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A REVIEW ON MICROBIAL REMEDIATION OF PESTICIDE CONTAMINATED ENVIRONMENTS

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ABSTRACT

Biodegradation is the biologically catalyzed modification of an organic chemical's structure. However, this modification can be through different metabolic pathways and does not necessarily mean a reduction in toxicity. Mineralization, one type of biodegradation, is defined as the conversion of an organic substance to its inorganic constituents, rendering the original compound harmless. Transformation is defined as any metabolically-induced change in the chemical composition of a compound. Bioremediation refers to the use of microorganisms to degrade contaminants that pose environmental and human risks. Bioremediation processes typically involve the actions of many different microbes acting in parallel or sequence to complete the degradation process. Both in situ (in place) and ex situ (removal and treatment in another place) remediation approaches are used. The versatility of microbes to degrade a vast array of pollutants makes bioremediation a technology that can be applied in different ecosystem conditions. Though it can be inexpensive and in situ approaches can reduce disruptive engineering practices, bioremediation is still not a common practice.

KEY WORDS

Biodegradation, Different Metabolic Payways, ex-situ remediation approaches

INTRODUCTION

Due to human activities, annually a vast number of pollutants are releasing into the environment. Globally, water and air are being polluted by more than one billion pounds of toxins, 1,000 new products are synthesized, and 60,000 to 95,000 chemicals are commercially used (29). Among these substances pesticides are used extensively, and its consumption reached to 2 million tonnes globally (1), 45% of pesticides utilize by Europe followed by USA 24% and 25% in rest of the world. In Asia, China uses highest percentage followed by Korea, Japan and India. In India use of pesticide is about 0.5 kg/hectare and large contribution is from organochlorine pesticides. Presently India is largest producer of pesticides in Asia and ranks 12th in world (5).

The pesticides are of chemical substances used as insecticides, fungicides, nematicides, rodenticides, molluscicides to control weeds, crop diseases and human and animal health safety. crop productivity was enhanced and vector-borne diseases were controlled due to utilization of pesticides (7). Regrettably, only 2-3% of pesticide is actually utilized, and the rest remains in soil and water causing environmental pollution leading to toxicity. Thus, pesticide residues remain in top soil, leading to toxicity in the soil-water compartment. The introduction of 2,4-D (2,4-dichlorophenoxy acetic acid), DDT (dichloro diphenyl trichloroethane) and BHC (benzene hexachloride) after second World War became popular enormously due to effectiveness against target and economical, however drastic utilization made pesticides genetically resistant, non-target organisms were harmed, and

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pesticide residues often appeared in soil-water (34) there by entering into the food chain, ultimately get accumulated in human body became toxic and affect the health.

Pesticides are usually classified by their chemical nature in the following major groups such as organochlorines, organophosphates, carbamates, pyrethroids and chlorophenols (12). The pesticide residues and chemical composition are given in the following table (27).

Table 1. Classification of pesticides based on their chemical composition (adapted from (27)).

Group	Chemical composition
Organochlorine	Carbon atoms, chlorine, hydrogen and occasionally oxygen. They are nonpolar and lipophilic.
Organophosphate	Possess central phosphorus atom in the molecule. In relation whit organochlorines, these compounds are more stable and less toxic in the environment.
Carbamates	Chemical structure based on alkaloid of plant, *Physostigma venenosum*.
Pyrethroids	Compounds similar to the synthetic pyrethrins (alkaloids obtained from petals of Chysanthemun cinerariefolium).
Botanical origin	Products derived directly from plants. Not chemically synthesized.
Biological	Viruses, microorganisms or their metabolic products.
Copper	Inorganic compounds of copper.
Thiocarbamates	Differ from carbamates in their molecular structure, containing an-S-group in its composition.
Organotin	Presence of tin as a central atom of the molecule.
Organosulfur	They have a sulfur central atom in the molecule, very toxic to mites or insects.
Dinitrophenols	They are recognized by the presence of two nitro groups (NO_2) bonded to a phenol ring.
Urea derivatives	Compounds which include the urea bound to aromatic compounds.
Diverse composition	Triazines, talimides, carboxyamide, trichloroacetic and trichloropicolinic acids derivatives, guanidines and naphthoquinones.

Although some of the pesticides use has been discontinued, the stabile nature of them in soil-water becomes importunate pollutant and the extreme toxic nature results in to the severe cases of pesticide poisoning, which is becoming the issue of concern nowadays (25). The environmental awareness increased, and pesticide regulatory measures developed to remediate, and to protect environment from contaminants in future. So, it is necessary to remediate pollutant sites and environment by developing bioremediation strategies (15).

