremediation, Fungal bioremediation*. John Wiley & Sons.

[40] Solomon, K. R., Williams, W. M., Mackay, D., Purdy, J., Giddings, J. M., and Giesy, J. P., 2014. *Properties and uses of chlorpyrifos in the United States*. Ecological Risk Assessment for Chlorpyrifos in Terrestrial and Aquatic Systems in the United States, pp. 13-34.

[41] Racke, K. D., 1993. "Environmental fate of chlorpyrifos." *Rev. Environ. Contam. Toxicol.,* pp. 1-150.

[42] Arbeli, Z. and Fuentes, C. L., 2007. "Accelerated biodegradation of pesticides, An overview of the phenomenon, Its basis and possible solutions; And a discussion on the tropical dimension." *Crop Protection,* vol. 26, pp. 1733-1746.

[43] Yang, L., Zhao, Y. H., Zhang, B. X., Yang, C. H., and Zhang, X., 2005. "Isolation and characterization of a chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol degrading bacterium." *FEMS. Microbiol. Lett.,* vol. 25, pp. 67-73.

[44] Yang, C., Liu, N., Guo, X., and Qiao, C., 2006. "Cloning of mpd gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil." *FEMS. Microbial. Lett.,* vol. 365, pp. 118-125.

[45] Barathidasan, K., Reetha, D., Milton, D. J., Sriram, N., and Govindammal, M., 2014. "Biodegradation of chlorpyrifos by co-culture of Cellulomonas fimi and Phanerochaete chrysosporium." *Afr. J. Microbiol. Re.,* vol. 8, pp. 961-966.

[46] Feng, F., Ge, J., Li, Y., He, S., Zhong, J., Liu, X., and Yu, X., 2017. "Enhanced degradation of chlorpyrifos in rice (Oryza sativa L.) by five strains of endophytic bacteria and their plant growth promotional ability." *Chemosphere,* vol. 184, pp. 505-513.

[47] El-Helow, E. R., Badawy, M. E., Mabrouk, M. E., Mohamed, E. A., and El-Beshlawy, Y. M., 2013. "Biodegradation of chlorpyrifos by a newly isolated." *Bacillus Subtilis Strain, Y242, Biorem. J.,* vol. 17, pp. 113-123.

[48] Fulekar, M. H. and Geetha, M., 2008. "Bioremediation of Chlorpyrifos by Pseudomonas aeruginosa using scale up technique." *J. Appl. Biosci.,* vol. 10, pp. 657-60.

[49] Chishti, Z. and Arshad, M., 2013. "Growth linked biodegradation of chlorpyrifos by agrobacterium Enterobacter sp." *Enterobacter sp. Int. J. Agric. Biol.,* vol. 15,

[50] Sabdono, A., 2013. "Biodegradation of chlorpyrifos by a marine bacterium Bacillus firmus strain associated with branching coral Acropora SP." *J. Coastal Res.,* vol. 10, pp. 115-123.

[51] Li, J., Liu, J., Shen, W., Zhao, X., Hou, Y., Cao, H., and Cui, Z., 2010. "Isolation and characterization of 3, 5, 6-trichloro-2-pyridinol-degrading Ralstonia sp. strain T6." *Bioresour. Technol.,* vol. 101, pp. 7479-7483.

[52] Anwar, S., Liaquat, F., Khan, Q. M., Khalid, Z. M., and Iqbal, S., 2009. "Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by Bacillus pumilus strain C2A1." *J. Hazard Mater,* vol. 168, pp. 400–405.

[53] Lu, P., Li, Q., Liu, H., Feng, Z., Yan, X., Hong, Q., and Li, S., 2013. "Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by Cupriavidus sp. DT-1." *Bioresour. Technol.,* vol. 127, pp. 337-342.

[54] Silambarasan, S. and Abraham, J., 2013. "Kinetic studies on enhancement of degradation of chlorpyrifos and its hydrolyzing metabolite TCP by a newly isolated Alcaligenes sp. JAS1." *J. Taiwan Inst. Chem. E.,* vol. 44, pp. 438-445.

[55] Feng, Y., Racke, K. D., and Bollag, J., 1997. "Isolation and characterization of a chlorinated-pyridinol-degrading bacterium." *Appl. Environ. Microbiol.,* vol. 63, pp. 4096-4098.

[56] Rani, M. S., Devi, K. V. L. P. S., Madhuri, R. J., Aruna, S., Jyothi, K., Narasimha, G., and Venkateswarlu, K., 2008. "Isolation and characterization of a chlorpyrifos-degrading bacterium from agricultural soil and its growth response." *Afr. J. Microbio. Res.,* vol. 2, pp. 26-31.

