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REVIEW ARTICLE

Bioremediation: An Eco-friendly Approach for Treating Pesticides

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ABSTRACT
With the increase in environmental pollution in today's life, new techniques need to be evolved for its treatment too. In this regard we try to rely much on sustainable ways to treat the pollution. Bioremediation is one such promising technique in which microorganisms are used for the treatment of environmental pollutants and can be defined as biological response to environmental abuse. It is also related with the biological regeneration of the previously polluted sites and with the cleaning of areas that have been polluted recently, as a result of production, storage, transport and use of chemicals. Among these chemicals pesticides are very crucial as their use has widely increased to protect the crops from reduction in yield and quality. Also the pesticides have become an important part of modern agriculture. But continuous application of pesticides leads to degradation of the atmosphere. Pesticides have become a major contaminant of air, water, soil and vegetables. Moreover, these can easily pass into living tissues resulting in Bioaccumulation. Thus, due to its ecofriendly and sustainable behavior, bioremediation techniques have provedattested to be a significant device in treating the sites that are polluted by chemical pesticides.
***Key Words –** Bioremediation, Pesticides and Eco-friendly Approach.*

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INTRODUCTION

With the increase in environmental contamination due to the continuous rise in population, industrialization and urbanization , a potential hazard to human health has also increased and a major contributor of this contamination are the pesticides which are widely used for the minimization of crop pests and thus protection of crops from yield losses. Pesticides have become a significant feature of modern agriculture due to their necessity in economical pest management and enhancement of product quality[1]. But unlimitedapplication of pesticide results in the degradation of the atmosphere, around 90% of the applied agricultural pesticides fail to reach their target organisms and disperse through air, soil and water. Out of the total volatile emission to the environment, 63% are pesticides [2]. Above all their ability to accumulate into the tissues of living organisms leading to bioaccumulation is the major concern. All these factors have responsible for environmental pollution and major steps will be taken to tackle this problem.

The conventional technique used for the treatment of these contaminants are effective but also have certain drawbacks like cost, complexity and pollution,also in many cases these techniques are not sufficient [3]. Therefore, the employment of an alternative method for the remediation of such contaminants is very necessary. On this regard, bioremediation is an effective and innovative solution for pollution abatement.

Basically, bioremediation is an emerging technology that uses microorganisms to remediate polluted sites. The advantages like cost effectiveness and ecofriendly approach have made this technique an alternative to physiochemical methods.Bacteria, yeast and fungi are the main biological agents used in bioremediation [4].

BACTERIAL AND FUNGAL REMEDIATION OF PESTICIDES

In bioremediation process, different microorganisms are used for the degradation of various pesticides. Actually it depends on the chemical nature of the pesticide. The selection of these microorganisms should be done carefully for the effective remediation as they can continue to exist within a narrow range of contaminants [5,6].

Bacteria mainly of the genus *Alcaligenes, Pseudomonas,Flavobacterium and Rhodococcus* are the potent degraders of pesticides [7,8,9,10]. Actinomycetes also show impressive ability to biodegrade pesticides. Studies show that these microorganisms produce various extracellular enzymes that cause the degradation ofdifferent types of complex organic compounds. These actinomycetes work under aerobic conditions and an extensive feature is the presence of monooxygenases and dioxygenases [11]. The major genera involved are *Clavibacter, Rhodococcus, Streptomyces, Arthrobacter and Nocardia.*

The most recent studies have shown the capacity of actinomycetes in the potential deterioration of pesticides. White rot fungi such as *Phanerochaete chrysosporium* and *Trametesversicolor* have played an significant role in biodegradation of pesticides like lindane, atrazine, metalaxyl, DDT , dieldrin, aldrin,mirex and chlordane,diuron,etc [12,13,14,15,16,17].

Pesticides exhibit a great variation in their chemical structure including triazinonones, striazines, organophosphates, carbamates, acetanilides etc. Due to this variation, their mineralization is difficult by single isolates, therefore, we need to use consortia of bacteria for the complete and effective degradation.

