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Biological approaches for degradation of insecticide

Priyanka Brahmane, SV Gatkal, Rashmika Kumbhar and CD Deokar

Abstract
In the last few decades, highly toxic organic compounds have been synthesized and released into the environment for direct or indirect application over a long period of time. Pesticides, fuels, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorophenols, and dyes are some of these types of compounds Some synthetic chemicals are extremely resistant to biodegradation by native flora (Rochkind-Dubinsky *et al*., 1987) compared with the naturally occurring organic compounds that are readily degraded upon introduction into the environment. Therefore, hazardous wastes and chemicals have become one of the greatest problems of modern worldwide society. The present paper reviews Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule (Raymond *et al*., 2001; Wiren-Lehr *et al*., 2002). Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide /insecticide degraders (De Schrijver and De Mot, 1999).therefore its benefits Recovery of a contaminated medium by using living organisms. Approach to enhance the degrading capability. Application in all types of contaminated fields. Effective process Genetic engineered micro-organisms and Eco-friendly technology.

Keywords: biodegradation, insecticide, microorganisms bacteria, fungi, enzyme

Introduction
In the last few decades, highly toxic organic compounds have been synthesized and released into the environment for direct or indirect application over a long period of time. Pesticides/Insecticide, fuels, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorophenols, and dyes are some of these types of compounds. Some synthetic chemicals are extremely resistant to biodegradation by native flora (Rochkind-Dubinsky *et al*., 1987) [8] compared with the naturally occurring organic compounds that are readily degraded upon introduction into the environment. Therefore, hazardous wastes and chemicals have become one of the greatest problems of modern worldwide society. Biological decomposition of pesticides/insecticide is the most important and effective way to remove these compounds from the environment. Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule (Raymond *et al*., 2001; Wiren-Lehr *et al*., 2002) [7, 10]. Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide /insecticide degraders (De Schrijver and De Mot, 1999) [4].

The effect caused by these Insecticides on soil, water, human health as follows
A) Soil effects
- Assimilated by plants
- Transported with soil eroding particles to water
- Accumulation in animal food chain

B) Water effects
- Sedimentation of hydrophobic pollutants
- Change in physicochemical characteristics

C) Human Health effects
- Mutagenicity
- Teratogenicity
- Carcinogenicity
- Allergenicity

Biodegradation
According to the definition by the International Union of Pure and Applied Chemistry, the

term biodegradation is "Breakdown of a substance catalyzed by enzymes *in vitro* or *in vivo*. In other words, defined as the ability of microorganisms to convert toxic chemicals (xenobiotics) to simpler non-toxic compounds by synthesis of certain enzymes. Biodegradation of xenobiotics can be affected by substrate specificity, nutrition source, température, pH etc.

Role of Microbes in Biodegradation

- Great versatility of microbes
  - ✓ Simpler,
  - ✓ Economical
  - ✓ More environmental friendly strategy
- Bacterial - efficient in
  - ✓ Biotransformation processes
- Strategies for obtaining energy from
  - ✓ Virtually every compound under oxic or anoxic conditions
- By using ultimate electron acceptors
  - ✓ Such as nitrate, sulfate and ferric ions.
  - ✓ Benzene ring next to glucosyl- to break resonance structure

Criteria for Biodegradation Strategies

Organisms must have necessary catabolic activity required for degradation of contaminant at fast rate to bring down the concentration of contaminant.

✓ The target contaminant must have bioavailability.
✓ Soil conditions must be favorable for microbial/plant growth and enzymatic activity.
✓ Cost of bioremediation must be less than other technologies of removal of contaminants.

How Microbes Use the Contaminant: Contaminants may serve as:

- **Primary substrate:** Enough available to be the sole energy source.
- **Secondary substrate:** Provides energy, not available in high enough concentration.
- **Co metabolic substrate:** Utilization of a compound by a microbe relying on some other primary substrate.

How to increase efficiency of biodegradation capabilities of microorganism

We are aware that the term biodegradation is often used in relation to ecology, waste management, biomedicine, and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products, this will mainly give attention to biodegradation in relation to bioremediation through describing processes (natural attenuation, bio stimulation and bio augmentation) utilizing degradation abilities of microorganisms in bioremediation and factors affecting this process. Microorganisms may be genetically engineered for many purposes. One such purpose is for the efficient degradation of pollutants. So, the second scope of this is to demonstrate the importance of some GEM in this process and to describe obstacles for testing GEM in the field, which must be overcome before GEM can provide an effective clean-up process at lower cost (Figure 4).

