BIOREMEDIATION OF PESTICIDE WASTE AT CONTAMINATED SITES

Kalpna Varshney

Assistant Professor, Department of Chemistry,

Faculty of Engineering and Technology,

Manav Rachna International Institute of Research & Studies, Faridabad, Haryana, India

Abstract

As a consequence of all the immoral and non-ethical environmental harming activities of man, natural resources have been intoxicated and bio magnification has reached its height over the past few decades. In turn, humanity itself got affected as some of these substances (pesticides), due to their persistent, non-biodegradable nature, get accumulated in adipose tissue, thus urging us to take urgent steps for remediation of pesticide waste. According to the data, India is the largest producer of pesticide in Asia. Majority of organochlorides and organophosphate are the cause of serious environmental issues due to Bio magnification. The creation of these noxious chemicals has forced to take instinctive measures to implement new green technologies like Bioremediation and Phytoremediation. These ultra-innovative technologies may practice as armament against pesticide contamination battle. This novice technology is eco-friendly, manageable, cost effective and safe Agro practices. Outcome of bioremediation is the complete mineralization of pollutants to H2O and CO2 without the accumulation of intermediates.

Key Words: Bioremediation, Phytoremediation, Pesticides, pollution, Green process

Introduction

The concept of Green Revolution has played a significant role for the consumption of variety of pesticides. Though pesticides have been emerged out to play very important role in human health and are very useful to protect our cash, fiber and food crops, yet they have their dark side also. (1) Pesticides can be classified are as per their toxicity, functional group and their persistence in the environment and target organisms.(2, Table-1) Therefore this boon soon turned into bane. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 persistent organic chemicals are pesticides. The persistent dose is dependent on the dosage of pesticides used at the time of application. This dose is harmful to the environment and also to the wildlife. For this reason, the perseverance of pesticides is not at all good for environment and life. Persistent organic pollutants, includes various organochlorine and organophosphate pesticides like aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzenes, mirex and toxaphene. Even insect repellents such as diethyltoluanide (DEET) are prospective to be harmful and non-degradable.

Table 1: Types of Pesticides and Examples [source : Vaccari et.al. (2006)]

Pesticide	**Examples**
Insecticide	
Organophosphorus	Diazinon, dichlorvos, dimethoate, malathion, parathion
Carbamate	Carbaryl, propoxur, Aldicarb methiocarb
Organochlorine	DDT, methoxychlor, toxaphene, mirex, Kepone
Cyclodienes	Aldrin, chlordane, dieldrin, endrin, endosulfan, heptachlor
Herbicides	Chlorophenoxy acids, hexachlorobenzene (HCB)
Nitrogen-based	Picloram, Atrazine, diquat, paraquat
Organophosphates	Glyphosate (Roundup)
Fungicide	
Nitrogen-containing	Triazines, dicarboximides, phthalimide
Wood preservatives	Creosote, hexachlorobenzene
Botanicals	Perethrin, permethrin
Antimicrobial	Chlorine, quaternary alcohols

Perseverance of pesticide in environment is because of either their physico-chemical properties or their nondegradability. Major abiotic factors heat, Light or moisture could lead to little degradation of pesticides. (2,3). However, their high cost and low efficiency of these technologies limit their use in pesticides degradation (4, 5, and 6). In India, usage of pesticides exceeds from 0.25 kg/hectare to 0.5 kg/hectare thus, the stocking of these noxious chemicals in soil has led to implement new green technologies like biodegradation, bioremediation and phytoremediation. With Bioremediation and phytoremediation technologies it is possible to reduce the pesticide contamination up to certain level. The process of bioremediation usually occurs in soil, whereby pesticides are broken down into less active/toxic compounds by fungi, bacteria, and other microorganisms that use pesticides as energy and carbon sources.(7). Bioremediation means to remove contaminant from the nondegradable waste product using microorganism. It means that organisms can gather on toxic substance or waste product, and decrease their presence and the adverse effects on environment but these are not able to remove them from environment.