TYPES OF BIOREMEDIATION-

On the basis of location where the soil is treated for pesticides, bioremediation is of two types :-

(i) In-situ bioremediation and (ii) Ex-situ bioremediation.

1. ***In-situ* bioremediation**:-

In this type of bioremediation, the polluted soil is treated at its prime location. The following treatments are included in this are :-

TABLE 1: TYPES OF PESTICIDES AND THEIR EFFECTS[55]

Pesticide	Class	Examples	Health effects
Insecticides	Organophosphates	Parathion,malathion,Methyl parathion,Chlorpyrifos,Diazinon,Dichlorvos, Phosmet,Fenitrothion tetrachlorvinphos and azinphos methyl	Neuropathy, Myopathy, Tremors, Irritability, Convulsions, inhibiting the enzyme acetyl cholinesterase,paralysis
	Carbamates	Aldicarb,Carbofuran(furadan), Phenoxycarb,Carbaryl(sevin), Ethienocarb and Fenobucarb	Inhibition of acetyl cholinesterase enzyme, Paralysis
	Organochlorines (dichlorodiphenylethanes and cyclodienes)	DDT,Dicofol, Heptachlor, Endosulfan, Chlordane,Aldrin, Dieldrin, Endrin, Mirex and Pentachlorophenol	Stimulation of the nervous system by disrupting the sodium/potassium balance of the nerve fiber,Tremors, Irritability, Convulsions,Hyperexcitable state of the brain, Cardiac arrhythmiatic and Reproductive problems
Herbicides	Phenoxy and benzoic acids,Triazines, Ureas and Chloroacetanylides	Chlorophenoxy acids, Hexachlorobenzene(HCB), Picloram, Atrazine, Simazine, Propazine, Diquat, Paraquat, Oxyfluorfen, Alachor,Fluroxypyr	Dermal toxicity , Carcinogenic effect, Damage to liver, Thyroid, Nervous system, Bones, Kidneys, Blood and Immune system
Fungicides	Substituted benzenes, Thiocarbamates, Thiophthalimides, Organomercury Compounds etc.	Chloroneb,chlorothalanil, Hexachloro benzene, Ferbam,Metamsodium, Thiram,Ziram,Ethylmercury	Damage to liver, Thyroid, Nervous system, Bones, Blood and Immune system, Carcinogenic property also.

Rodenticides	Coumarins,1,30-indandions	Warfarin,Coumatetralyl, Difenacoum,Flocoumafen,Bromadiolone,Diphacinone,Chlorophacinone, Pindone	
Nematicides		Aldicarb,Dibromochloropropane	
Bactericides		Metiram,Difolatan	

TABLE 2: MICROORGANISMS HAVING POTENTIAL FOR REMEDIATION OF PESTICIDES

Microorganism involved in the degradation	Pesticide
Pseudomonas	Cypermethrin,Oxyfluorfen,Chlorphyrifos, Iprodione (fungicide),Atrazine [8,38,39,40,23]
Bacillus	Lindane,Endosulfan,Oxyflurfen [41,42,43]
Rhodococcus	Metamitron [44,45]
Arthrobacter	Metamitron,Atrazine [44]
Staphylococcus	Endosulfan [42]
Stenotrophomonas	Tetrachlorvinphos,Chlorpyriphos [46,47]
Bjerkandera	Terbufos,Azinphosmethyl,Phosmet and Tribufos [48]
Pleurotus	Terbufos,Azinphosmethyl,Phosmet and Tribufos [48]
Proteus	Tetrachlorvinphos [46]
Vibrio	Tetrachlorvinphos [46]
Yersinia	Tetrachlorvinphos [46]
Serratia	Tetrachlorvinphos [46]
Synechocstis(cyanobacterium)	Chlorpyrifos [49]
Brucella	Chlorpyrifos [50]
Trichoderma	Malathion [51]
Micococcus	Cypermetherin [52]
Sphingomonas	Oxyfluorfen [53]
Enterobacter	Chlorpyrifos [54]

a) Bioventing :-

In this technique, oxygen and/or nutrients are added to the soil so as to accelerate the rate of bioremediation [18]. The two most commonly added nutrients are nitrogen and phosphorous [19]. The technique works well in case of well drained, medium and coarse textured soils.

b) Biosparging:-

It is a technique in which air is injected under pressure below the water table so as to enhance the amount of ground water oxygen .thus, enhancing the degradation of contaminants by bacteria present in nature.