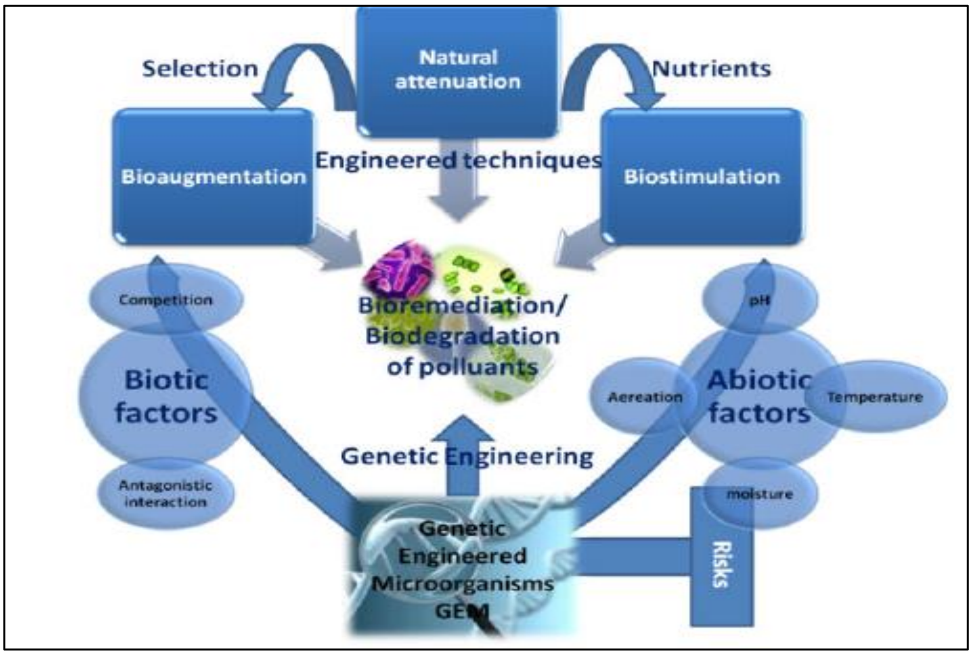


Fig 4: Bioremediation of pollutants utilizing biodegradation abilities of microorganisms include the natural attenuation, although it may be enhanced by engineered techniques, either by addition of selected microorganisms (bio augmentation) or by bio stimulation, where nutrients are added. Genetic engineering is also used to improve the biodegradation capabilities of microorganisms by GEM. Nevertheless, there are many factors affecting the efficiency of this process and risks associated to the use of GEM in the field

Microorganisms

1. Aerobic bacteria

Examples: *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus,* and *Mycobacterium.* Shown to degrade pesticides and hydrocarbons; alkanes and polyaromatics. May be able to use the contaminant as sole source of carbon and energy.

2. Methanotrophs

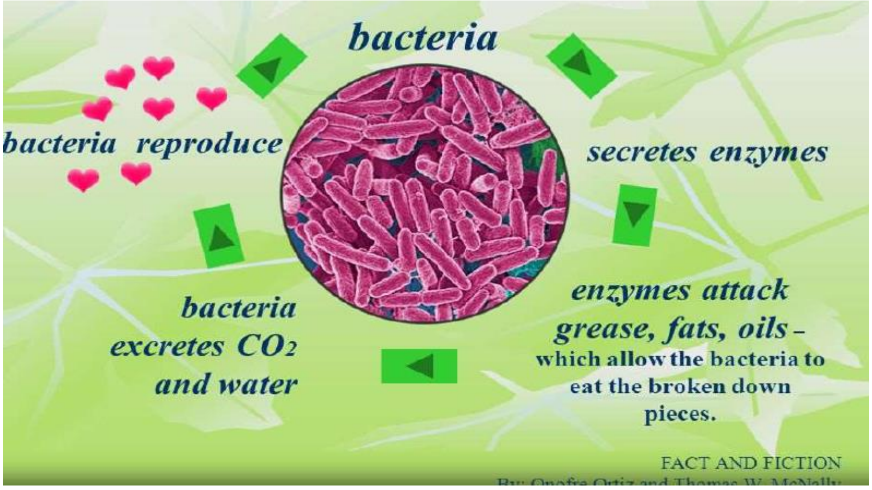
Aerobic bacteria that utilize methane for carbon and energy. Methane monooxygenase has a broad substrate range. active against a wide range of compounds (e.g. chlorinated aliphatic such as trichloroethylene and 1, 2-dichloroethane)

3. Anaerobic bacteria

Not used as frequently as aerobic bacteria. Can often be applied to bioremediation of polychlorinated biphenyls (PCBs) in river sediments, trichloroethylene (TCE) and chloroform.