The key factor in bioremediation is to reduce the pesticide toxicity levels to undetectable amount, or to that level which is not harmful. There are various guidelines issued by regulatory authorities regarding this (8). For the successful bioremediation a strong and capable bacterial strain is required which can degrade the toxic substance to harmless one. After this the bioremediation is conjointly completed by biodegradation of a pesticide that involves the oxidation of parent compound to carbon dioxide and water. During the oxidation of the pesticides there is transfer of electrons from pesticides to any electron acceptor. Amongst all electron acceptors oxygen is usually the most capable one, Other electron acceptors include nitrate, iron, and sulfate which are used in anaerobic conditions. This process is enhanced by enzymes produced by a microbial cell or enzyme found external to the cell. Lack of a suitable enzyme is one of the common reasons for perseverance of any pesticide. (8, 9)
Bioremediation is blend of two process Bio augmentation and biostimulation. In Bioaugmentation additional microorganisms are added to contaminated site with pesticides so that this can be reinforced the natural bio process. While in bio stimulation the nutrients or food is supplied to that contaminated site so that the parent microorganism population can survive and grow more for their intended work.

Types of Bioremediation Methods

Bioremediation can be done either in-situ or ex-situ methods. In in- situ bioremediation the microorganisms are applied for treatment i.e. for the degradation of hazardous pesticides present in the under the soil surface and during this no digging of polluted soils or sediments occurs (10).
Ex situ methods involves physical removal or excavation of contaminated soil for bioremediation (11)

- In-Situ
    - Bioventing
    - Bioslurping
    - Biosparging
    - Bioaugmentation
    - Phytoremediation
- Ex-situ
    - BioPile
    - Land farming
    - Composting
    - Bioreactors

Some of the examples of in situ and ex situ bioremediation are given below:

Bioventing

It characteristically applies low air flow so that sufficient amount of oxygen can be supplied to microorganisms. This method shows substantial potential of stabilizing or removing pesticides from soil. In traditional bioventing systems, oxygen is transported by an electric blower to subsurface soil to flourish microbial activity. In passive bioventing technique natural air is used transport oxygen to the subsoil via bioventing wells. Single way valve, is used to permit air to enter in sub soil. When atmospheric pressure falls below the subsurface pressure, the valve is closed down, and traps the air in the sub surface that results in the increase concentration of oxygen at this place.

Biosparging

This method has been in use since 1985, biosparging is applied for the remediation of volatile organic pesticides and other VOCs liquefied in the soil near groundwater table. It uses native microorganisms to bioremediate VOCs. In this technoque, air and nutrients (if required) are inoculated into soil.

Bioslurping

It is a blend of two processes bioventing and vacuum-enhanced free-product recovery. Bioslurping is an exceptional method in in-situ treatment as it also remediates floating waste products on top of the groundwater. This technique applies a vacuum to extract, soil vapor, water, and free product from the subsurface. Finally these products are separated out and then treated for biodegradation.

Bioventing, biosparging, and bioslurping are simply operational if the contaminated site soil is homogeneous.

Bioaugmentaion: Additional microorganisms are added to contaminated site with pesticides so that this can be reinforced the natural bio process.

Phytoremediation: Phytoremediation is a kind of bioremediation method in which plants are used to, transport, and/or damage pesticides or toxicants in the soil and underground water. Phytoremediation is used for the remediation of heavymetals, radioactive materials, pesticides, hydrocarbons and volatile organic compounds (VOCs).

Ex-situ methods

Biopile

Through this method soil is excavated and piles are formed above the ground, followed by addition of nutrient supplement and capable bacteria strains, and sometimes oxygen is also supplied to increase bioremediation (12). The biopiling starts with laboratory tests of soil that will decide the biological degradation tendency of the soil sample. Then the soil sample is cleaned by removing plastic and other solid materials mixed into it. Then it is homogenized and mixed with bacteria, oxygen and nutrients for the process to start.

Bioreactor

Bioreactor is a specific container or vessel in which contaminated soil and other raw materials are feed to produce the desired remediated products following a series of biological reactions. Bioreactors can be operated in different ways, which include: batch, fed-batch, sequencing batch, continuous and multistage. The choice of mode can be decided only on the financial basis. Polluted soil with pesticides can be fed into a bioreactor either in solid powder or in slurry form. The conditions inside the bioreactors can be modified as per our need like, oxygen content, bacterial strain, nutrients for enhancement of the bioremediation process (13).

