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Bioremediation of Chlorpyrifos Contaminated Soil by Microorganism

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Abstract— *India is agricultural based country where 70% of the population survives on it. In order to increase the production of field various pesticides are used. Chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) is an organophosphate pesticide which is widely used as insecticide for crop protection. But due to its persistent nature into the environment, it is leading to various hazards including neurotoxic effects, cardiovascular diseases and respiratory diseases. Bioremediation is a technology to eliminate chlorpyrifos efficiently from the environment. In bioremediation of chlorpyrifos the potential degradative microorganisms possess opd (organophosphate degrading) gene which hydrolyses the chlorpyrifos and utilizes it as a sole carbon source.Thus the present review discusses about how through bioremediation the pesticide chlorpyrifos can be degraded using potential soil microorganisms.*

Keywords— Pesticides, Organophosphate, Chlorpyrifos, opd gene, Bioremediation.

I. IMPACT OF MODERN AGRICULTURE ON ENVIRONMENT

Until about four decades agoin agricultural systems, crop yields depended totally on internal resources like recycling of organic matter, biological control mechanisms and rainfall patterns. Agricultural yields were modest, but stable. To safeguard the production, variety of crops were grown in space and time in a field as insurance against pest outbreaks or severe weather. Inputs of nitrogen were gained by rotating major field crops with legumes. In turn rotations suppressed insects, weeds and diseases by effectively breaking the life cycles of these pests. In these types of farming systems the link between agriculture and ecology was quite strong and signs of environmental degradation were seldom evident. But as agricultural modernization progressed, the ecology-farming linkage was often broken as ecological principles were ignored or overridden. In fact, several agricultural scientists have arrived at a general consensus that modern agriculture confronts an environmental crisis. Evidence has accumulated showing that whereas the present capital- and technology intensive farming systems have been extremely productive and competitive; they also bring a variety of economic, environmental and social problems (Altieri and Rosset, 1995).

The very nature of the agricultural structure and prevailing policies in a capitalist setting have led to an environmental crisis by favouring large farm size, specialized production, crop monocultures and mechanization. Today as more and more farmers are integrated into international economies, the biological imperative of diversity disappears due to the use of many kinds of pesticides and synthetic fertilizers, and specialized farms are rewarded by economies of scale. In turn, lack of rotations and diversification take away key self-regulating mechanisms, turning monocultures into highly vulnerable agro-ecosystems dependent on high chemical inputs. Also, fields that in the past contained many different crops, or a single crop with a high degree of genetic variability, are now entirely devoted to a genetically uniform single crop. The specialization of farms has led to the image that agriculture is a modern miracle of food production. However, excessive reliance on farm specialization has negatively impacted the environment (Altieri and Rosset, 1995).

II. USE OF PESTICIDES IN CROP PROTECTION

Pesticides are those substances which are used to control, destroy, repel or attract pests in order to minimise their detrimental effects. Pests are those organisms like weeds, insects, bacteria, fungi, viruses and animals which adversely affect our way of life. Pests can reduce the quality and quantity of food produced by lowering production and destroying stored produce; they can harm our animals (like fleas, worms and diseases); they compete with humans for food and affect the health, welfare and way of life of people; they can destroy buildings (termites) and are a major cause of land degradation (noxious weeds, rabbits, feral pigs, etc). Pest activity greatly increases the costs of farming. Pesticides therefore are used in many situations such as livestock farming, cropping, horticulture, forestry, home gardening, homes, hospitals, kitchens, road-sides, recreational and industrial areas (Jayashree and Vasudevan, 2007).

Pesticides may be derived from inorganic sources (copper, sulphur), natural organic sources (plants) or be organic compounds synthesised in a laboratory.Many of the

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earliest pesticides were either inorganic products or derived from plants, for example burning sulphur to control insects and mites. Other early insecticides included hellebore to control body lice, nicotine to control aphids, and pyrithrin to control a wide variety of insects. Lead arsenate was first used in 1892 as an orchard spray while about the same time it was accidentally discovered that a mixture of lime and copper sulphate controlled downy mildew, a serious fungal disease of grapes. It is still one of the most widely used fungicides. Many of these early chemicals had disadvantages. They were often highly toxic, were very persistent, posing a threat to the environment (Jayashree and Vasudevan, 2007).