[57] Jabeen, H., Iqbal, S., and Anwar, S., 2015. "Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by a novel rhizobial strain Mesorhizobium sp. HN3." *Water Environ. J.,* vol. 29, pp. 151-160.

[58] Xu, G., Li, Y., Zheng, W., Peng, X., Li, W., and Yan, Y., 2007. "Mineralization of chlorpyrifos by co-culture of Serratia and Trichosporon SP." *Biotechnol. Lett.,* vol. 29, pp. 1469-1473.

[59] Harms, H., Schlosser, D., and Wick, L. Y., 2011. "Untapped potential, Exploiting fungi in bioremediation of hazardous chemicals." *Nat. Rev. Microbiol.,* vol. 9, pp. 177-192.

[60] Ivashina, S. A., 1986. "Interaction of Dursban with soil micro-organisms, Agrokhimia." pp. 75-76.

[61] Jones, A. S. and Hastings, F. L., 1981. "Soil microbe studies. In, Hastings F.L., Coster J.E., Field and laboratory evaluations of insecticides for southern pine beetle control. USDA, Southern Forest Experiment Station." *Forest Service, SE.,* vol. 21, pp. 13-14.

[62] Fang, H., Xiang, Y. Q., Hao, Y. J., Chu, X. Q., Pan, X. D., Yu, J. Q., and Yu, Y. L., 2008. "Fungal degradation of chlorpyrifos by Verticillium sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi." *Int. Biodeter. Biodegr.,* vol. 61, pp. 294-303.

[63] Lal, S. and Lal, R., 1987. "Bioaccumulation, Metabolism, And effects of DDT, Fenitrothion, And chlorpyrifos on Saccharomyces cerevisiae." *Arch. Environ. Contam. Toxicol.,* vol. 16, pp. 753-757.

[64] Bumpus, J. A., Powers, R. H., and Sun, T., 1993. "Biodegradation of DDE (1, 1-dichloro-2, 2-bis (4-chlorophenyl) ethene) by Phanerochaete chrysosporium." *Mycol. Res.,* vol. 97, pp. 95-98.

[65] Chen, S., Liu, C., Peng, C., Liu, H., Hu, M., and Zhong, G., 2012. "Biodegradation of chlorpyrifos and its hydrolysis product 3, 5, 6-trichloro-2-pyridinol by a new fungal strain." *Cladosporium Cladosporioides Hu-01 Plos One*, vol. 7, p. 47205.

[66] John, E. M., Sreekumar, J., and Jisha, M. S., 2016. "Optimization of chlorpyrifos degradation by assembled bacterial consortium using response surface methodology." *Soil Sediment Contam.*, vol. 25, pp. 668-682.

[67] Sethunathan, N. and Yoshida, T., 1973. "A Flavobacterium sp. that degrades diazinon and parathion." *Can. J. Microbiol.*, vol. 19, pp. 873-875.

[68] Sasikala, C., Jiwal, S., Rout, P., and Ramya, M., 2012. "Biodegradation of chlorpyrifos by bacterial consortium isolated from agriculture soil." *World J. Microbiol. Biotechnol.*, vol. 28, pp. 1301-1308.

[69] Najavand, S., Lotfi, A. S., Mohsenifar, A., Arjmand, S., and Mota, A., 2012. "Expression and characterization of bacterial organophosphorus hydrolase in Pichia pastoris with the intent to degrade organophosphate neurotoxins." *Pathobiology Res.*, vol. 15, pp. 61-72.

[70] DeFrank, J. J. and Cheng, T. C., 1991. "Purification and properties of an organophosphorus acid anhydrase from a halophilic bacterial isolate." *J. Bacteriol.*, vol. 173, pp. 1938-1943.

[71] Ningfeng, W., Minjie, D., Guoyi, L., Xiaoyu, C., Bin, Y., and Yunliu, F., 2004. "Cloning and expression of ophc2, a new organphosphorus hydrolase gene." *Chin. Sci. Bull.*, vol. 49, pp. 1245-1249.

[72] Serdar, C. M. and Gibson, D. T., 1985. "Enzymatic hydrolysis of organophosphates: cloning and expression of a parathion hydrolase gene from Pseudomonas diminuta." *Nat. Biotechnol.*, vol. 3, p. 567.

[73] Yang, H., Carr, P. D., McLoughlin, S. Y., Liu, J. W., Horne, I., Qiu, X., Jeffries, C. M. J., Russell, R. J., Oakeshott, J. G., *et al.*, 2003. "Evolution of an organophosphate-degrading enzyme, A comparison of natural and directed evolution." *Protein Eng.*, vol. 16, pp. 135-145.

[74] Cui, Z. L., Li, S. P., and Fu, G. P., 2001. "Isolation of methyl parathion degrading strain M6 and cloning of the methyl parathion hydrolase gene." *Appl. Environ. Microbiol.*, vol. 67, pp. 4922–4925.