STRATEGIES FOR REMEDIATION OF PESTICIDES

Before choosing the method should consider the type of contaminants and source to be treated, sometimes multiple contaminant such as byproducts, raw material and solvents, sources may be soil, sediments, sludges, ground water or surface water to be treated. The ideal method should treat compound without forming any intermediate compounds (10). From the decade different methods have been approached, known to be Low Temperature Thermal Desorption (41), Incineration (19), Bioremediation (11) and Phytoremediation (8) (Table 2).

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Table 2. Technologies efficiency for the treatment contaminant by pesticides

Technology	Time (months)^2	Source	Efficiency
Low Temperature Thermal Desorption	0.75	Soil, Sludge and Sediment	82% to >98%
Incineration	1	Soil, Sludge and Sediment	>99%
Bioremediation	3.1	Soil, Sludge, Sediment and Groundwater	up to 99.8%
Phytoremediation	--	Soil, Sludge, Sediment and Groundwater	up to >80%

1. ***Bioremediation of Pesticides***

The mechanisms which use chemical and physical methods are effective for organic pesticides. However, these methods have drawbacks such as cost, handling, and transportation of harmful materials and also leave intermediate products. In addition, these methods alone not sufficient, extra methods are required as the contaminants are of mixed in many cases (24). Biological mechanism or bioremediation is effective technology which results in the complete conversion of pesticide residues into CO_2 and H_2O or less harm full components, is economic and environmentally friendly as compared. The microorganisms can be used in different ways, through enhanced natural attenuation, application of non-native or genetically modified organisms into contaminated sites (bioaugmentation) and by addition of nutrients or electrons to improve the remediation capacity (biostimulation). Bioremediation of pesticides can perform using different technologies such as bioremediation using microorganisms, using plants called phytoremediation and vermiremediation using earthwarms.

The most important biological agents in bioremediation are bacteria and fungi, which can utilize pesticides as nutrients and electron to survive. The micro biota in soil develop the ability to degrade and generate a mechanism for pesticide bio remediated site, microbial diversity in site together with pollutant nature, parameters like soil pH, moisture, nutritional state, temperature, oxidation-reduction potential influences the efficiency of bio degradation (22). Advanced analytical and molecular techniques profound our knowledge of microbial degrading mechanism, occurrence and identity of target microorganism that effect destruction of pesticides (17). A single microorganism does not possess the capability to degrade organic compounds of pesticides, mixed microbes have the potential to degrade hence, more than one organism is necessary hence, the genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas.

2. ***Microbial Degradation of Organochlorine Pesticides***

The pesticides Dichlorodiphenyltrichloroethane (DDT) and dieldrin are recalcitrant and accumulate for a long time in environment (18), the bacterial species of Bacillus, Pseudomonas, Arthrobacter and Micrococcus were found to be potent organochlorine degrading bacteria (21). The fungal strains *Penicillium miczynskii*, *Aspergillus sydowii*, *Trichoderma* sp., *Penicillium raistrickii*, and *Bionectria* sp. were also found to be more effective microorganisms against organochlorine (26). A study on white rot fungi on pesticide heptachlor and heptachlor epoxide was carried out, among 18 species *P. tremellosa*, *P. brevispora* and *P. acanthocystis* species removed and the metabolic pathway was also proposed (43). The dynamic enzymatic system of bacteria degrades the endosulfan by eliminating its functional groups, *Mycobacterium tuberculosis* enzyme degrades beta-endosulfan to more toxic end product (42). However, monooxygenase encoded by Arthrobacter sp. KW yields into nontoxic endosulfan diol. The bacterial species *Pseudomonas aeruginosa* and *Burkholderia cepaeia* hydrolyze endosulfan yields the less toxic metabolite endosulfan diol (20).

3. ***Organophosphorous Pesticides and Bioremediation***

Flavobacterium sp. ATCC 27551 was found to be the first bacterial isolate which can degrade organophosphorus compounds (35), and the enzymatic system degradation pathway also studied (37).The genes involved in pesticide degradation are present in plasmid DNA know as catabolite plasmid, the bacterial species *Klebsiella*, *Rhodococcus*, *Pseudomonas*, *Flavobacterium* and *Acinitobacter* contains complex metabolic pathway coding genes are present in catabolite plasmid (32). Chlorpyrifos, OP pesticide, can be degraded by *Psudomonas putida* strain MAS-1 rapidly by utilizing as a carbon source, it was observed that in 24hr of incubation 90% was degraded. *Pseudomonas* isolated from waste water were able to utilize chlorpyrifos as carbon source (3).