III. USE OF CHLORPYRIFOS IN CROP PROTECTION

Chlorpyrifos is a broad-spectrum insecticide. It is a type of organophosphorus insecticide. Chemically, it is *O,O-diethyl-O-(3,5,6-trichloro-2- pyridinol) phosphorothioate*. It is used in field protection of corn, cotton, peaches, apple etc. Termites and insects are susceptible to chlorpyrifos[3]. While originally used primarily to kill mosquitoes, Chlorpyrifos is effective in controlling cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice. It is used as an insecticide on grain, cotton, field, fruit, nut and vegetable crops, and well as on lawns and ornamental plants. It is also registered for use in domestic dwellings, farm buildings, storage bins, and commercial establishments. Chlorpyrifos acts on pests primarily as a contact poison, with some action as a stomach poison. It is available as granules, wettable powder, dustable powder, and emulsifiableconcentrate (Mallick *et al.*, 1999).

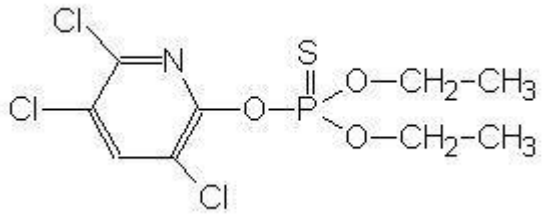

Fig.1. Chemical structure of Chlorpyrifos

IV. PROPERTIES OF CHLORPYRIFOS

The physical and chemical properties of a pesticide plays significant role indetermining its environmental fate and transport. The physical and chemicalproperties of chlorpyrifos are as mentioned below (Ajaz *et al.*, 2005):

Chemical Name - O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate
Molecular Formula - $C_9H_{11}Cl_3NO_3PS$
Rel. Molecular Mass - 350.62
Density - 1.38 g/cc at 46°C
Boiling point - Decomposes before boiling. Thermal decomposition occurs between 160-180°C
Melting point - 41.5 – 44°C

V. HAZARDS OF CHLORPYRIFOS

Chlorpyrifos is moderately toxic to humans. It primarily affects the nervous system through inhibition of cholinesterase, an enzyme required for proper nerve functioning. Poisoning from chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system. It is also a skin and eye irritant. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, and slow heartbeat. Very high doses may result in unconsciousness, incontinence, and convulsions or fatality (Eaton, 2008). Transport of chlorpyrifos to human results in neural disorders, inhibition of DNA synthesis, interference with gene transcription, altered function of neurotrophicsignaling cascade and synaptic function (Lakshmi *et al.*, 2009).

VI. SOIL PERSISTENCY AND ENVIRONMENT FATE OF CHLORPYRIFOS

Chlorpyrifos is moderately persistent in soils. The half-life of chlorpyrifos in soil is usually between 60 and 120 days, but can range from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions. Chlorpyrifos was less persistent in the soils with a higher pH. Soil half-life was not affected by soil texture or organic matter content. In anaerobic soils, the half-life was 15 days in loam and 58 days in clay soil. Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes. Chlorpyrifos adsorbs strongly to soil particles and it is not readily soluble in water. It is therefore immobile in soils and unlikely to leach or to contaminate groundwater (Ajaz *et al.*, 2005).

TCP*(3,5,6-trichloro-2-pyridinol)*is the principle metabolite of chlorpyrifos, adsorbs weakly to soil particles and appears to be moderately mobile and persistent in soils. The US EPA considers that there is insufficient data to fully assess the environmental fate of Chlorpyrifos. Chlorpyrifos is tightly adsorbed by soil and not expected to leach significantly. Volatilization from soil surface will contribute to loss. Depending on soil type, microbial metabolism of Chlorpyrifos may have a half-life of up to 279 days. Higher soil temperatures, lower organic content and lower acidity increases degradation of chlorpyrifos. Chlorpyrifos inhibits nitrification and nitrogen fixation marginally, many bacterial strains were unable to degrade

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it but some microorganismscan use chlorpyrifos as their only source of carbon and nitrogen (Ajaz *et al.*, 2005).