Land farming

Land farming is the simplest and economic bioremediation technique as it required very less instrumentation for operation. There is always a debate regarding its in-situ or ex-situ categorization pesticide depth in soil is very important to decide whether land farming can be carried out ex situ or in situ. When the soil is excavated and/or tilled then the method of treatment will decide its in-situ or ex-situ nature. When digged polluted soil is treated on-site, it can be regarded as in situ. As per Nikolopoulou 2013 when a pesticide lies <1 m below ground surface, bioremediation can be done without excavation, while pollutant lying >1.7 m requires to be transported to ground surface for effective bioremediation (14).Land farming is usually used for remediation of hydrocarbon-polluted sites including polyaromatic hydrocarbons (15)

Composting

It consists of excavating the soil and then mixing organic matter such as wood, hay, manure, and plant waste with the contaminated soil. The important aspect in composting to maintain a temperature range of 54 to 650C. This can be done by the indigenous microorganisms while degrading the toxicants. Composting is most effective technique to remove PAH, TNT, and RDX (16).

Factors affecting bioremediation process

Bioremediation is a complex process and governed by various factors. These factors include the presence of a capable microbial strain, the accessibility of pesticides and toxicants to the microbial population, temperature,pH, moisture and oxygen content. (17,18)

Microbial strains

These can be isolated roughly from any environmental circumstances. They will then adjust themselves in any conditions and grow at extreme temperature conditions, in water, either in aerobic or anaerobic conditions. Provided proper nutrients and carbon sources are present. Anaerobic bacteria are not as frequently used as aerobic bacteria. Fungi such as the white rot fungus Phanaerochaete chrysosporium have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs.

Table2: Types of Fungus used for Bioremediation of pesticides (source: Watanabe et.al. (2001)

Species of fungi	**Potential for degrading pesticide**
Flammulina velupites, Stereum hirsutum,Coriolus versicolor,Dichomitus squalens, Hypholoma fasciculare, Auricularia auricula,Pleurotus ostreatus, Avatha discolor and Agrocybe semiorbicularis	triazine, phenylurea, dicarboximid, chlorinated organophosphorus compounds
White-rot fungi	Heptachlor atrazine, terbuthylazine, lindane, metalaxyl, chlordane mirex,gammahexachlorocyclohexane (g-HCH), dieldrin, diuron, aldrin, DDT, etc.,

Table3: **Types of Bacteria used for Bioremediation of pesticides (source: Lakshmi Tewari et al.)**

Species of Bacteria	Potential for degrading pesticide
Alcaligenes denitrificans	Fluoranthene(PAH)
Arthrobacter sp.	Carbofuran, Parathion
Arthrobacter sp.	EPTC, Pentachlorophenol, glyphosate
Bacillus sphaericus	Urea herbicides, Parathion
Brevibacterium oxydans	DH35A Cyclohexylamine
Burkholderia sp.	P514 1,2,4,5-Te CB
Clostridium Quinoline,	Glyphosate
Corynebacterium nitrophilus	Acetonitril, Carboxylic acid
Dehalococcoides ethanogenes	Trichloroethylene (TCE)
Desulfovibrio sp.	Nitroaromatic compounds
Flavobacterium sp.	Pentachlorophenol, Parathion
Alcaligenes faecalis	Arylacetonitrils

Environmental Factors (21,22,23)

Microbial growth and activity are readily affected by pH, temperature, and moisture.

Temperature

affects biochemical reactions rates, and the rates of many of them double for each 10 °C rise in temperature. Above a certain temperature, however, the cells degenerate.

pH

Although microorganisms have been also isolated in extreme conditions, most of them grow optimally over a narrow pH range, so that it is important to achieve optimal conditions. If the soil has too much acid it is possible to rinse the pH by adding lime. Range of pH from 6.5 to 8.5 is normally most favorable for biodegradation.

Oxygen

Often the most important factor limiting rates of biodégradation in the environment is the availability of molecular oxygen. The initial enzymes of aerobic attack on hydrocarbons are oxygenases, which have an absolute requirement for molecular oxygen. Delivering air or oxygen to contaminated soils can be difficult for a number of reasons: the soil porosity may not be favorable, and therefore mass transfer from the gas phase to the aqueous phase will be limited. Also, the relatively low solubility of oxygen in water is a primary limiting factor.