4. ***Biodegradation of Carbamates***

Carbamates are polar and soluble in water and thermo tolerant. Due to their broad-spectrum activity they are being using as pest control since 1950s. These are carbamate pesticides are organic compounds derivatives of carbamic acid and esters of carbamates, these compounds inhibit acetylcholine esterase and found to be most poisonings pest in rural environment by transforming into products by hydrolysis, photolysis, biotransformation and matabolic reactions of human and animals (38). The carbamete degrading bacteria hydrolyse the methylcarbamate linkage by an enzyme carbofuran hydrolase, which is synthesized by mcd gene, located in catabolite plasmid of *Achromobacter* sp (40). Other microorganisms such as *Pseudomonas*, *Mesorhizobium*, *Ralstonia*, *Rhodococcus*, *Ochrobactrum*, and *Bacillus* are also potent carbamate pesticide degraders (9). Fungal strains such as *Aspergillus niger* produces novel hydrolase can degrade N-methylcarbamate insecticides (30).

5. ***Genetically Engineered Microorganisms***

In early days, the bacterial genes coding for catabolic activity of organism was isolated and cloned, later by the application of genetic engineering in biodegradation opened an era by developing genetically engineered microorganism (GEM). It is defined as microorganism whose genetic material is altered by exchanging between organisms. GMS have potential to remediate soil, groundwater and activated sludge, exhibiting the enhanced degrading capabilities of a wide range of chemical contaminants (33). The *Pseudomonas* strain contains multi plasmid can oxidize aliphatic, aromatic, terpenic and polyaromatic hydrocarbons (16). The strain of *Pseudomonas putida* contains XYL and NAH plasmids and CAM and OCT recombinant plasmid, these bacteria can grow on crude oil and can metabolize hydrocarbons and it also known as superbug (33). The inoculation of GEM during culturing can stop the problems associated with competition in mixed culture. However, there is a controversy also surrounding the release into the environment and field testing are required to issue the safety then only ecological damage can be resolved.

6. ***Enzymes Involved in Biodegradation***

The process of bioremediation is actually involving the microorganisms and enzymes produced by them for detoxification of harmful compounds, hence it is impartment to select the organisms producing potential enzymes relevant to the different hydrocarbon degradation. As there are many limitations has been appeared with usage of whole microorganisms for the degradation usage of enzymes separated from their cells grasp interest and came in to exist in the recent years. Different types of enzymes such as oxidoreductases, hydrolases, oxygenases and laccases have been involved in the degradation of hydrocarbons and other pollutants. Based on the mode of degradation and ability to degrade, enzymes are classified in to oxidoreductases and hydrolases. Aerobic degradation of pollutants involves oxygenases, these enzymes catalyse degradation by introducing one or two oxygen molecules for initial attack followed by cleavage of ring in the aromatic compounds. Mono oxygenases perform the degradation by introducing one oxygen molecule to the aromatic compounds and increase the solubility. Mono oxygenases not only attach oxygen molecule, but also perform other reactions includes dehalogination, denitrification, desulpharizatio and hydroxylation of the aromatic compounds (2), these are of two types, flavin dependant monooxygenases and P450 monooxygenase. Cytochrome P450 family of monooxygenase enzymes are used in many industries to degrade contaminates released. Dioxygenases degrade pollutants by adding two oxygen molecules in the aromatic ring. Based on the mode of action dioxygenase are classified in to two types, an aromatic ring hydroxylation dehydrogenase (ARHD) which introduce two molecules of oxygen in to aromatic ring to degrade chemical compounds and aromatic ring cleavage dioxygenases (ARCD) which actually cleaves aromatic ring (28). Along with oxygenases, other enzymes include laccases, peroxidases, lipases, cellulases, carboxilestarases, phosphotriestarases and haloalkanedehalogenases also produced by different kinds of microbes used to degrade pollutants. However, low level of enzyme production by the organisms and degradation of only few kinds of compounds by each enzyme led the scientists towards genetic modifications. These modifications involves transformation of genes which involved in enzyme production into the target organisms, and manipulation of basic amino acid structure of the particular enzymes can improve the degradation.

CONCLUSION

Growth and survival of microbial populations are altered by several ecological factors like temperature, structure and composition of the pesticide, type of medium and nutrient availability during the process of bioremediation. These factors affect the application of bioremediation as a process of clean-up. This can limit the scope and effectiveness of bioremediation. Thus, bioremediation is not a complete remedial mechanism for removing pesticide contamination.

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