VII. FATE IN HUMANS AND ANIMALS

Chlorpyrifos is readily absorbed into the bloodstream through the gastrointestinal tract if it is ingested, through the lungs if it is inhaled, or through the skin if there is dermal exposure. In humans, chlorpyrifos and its principal metabolites are eliminated rapidly. Chlorpyrifos is eliminated primarily through the kidneys. It is detoxified quickly in rats, dogs, and other animals. Chlorpyrifos is moderately to very highly toxic to birds. Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms. Cholinesterase inhibition was observed in acute toxicity tests of fish exposed to very low concentrations of this insecticide. Aquatic and general agricultural uses of chlorpyrifos pose a serious hazard to wildlife and honeybees (Cho *et al.*, 2004).

VIII. BIODEGRADATION OF CHLORPYRIFOS BY MICROORGANISM

Availability of different pesticides in field provides exposure of several different kinds of microorganisms to pesticides. Most of the organisms die under toxic effect of pesticides but few of them evolve in different ways and use pesticide compounds in metabolism. Several reports are available indicating degradation of different pesticides when they are available in nature in excess (Horvath, 1972, Hussain *et al.*, 2007 and Lakshmi *et al.*, 2009). Successful removal of chlorpyrifos by the addition of bacteria (bioaugmentation) had been reported (Singh *et al*., 2004).

Degradation strategies exhibited by microbes include: co metabolize the biotransformation of a molecule coincidental to the normal metabolic functions of the microbe; catabolism- the utilization of the molecule as a nutritive or energy source; and extracellular enzymes (phosphatases, amidases and laccases) – secreted into the soil, which can act on the molecule as a substrate. Three basic types of reactions can occur: degradation, conjugation, and rearrangements, and all of which can be microbially mediated. Complete degradation of a chemical in the soil to carbon dioxide and water involves many different types of reactions. Microorganisms are key players in determining the environmental fate of novel compounds because they can be used as carbon and energy sources by microorganisms (Singh, 2008).

1. Biodegradation of chlorpyrifos by Bacteria

Bacteria use natural organics such as proteins, carbohydrates, and many others as carbon and energy sources. Many of the xenobiotic compounds of environmental concern are naturally occurring relatives of these organics. For other xenobiotics, repeated exposure has resulted in the adaptation and evolution of bacteria capable of metabolizing these man-made compounds (Zhang *et al.*, 2005). Microbial degradation of organophosphate pesticides like chlorpyrifos is of particular interest because of the high mammalian toxicity of such compounds and their widespread and extensive use.

Chlorpyrifos has been shown to be degraded co-metabolically in liquid media by bacteria [14]. *Pseudomonas aeruginosa* is the most common Gram negative bacterium found in soil. Isolates of this bacterium have been found to have potential to degrade chlorpyrifos (Fulekar and Geetha, 2008). Enhanced degradation of chlorpyrifos by *Enterobacter* strain B-14 was reported (Singh *et al*., 2004). Yang *et al*., (2005), isolated *Alkaligenes faecalis* DSP3, which is also capable of degrading chlorpyrifos and results in the formation of by product 3, 5, 6-trichloro-2-pyridinol (TCP) (Rani, *et. al*., 2008). A chlorpyrifos degrading *Flavobacterium sp.* is reported by Jilani and Khan (2004).

A few chlorpyrifos-degrading bacteria, including *Enterobacter* strain B-14, *Stenotrophomonas* sp. YC-1, and *Sphingomonas* sp. Dsp-2, have been studied. The metabolism of chlorpyrifos by microorganism in soil has been reported by many scientists. Chlorpyrifos gets oxidized to exon analogue [O,Odiethyl-O- (3,5,6-trichloro-2-pyridinyl) phosphate, III] of insecticide and finally into 3,5,6-trichloropyridinol (II) (Mukherjee *et. al*., 2004). Various *opd* (organophosphate degrading) genes have been isolated from different microorganisms from different geographical regions, which have been shown to hydrolyze chlorpyrifos (Hussain *et al*., 2007).