Moisture content

All soil microorganisms require moisture for cell growth and function. Availability of water affects circulation of water and soluble nutrients into and out of microorganism cells. However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration. Moisture range of 50-80% is optimal for biodegradation.

Nutrients

Although the microorganisms are present in contaminated soil, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated. Biostimulation usually involves the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the pesticides. Inorganic nutrients such as nitrogen and phosphorus are necessary for microbial activity and cell growth

Soil Type

Some contaminants can stick to soil particles and microorganisms are unable to use them for biodegradation. Therefore, under these circumstances, bioavailability of contaminants does not solely depend on the characteristics of the contaminant but also on soil type

Table 4: Factors affecting the process of bioremediation of pesticides [source: Shanahan P (2004) Bioremediation. Waste Containment and Remediation Technology. Springer.

Factor	**Conditions required**
Micro organisms	Aerobic or Anaerobic
Natural biological processes of micro organisms	Catabolism and Anabolism
Environmental factors	Oxygen content Temperature, pH, Electron acceptor/ donor
Nutrients	Carbon, Nitrogen, oxygen etc.,
Soil moisture	25-28 % of water holding capacity
Type of soil	Low clay or slit content

Advantages

Environmentally friendly and cost saving features are amongst the major advantages of bioremediation compared to both chemical and physical methods of remediation. One of the major advantages of bioremediation techniques is that they do not require extensive preliminary assessment of polluted site prior to remediation; this makes the preliminary stage short, less laborious and less expensive.

Limitations of Bioremediation

Despite of several advantages there are few limitations also in bioremediation method. One of the key limitations is the type of microorganism. Bioremediation is not done by microorganism for any charity rather; it is for its own survival and food need. So when the organism is doing its job under environmental condition, it is ok, if conditions are altered then bioremediation may affect. When using bacteria and fungi, nutrients are required to be added for the microorganism and these are given in the form of fertilizers, this may bother to other soil organisms when done in situ method. Other very important limitation is the cost i.e. cost benefit ratio (23). It's very difficult for government and even for industry to spend large amounts of money for bioremediation. Industry in particular always likes to keep their costs of products and services down for gain in the market. An additional problem is that environmental disruption may be caused by both *ex situ* and *in situ* methods as these may cause damage to the environmental balance. The long-term effects of introducing naturally occurring non-native bioremediation organisms into an area are not completely beneficial always.

Conclusion

Standard methods of directly comparing bioremediation techniques should be developed. The compatible microorganisms for specific contaminants and pesticides should be identified specifically. Cost effectiveness is another parameter which needs to be keep in mind whenever we are choosing bioremediation approaches. The notable step to a effective bioremediation is site categorization, that helps to choose the most appropriate and achievable bioremediation technique (ex situ or in situ). Ex situ bioremediation techniques are more expensive because of extra costs involved in it like excavation and transportation of soil. While, in situ techniques have no added cost require for digging of soil; however, sometime cost of setting up of equipments on the site, coupled with inability to effectively visualize and control the subsurface of polluted site may render some in situ bioremediation techniques inefficient. The other physicochemical characteristics of polluted site(s) including type of soil, contaminant depth and type, distance of site relative to habilitated site and effective features of each bioremediation technique should be combined in determining the most appropriate and capable method to effectively treat contaminated sites. (24)

References:

1. Dollacker A. 1991 Pesticides in third world Pilank Nachor. Bayer,44(1): 89-99.
2. Kempa, E. S. 1997. Hazardous wastes and economic risk reduction: Case study, Poland. Int. J. Environ. Pollut., 7: 221–248.
3. Wehtje, Glenn & H. Walker, Robert & N. Shaw, Joey. 2009. Pesticide retention by inorganic soil amendments. Weed Science. 48. 248-254. 10.1614/0043-1745.
4. Dijkgraaf, E. and Vollebergh, H. R. J. 2004. Burn or bury? A social cost comparison of final waste disposal methods. Ecol. Econ., 50: 233–247.

5. Jin, J. J., Wang, Z. S. and Ran, S. H. 2006. Solid waste management in Macao practices and challenges. Waste Manage., 26: 1045–1051.

6. Sibanda MM, Focke WW, Labuschagne FJ, Moyo L, Nhlapo NS, Maity A, 2011..Degradation of insecticides used for indoor spraying in malaria control and possible solutions. Malaria journal.,10:307. Epub 2011/10/20.