In one study, it is found that within a remedial period of 10 months at a temperature of 18^{o} C, biosparging was able to remove more than 70% of B7EX [20]. Thus, indicating it as a promising technology to treat B7EX contaminated ground water.

c) Bioaugmentation :

The process of bioaugmentation involves the import of microorganisms to a contaminated site to increase the rate of degradation. But it has certain limitations like competition of the introduced microorganisms with the indigenous population for development.

Ex-situ Bioremediation:-

In Ex-situbioremediation, the contaminated soil is excavated and treated at another location.The following are the treatments included in it.

a) **Landfarming:-**

In this process, the contaminated soil is dugout and separated mechanically through sieving. The polluted site is then placed in layers over the clean soil, allowing the detoxification,degradation and immobilization of contaminants by natural processes [21]. The contaminated soil layer is covered by a synthetic, concrete or clay membrane. Considerate amount of oxygen, nutrients and moisture along with the pH (7.0) are also maintained to aid the remediation process.

b) **Composting:-**

When organic wastes are treated with by microorganisms for degradation at elevated temperatures (55 C to 65 C), thus is known as composting. The temperature is increased due to the heat released in the degradation process results in increased solubility of contaminants with higher metabolic activity in composts.

c) **Bioreactors:-**

It is a method in which contaminated soil, water and nutrients are mixed and a mechanical bioreactor is used to agitate the mixture which stimulates the action of microorganisms. It is a quick process[21] which is best suited for clay soils.

FACTORS AFFECTING BIOREMEDIATION IN SOIL:

Various factors such as soil type, moisture, pH, temperature and organic matter influences the effectiveness of bioremediation strategies:

a) Moisture content:

Water is a major requirement of microorganisms as it is required for their growth and dispersal of nutrients and by products across the cell membrane during the process of biodegradation [22]. The movement of bacteria is restricted in low moisture conditions and on the other hand, oxygen transport is limited due to high moisture.

b) Concentration of nutrients and oxygen:

Availability of oxygen and nutrients affects the microbial efficiency of degradation to a greater extent. A recent study has shown the increment in biodegradation of oxyflourfen, an herbicide by the addition of NPK fertilizers [23].The concentration of organic matter in soil also plays a vital role in degradation of fluroxpyr [24, 25]. The favorable oxygen concentration required for effective bioremediation is >0.2mg/L and >10% air filled pore space for aerobic degradation.

c) pH:

The availability of nutrients is affected by the soil pH which in turn also affects the microbial efficiency. Also certain microbial species can exist within a narrow range of pH.

d) Temperature:

Temperature controls the rate of enzymatic reactions within microorganisms thus influencing the rate of biodegradation.There is a limit of temperature that the microbes can withstand.Recently, it is concluded that 25 C-35 C is the optimum temperature for microbial activity [25].

MERITS OF BIOREMEDIATION-

1. Bioremediation is a naturally occurring process and is accepted and perceived by the people as a process for treating contaminated soil. The biodegradative microbial population increases when the polluted waste materialis present and declines when this polluted waste is degraded. The end products of this bioremediation process are carbon dioxide, water and cell biomasses which are harmless.
2. It is possible to degrade the hazardous waste material completely using bioremediation technique. Through bioremediation many hazardous compounds can be transformed to harmless products. The chance of future liability associated with treatment and disposal of hazardous waste material is also eliminated.
3. The target hazardous waste and pollutants can be degraded without transferring contaminants from one place to another.
4. Bioremediation can be carried out, without a major disruption of normal activities. For this we have to transport massive amounts of waste offsite along with the potential threat to human health andatmosphere that can takes place during transportation is also eliminated.
5. The other techniques for cleanup of hazardous waste, other than bioremediation are more expensive as compared to bioremediation.