Chlorpyrifos has been shown to be degraded co metabolically in liquid media by bacteria, and various *opd* genes have been isolated from different microorganisms from different geographical regions, some of which have been shown to hydrolyze chlorpyrifos. Chlorpyrifos has been reported to be degraded co metabolically in liquid media by *Flavobacterium* sp. and also by an *Escherichia coli* clone with an *opd* gene (Singh *et. al.*, 2004). Enhanced degradation of chlorpyrifos by *Enterobacter* strain B-14 was reported by Singh *et al*., (2004). Six chlorpyrifos-degrading bacteria were isolated using chlorpyrifos as the sole carbon source by an enrichment procedure (Rani, *et. al*., 2008).

Chlorpyrifos hydrolysis was greatly accelerated under low moisture conditions, both in acidic and alkaline soils (Ajaz, *et al.*, 2005). *Arthrobacter* sp. strain B-5 hydrolyzed chlorpyrifos at rates dependent on the substrate. Chlorpyrifos (10 mg/L) was completely degraded in the mineral salts medium by *Flavobacterium* sp. ATCC 27551 for 24 h and by *Arthrobacter* sp. for 48 h, respectively.The

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rapid degradation of chlorpyrifos, added to a mineral salts medium as a sole carbon source or applied to the soil, by the *Flavobacterium* sp. ATCC 27551 (isolated from diazinon-retreated rice fields) and the *Arthrobacter* sp. (isolated from a flooded soil retreated with methyl parathion) was reported (Xu, 2007).

The degradation of chlorpyrifos was reported in mineral salt medium by an *Arthrobacter* species that was initially isolated from methyl parathion-enriched soil (Mallick *et al*., 1999). Both *Flavobacterium* sp. ATCC 27551 and *Arthrobacter* sp. effected very rapid degradation of chlorpyrifos, added to the mineral salts medium as a sole carbon source (Mallick *et al*., 1999). Pseudomonas *aeruginosa*, *Bacillus cereus*, *Klebsiella sp.*, and S*erratiamarscecens* obtained from consortia showed 84, 84, 81, and 80% degradation of chlorpyrifos (50 mg/L) in liquid medium after 20 days and 92, 60, 56, and 37% degradation of chlorpyrifos (50 mg/L) in soil after 30 days. Some recent reports indicate bacterial degradation of chlorpyrifos by *Flavobacterium*sp. ATCC 27551 and *Arthrobacter* sp., isolated from contaminated sources, which degrade chlorpyrifosco-metabolically, and *Enterobacter* strain B-14, *Alcaligenes faecalis,* and *Klebsiella sp.*, which degrade and utilize chlorpyrifos as sole carbon source (Jilani S. and Khan, 2004). *Bacillus sp*. And *Micrococcus sp.* possess potential to degrade chlorpyrifos (Getzin, 1981; Gomez *et al*., 2007).

2. Biodegradation by Fungi

Chlorpyrifos has also been reported to be effectively degraded by two soil fungi, *Trichoderma viride* and *Aspergillus niger* [16]. Several chlorpyrifos-degrading fungi, such as *Phanerochaete chrysosporium, Aspergillus terreus,* and *Verticillium* sp. DSP have also been reported [17]. *Verticillium* sp. and *Brassica chinensis* are reported for degradation of chlorpyrifos in culture medium ranging from 1 to 100 mg/L. Methods of *in-situ* bioremediation of *Verticillium* sp. are also developed and achieved good results (Arisoy, 1998; Trejo and Quintero, 2000; Bhalerao and Puranik, 2007). Fungal degradation of chlorpyrifos was reported by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil (Xu, 2007).