7. Environmental Protection Agency. In situ treatment technologies for contaminated soil. Environmental Protection Agency, 2006 Contract No.: EPA 542/F-06/013.

8. Sarfraz Hussain , Tariq Siddique , Muhammad Arshad & Muhammad Saleem,2009. Bioremediation and Phytoremediation of Pesticides: Recent Advances, Critical Reviews inEnvironmental Science and Technology, 39:10, 843-907, DOI: 10.1080/10643380801910090.

9. Uqab B, Mudasir S, Nazir R 2016 Review on Bioremediation of Pesticides. J Bioremed Biodeg 7:343. doi:10.4172/2155-6199.1000343).

10. Smith E, Thavamani P, Ramadass K, Naidu R, Srivastava P, Megharaj M. 2015. Remediation trials for hydrocarbon-contaminated soils in arid environments: evaluation of bioslurry and biopiling techniques. Int Biodeterior Biodegradation. 101:56–65. doi: 10.1016/j.ibiod.2015.03.029.

11. Philp JC, Atlas RM. Bioremediation of contaminated soils and aquifers. In: Atlas RM, Philp JC, editors. 2005 Bioremediation: applied microbial solutions for real-world environmental cleanup. Washington: American Society for Microbiology (ASM) Press; pp. 139–236.

12. Whelan MJ, Coulon F, Hince G, Rayner J, McWatters R, Spedding T, Snape IChemosphere. 2015 Jul; 131():232-40.

13. Mohan SV, Sirisha K, Rao NC, Sarma PN, Reddy SJ.2004 Degradation of chlorpyrifos contaminated soil by bioslurry reactor operated in sequencing batch mode: bioprocess monitoring J Hazard Mater. 10; 116(1-2):39-48.

14. Nikolopoulou M, Pasadakis N, Norf H, Kalogerakis N. 2013Enhanced ex situ bioremediation of crude oil contaminated beach sand by supplementation with nutrients and rhamnolipids.Mar Pollut Bull. 77(1-2):37-44.

15. Silva-Castro et al. 2012; Silva-Castro GA, Uad I, Gónzalez-López J, Fandiño CG, Toledo FL, Calvo C. Application of selected microbial consortia combined with inorganic and oleophilic fertilizers to recuperate oil-polluted soil using land farming technology. Clean Technol Environ Policy. 2012;14:719–726. doi: 10.1007/s10098-011-0439-0

16. Christopher Chibueze Azubuike, Chioma Blaise Chikere, Gideon Chijioke Okpokwasili, 2016. World J Microbiol Biotechnol 32:180.

17. Van Deuren, J., Lloyd, T., Chhetry, S., Raycharn, L., Peck, J., 2002 Remediation Technologies Screening Matrix and Reference Guide, Federal Remediation Technologies Roundtable, 4

18. Madhavi Gaonkar Naik and Mohini Dilip Duraphe2012. Review paper – Parameters affecting Bioremediation Vol 2/Issue 3/Jul-Sept 2012. International journal of lifescience and Pharma Research

19. Watanabe K, Kodoma Y, Stutsubo K, Harayama S 2001. Molecular characterization of bacterial populations in petroleum contaminated groundwater discharge from undergoing crude oil storage cavities. Applied and Environmental Microbiology 66: 4803-4809

20. Lakshmi Tewari, Jitendra Saini and Arti Bioremediation of Pesticides by Microorganisms: General Aspectsand Recent Advances2012. In book: Bioremediation of Pollutants Publisher: I.K. International Publishing House Pvt. Ltd.

21. Boopathy, R. 2000. Factors Limiting Bioremediation Technologies. Bioresource Technology, 74, 63-67. http://dx.doi.org/10.1016/S0960-8524(99)00144-3.

22. Ahmad M. and Ahmad, I.,2014 Bioremediation of Pesticides. Biotechnology book. Chapter: 5, Publisher: Studium Press LLC, U.S.A.

23. V. Mary kensa.2011 Bioremediation - an overview. Jr. of Industrial Pollution Control 27(2) pp 161-168.

24. Bioremediation of Contaminated Soils: A Comparison of In Situ and Ex Situ Techniques Jera Williams, https://tge.public.iastate.edu/courses/ce521/jera.pdf