DEMERITS OF BIOREMEDIATION-

1. It is not necessary that all compounds are susceptible to rapid and complete degradation, as bioremediation is limited to biodegradable compounds.
2. It also found in certain studies that the end products of bioremediation process may be more persistent or toxic than the parental compound.

3. High specificity is a feature of biological process. Presence of metabolically active microbial populations, suitable environmental growth conditions and appropriate levels of nutrients and waste material are important factors necessary for the successful bioremediation.
4. It is very difficult to carry out experiments from bench and pilot scale studies to full scale field operation.
5. The various types of waste material may be present as solids, liquids and gases.
6. The process of bioremediation is of longer duration than other treatment options.

RECENT DEVELOPMENTS IN PESTICIDES BIOREMEDIATION:

Several microorganisms have been isolated and identified, having capabilities of degrading the different types of pesticides and hazardous chemicals. Recently, research has performed to isolate and characterized those microorganisms, that are responsible for the degradation of carbfuran, carbarly and baygon[26-34].It was also found that the gene responsible for degradation of pesticides and hazardous chemicals were present on the plasmids.

It was noted that sequences of Esd gene has same homology to monooxygenase family that requires reduced favin, presented by a separate flavin reductase enzyme, found in *Mycobacterium smegmatis*as co-substrates [35]. The capability of Esd gene to catalyze the oxygenation of β-endosulfan to endosulfanmonoaldehyde to endosufan hydroxyether but it has incapable to degrade either α-endoslfan or the metabolites of endosulfan and endosulfansulphate. Coding enzyme of the gene Ese, from the monooxygenase family has also been reported [36], which has capability of degrading both endosufan α and β using *Arthrobacter* species.

Construction of potent superbug can be possible through considering the gene of interest and desired enzyme, to achieve the result at fast rate in short period of time. In a review, degradation of HCH and distribution of lin gene in *Sphingomonads*has also done [37]. In S. indium B90A, non-identical linA genes were found. For the dehydrochlorination of γ-HCH, the first two steps are carried out by the linA encoded HCH dehydrochlorinase (LinA).From the above literature it has been clear that understanding of molecular genetics, diversity and distribution of lin gene has necessary for the development of bioremediation technology.

GENETICALLY ENGINEERED MICROORGANISMS (GEMS)

A wide variety of toxic chemical compounds have been degraded by using different microorganisms and their enzymatic activity can be increased by using various genetic engineering techniques. Genetically engineered microorganisms have certain advantages such as rapid growth affinity, fast growth rate and resistance to toxicity. This overcomes the limitations of bioremediation. However, the potential results of releasing such genetically engineered microorganisms into the environment cannot be predicted practically because the conditions of the field are not always optimal, also, there are indigenous communities. These artificially introduced genes have the ability to persist in the environment for example, the phenol degrading plasmid has been found in soil after 6 years of the addition of genetically engineered microorganisms. Therefore, there exists a debate concerning the persistence, safety, contaminant and potential ecological damage related with the release of genetically engineered microorganisms in the environment. Due to these limitations, the utilization of genetically engineered microorganisms for large scale bioaugmentation is restricted.

Suicidal microorganisms have been designed to overcome the problem of continues and long term ecological damage. These cells die in the absence of pollutant.

CONCLUSION

Bioremediation, which is a technique to clean up the pollution by enhancing the nature's biodegradation process, is gaining significant attention these days. This technology offers acompetent and more economical method for the treatment of contaminated ground water and soil. With greater knowledge and experience, technologies are also improving and bioremediation has gained victory in dealing with different types of contaminated sites. But, unluckily, the concepts of bioremediation including its principles, techniques, pros and cons are not widely known or understood. Therefore the perception of the microbial communities, their response to natural environment and pollutants and the genetics should be developed by conducting field trials of the new bioremediation techniques. And then bioremediation can be used as a management tool to deal with the environmental pollution with increased success rates.

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