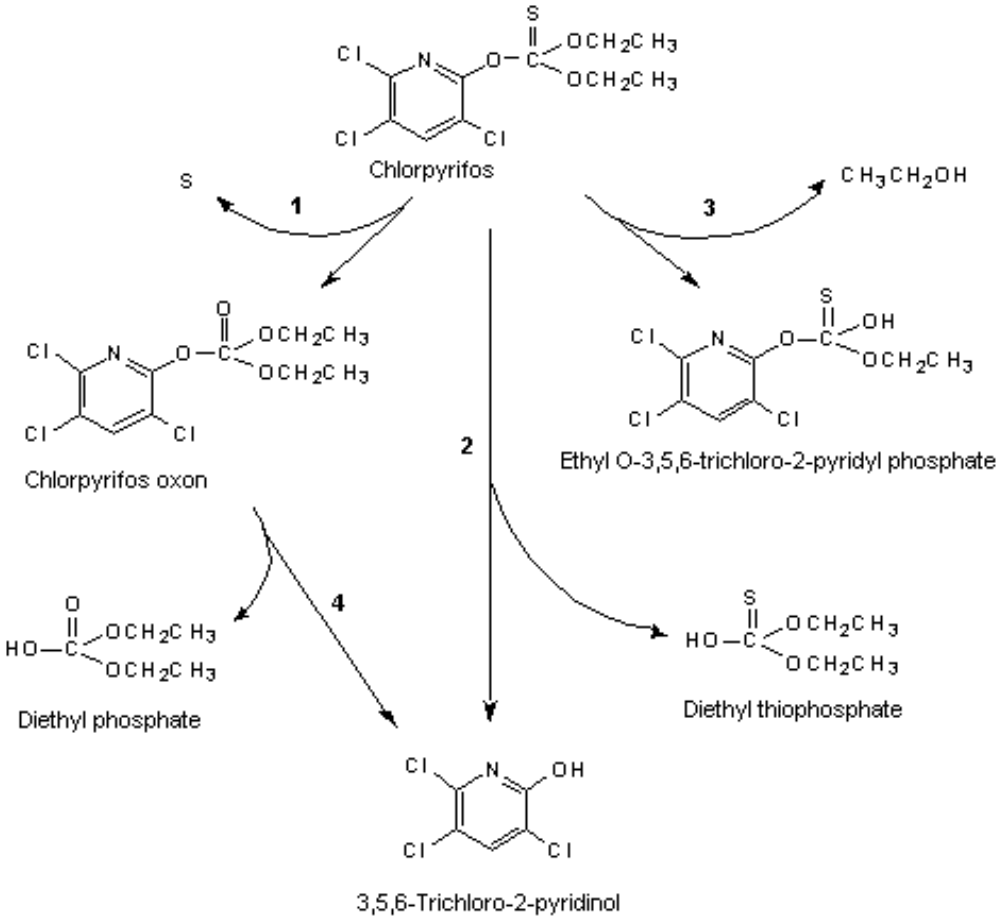

Fig.2: Pathway of Biodegradation of chlorpyrifos by microorganism (Smith *et al.*, 1967).

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A fungal strain capable of utilizing chlorpyrifosas sole carbon and energy sources from soil and degradation of chlorpyrifos in pure cultures and on vegetables by this fungal strain and its cell-free extract is also reported. This strain was identified as an unknown species of *Verticillium.* It opens a new research direction for development of novel bioremediation process .The chlorinated pyridinyl ring of chlorpyrifos undergoes cleavage during biodegradation by *P. chrysosporium*. But the degradation of chlorpyrifos proves more efficient by mixed populations than by pure cultures of fungi. Mixed population of fungi, such as *Alternaria alternata*, *Cephalosporium* sp., *Cladosporium cladosporioides*, *Cladorrhinum brunnescens*, *Fusarium* sp., *Rhizoctonia solani, and Trichoderma viride*, reveal the degradation of chlorpyrifos in liquid culture more efficiently (Singh *et al*., 2004).