[75] Khalid, M., Rasul, S., Hussain, J., Ahmad, R., Zia, A., Bilal, M., Pervez, A., and Naqvi, T. A., 2016. "Biodegradation of Organophosphorus Insecticides, Chlorpyrifos, by Pseudomonas putida CP-1." *Pak. J. Zool.*, vol. 48,

[76] Jaiswal, S., Bara, J. K., Soni, R., and Shrivastava, K., 2017. "Bioremediation of chlorpyrifos contaminated soil by microorganism." *Int. J. Environ. Agricul. Biotechnol.*, vol. 2,

[77] Serdar, C. M., Gibson, D. T., Munnecke, D. M., and Lancaster, J. H., 1982. "Plasmid involvement in parathion hydrolysis by Pseudomonas diminuta." *Appl. Environ. Microbiol.*, vol. 44, pp. 246-249.

[78] Horne, I., Sutherland, T. D., Harcourt, R. L., Russell, R. J., and Oakeshott, J. G., 2002. "Identification of an OPD (Organophosphate Degradation) gene in an Agrobacterium isolate." *Appl. Environ. Microbiol.*, vol. 68, pp. 3371-3376.

[79] Guha, A., Kumari, B., Bora, T. C., and Roy, M. K., 1997. "Possible involvement of plasmids in degradation of malathion and chlorpyrifos." *Micrococcus sp. Folia Microbiol.*, vol. 42, pp. 574-576.

[80] Chino-Flores, C., Dantán-González, E., Vázquez-Ramos, A., Tinoco-Valencia, R., Díaz-Méndez, R., Sánchez-Salinas, E., Castrejón-Godínez, M. L., Ramos-Quintana, F., and Ortiz-Hernández, M. L., 2012. "Isolation of the opdE gene that encodes for a new hydrolase of Enterobacter sp. capable of degrading organophosphorus pesticides." *Biodegradation*, vol. 23, pp. 387-397.

[81] Yang, C., Cai, N., Dong, M., Jiang, H., Li, J., Qiao, C., Mulchandani, A., and Chen, W., 2008. "Surface display of MPH on Pseudomonas putida JS444 using ice nucleation protein and its application in detoxification of organophosphates." *Biotechnol. Bioeng.*, vol. 99, pp. 30-37.

[82] Cao, L., Xu, J., Wu, G., Li, M., Jiang, J., He, J., Li, S., and Hong, Q., 2013. "Identification of two combined genes responsible for dechlorination of 3, 5, 6-trichloro-2-pyridinol (TCP)." *Cupriavidus Pauculus P2 J. Haz. Mat.*, vol. 260, pp. 700-706.

[83] Wang, A. A., Mulchandani, A., and Chen, W., 2002. "Specific adhesion to cellulose and hydrolysis of organophosphate nerve agents by a genetically engineered Escherichia coli strain with a surface-expressed cellulose-binding domain and organophosphorus hydrolase." *Appl. Environ. Microbiol.*, vol. 68, pp. 1684-1689.

[84] Xie, H., Zhu, L., Ma, T., Wang, J., Wang, J., Su, J., and Shao, B., 2010. "Immobilization of an enzyme from a Fusarium fungus WZ-I for chlorpyrifos degradation." *J. Environ. Sci.*, vol. 22, pp. 1930-1935.

[85] Qiao, C. L., Yan, Y. C., Shang, H. Y., Zhou, X. T., Zhang, Y., and Qiao, C., 2000. "Biodegradation of pesticides by immobilized cells of recombinant." *Escherichia coli*,

[86] Fuentes, M. S., Briceño, G. E., Saez, J. M., Benimeli, C. S., Diez, M. C., and Amoroso, M. J., 2013. "Enhanced removal of a pesticides mixture by single cultures and consortia of free and immobilized Streptomyces strains." *Biomed. Res. Int.*,

[87] Wang, X., Yao, M. Q., Liu, L., Cao, Y., and Bao, J., 2016. "Degradation of chlorpyrifos in contaminated soil by immobilized laccase." *J. Serb. Chem. Soc.*, pp. 66-66.