IX. ENZYMATIC ACTIVITY IN CHLORPYRIFOS BIODEGRADATION

Organophosphorus hydrolase is one of the important hydrolytic enzymes in detoxification technology that hydrolyze chlorpyrifos pesticides containing P–O, P–F and P–S bonds. The OPH enzymes, including O-Phenylenediamine Dihydrochloride (OPD), Methyl Parathion Hydrolase (MPH) Mevalonate Pyrophosphate Decarboxylase (MPD) etc., were identified for the hydrolysis for chlorpyrifos (Meysami and Baheri, 2003). The degradation of chlorpyrifos induced Organophosphorous Phosphatase (OPP) production and concentration were 28 times higher in the extracellular than inside the cells. The chlorpyrifos degradation efficiency for *L. fermentum*, *L. lactis* and *E. coli* were reported to 70 per cent, 61 per cent and 16 per cent with 3,5,6-trichloro-2-pyridinol (TCP), chlorpyrifos-oxon and diethyl-phosphatase end products respectively. Purification and characterization of a novelchlorpyrifos hydrolase from the fungi *Cladosporium cladosporioides* Hu-01 was done (Bhagobaty *et al.*, 2007).

X. GENES INVOLVED IN CHLORPYRIFOS BIODEGRADATION

The organophosphate-degrading *opd, mpd* genes were isolated from species that were capable to degrade chlorpyrifos. Most of them were plasmid based or located on the chromosome. The *opd* gene from *Agrobacterium radiobacter* was located on the chromosome. Identification of a novel phosphortriesterase enzyme from the coding of gene differs from organophosphate degradative gene (*opd*) in*Enterobacter* strain (Yang et al., 2005).

XI. GENETIC ENGINEERING IN CHLORPYRIFOS BIODEGRADATION

The genetically engineered bacteria with high detoxification potential were developed by gene engineering and enzyme engineering. Xu *et al.,* (2007), reported optimum pH of 7 and inoculum volume of 50 ml/kg on chlorpyrifos residues degradation by mutagenic bacteria DX1 in soil. Kapoor and Rajagopal (2011), studied the degradation of organophosphate pesticides by recombinant organophosphorus hydrolase (Dhanya, 2014).

Caoa *et al*., (2013), cloned a novel 6012 bp gene cluster from TCP-degrading strain P2 responsible for dehalogenation of 3,5,6-trichloro-2-pyridinol (TCP). The gene cluster consisted a monooxygenase gene (*tcpA1*), a flavinreductase 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. Cloned gene clusters from *Ralstonia sp.* T6 involved in 3,5,6-trichloro-2- pyridinol degradation. The *tcpRXA* genes constitute a gene cluster consisting FADH2- dependent monooxygenase gene*tcpA*, LysR family transcriptional regulator (*TcpR*) and flavinreductase (*TcpX*). T6-ΔtcpA-com, the complementation strain for the mutant strain T6- ΔtcpA, recovered the ability to degrade TCP, and the strain *E. coli* DH10B-tcpRXA, which expressed the tcpRXA gene cluster, had the ability to transform TCP to the green intermediate metabolite 3, 6-dihydroxy pyridine- 2,5-dione (DHPD) (Dhanya, 2014).

The cloning of *mpd gene* from chlorpyrifos degrading bacterial strains to *Escherichia coli* helps in developing its biodegradation capability. Wang *et al.,* (2002), cloned *Escherichia coli* with *opd gene* that degradechlorpyrifos co-metabolically. Yang *et al.,*(2005), cloned the *mpd* gene from chlorpyrifos– degrading bacterium *Stenotrophomonas* isolated using chlorpyrifos as the sole source of carbon by enrichment method that degraded 100 mg/l of chlorpyrifos within 24 hour to DETP and TCP. The thermostability and acidic stability of MPH have been improved bysite-directed mutation. Yang *et al.,* (2005), engineered *P. putida* JS444 with altered specificity of MPH enhance thedegradation of chlorpyrifos (Dhanya, 2014).

XII. CONCLUSION

The organophosphate pesticide chlorpyrifos used against pests not only protects crops but also causes havoc in the environment by its accumulation. Bioremediation is emerging as a beneficial tool in order to create pesticide free environment. The potential microorganisms have the ability to degrade pesticide to the fullest. But still there

needs more research to be done in order to bring the technique into field practice and make it more efficient. For large scale culture of such bacterial isolates to be used for bioremediation purpose, it is essential to determine the optimum growth conditions like temperature and pH. These isolated strains of bacteria are highly adapted to existing environment conditions and thus could be effectively utilized for bioremediation and metabolic detoxification of chlorpyrifos.

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