List of Figures

Fig-1. Chemical formula of chlorpyrifos

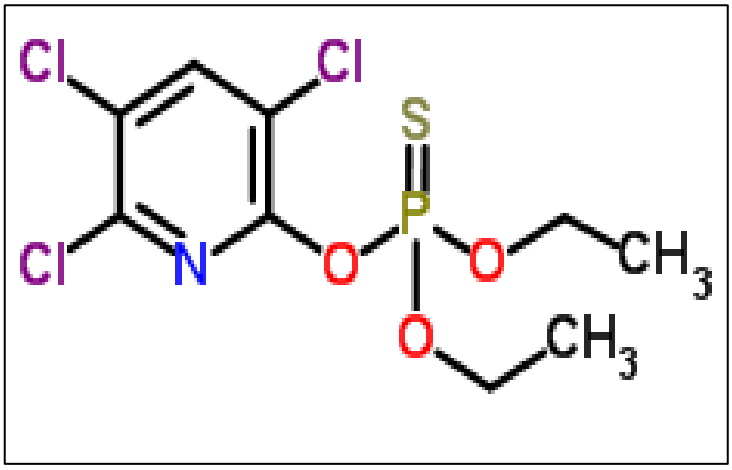

Fig-2. Effect of Chlorpyrifos on the AChE (Gilani et al., 2015)

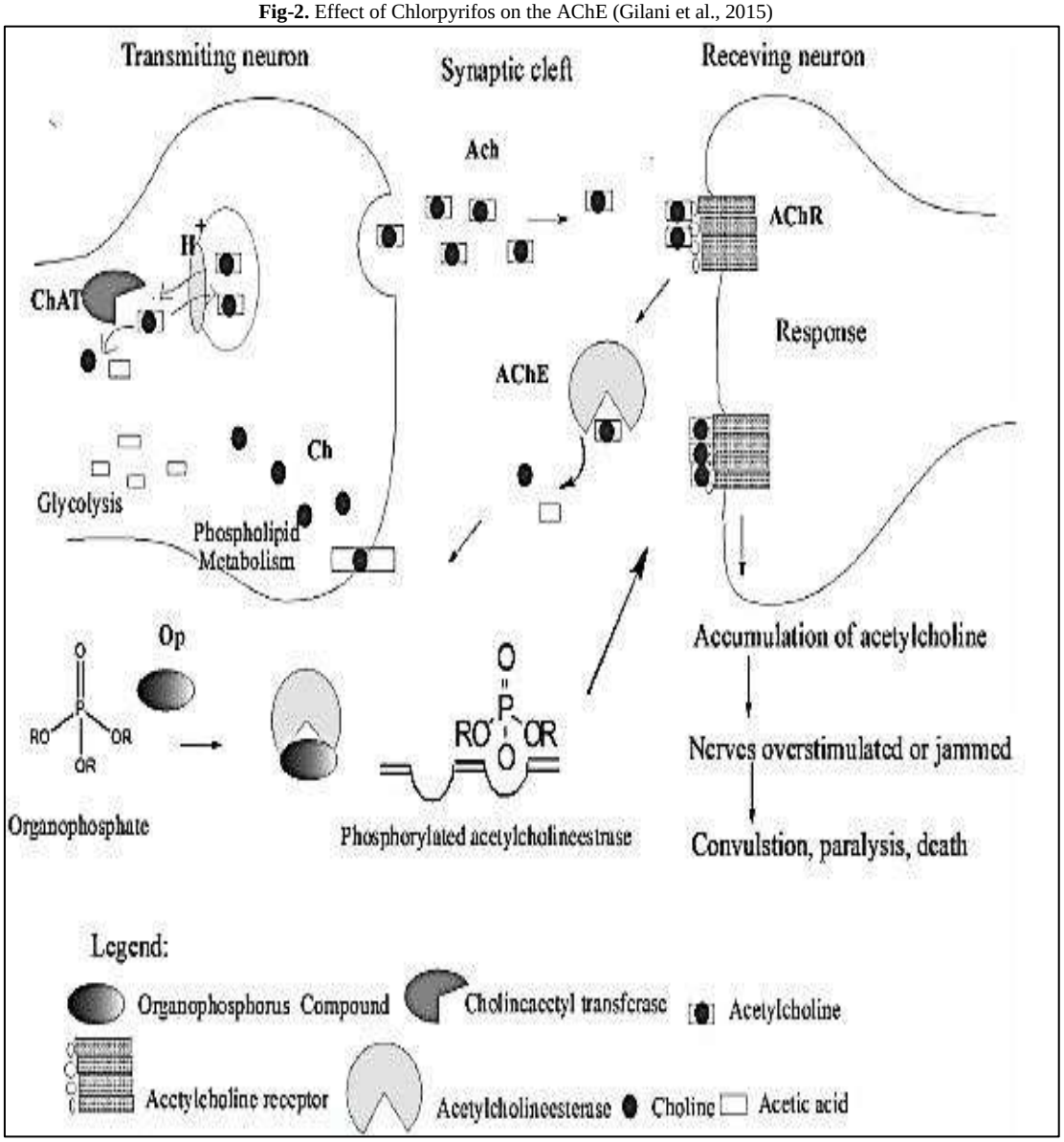

Fig-3. Proposed pathway of Chlorpyrifos degradation