Role of bacteria

Most bacterial species which degrade pesticides/insecticide belong to the genera *Flavobacterium, Arthrobacter, Azotobacter, Burkholderia* and *Pseudomonas*. The nature of degradation varies among species and the target compound. *Pseudomonas sp.* and *Klebsiella pneumoniae* have been shown to possess hydrolytic enzymes that are capable of breaking down s-triazine herbicides, such as atrazine. Similarly, a number of enzymes such as oxygenases, hydroxylases, hydrolases and isomerases present in *Pseudomonas* and *Alcaligene* ssp. have been shown to degrade herbicide *Acinetobacter* sp, *Bacillus* sp, *Pseudomonas* sp, *Alcaligenes* spp, and *Serratia* spp. were found as aerobic heterotrophs, and *Pseudomonas* spp. And *Bacillus* spp. was identified as petroleum utilizers in cow dung. Petroleum utilize sintotal aerobic heterotrophs ranged from 6.38% to 20% (Akinde and Obire, 2008).



Fungi

Ostreatus, Stereumhirsutum, and *Avatha discolor* have shown their ability to degrade various pesticide groups like phenylamide, triazine, phenylurea, dicarboximide, chlorinated**:** Able to degrade a diverse range of persistent or toxic environmental pollutants.

Role of fungi

Fungi degrade pesticides / insecticide by introducing minor structural changes to the insecticide rendering it nontoxic and are released to soil, where it is susceptible to further biodegradation by bacteria (Gianfreda and Rao, 2004). Several fungi such as *Agrocybesemior bicularis, Auricularia auricula, Coriolus versicolor, Dichomitus squalens, Flammulina velupites, Hypholoma fasciculare, Pleurotus* and organ phosphorus compounds (Bending *et al.,* 2002). According to Quintero *et al*.2007 Several classes of pesticides such *lindane, atrazine, diuron, terbuthylazine, metalaxyl, DDT gammahexachlorocyclohexa*ne (g-HCH), dieldrin, aldrin, heptachlor.

Enzymes invoved in biodegrdation

Enzymes are biological catalysts that facilitate the conversion of substrates into products by providing favorable conditions that lower the activation energy of the reaction. They have active sites.

Mechanism of enzyme action

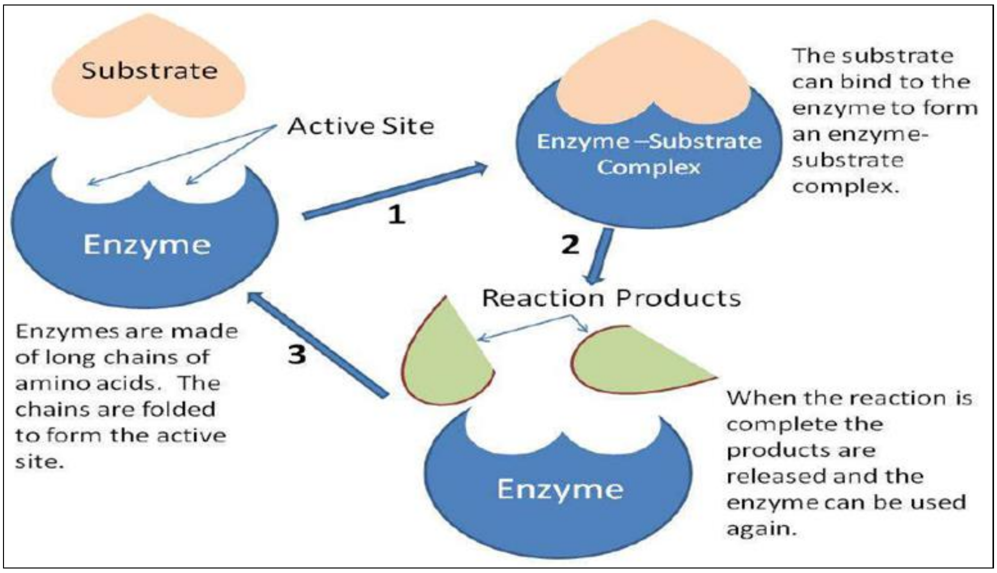
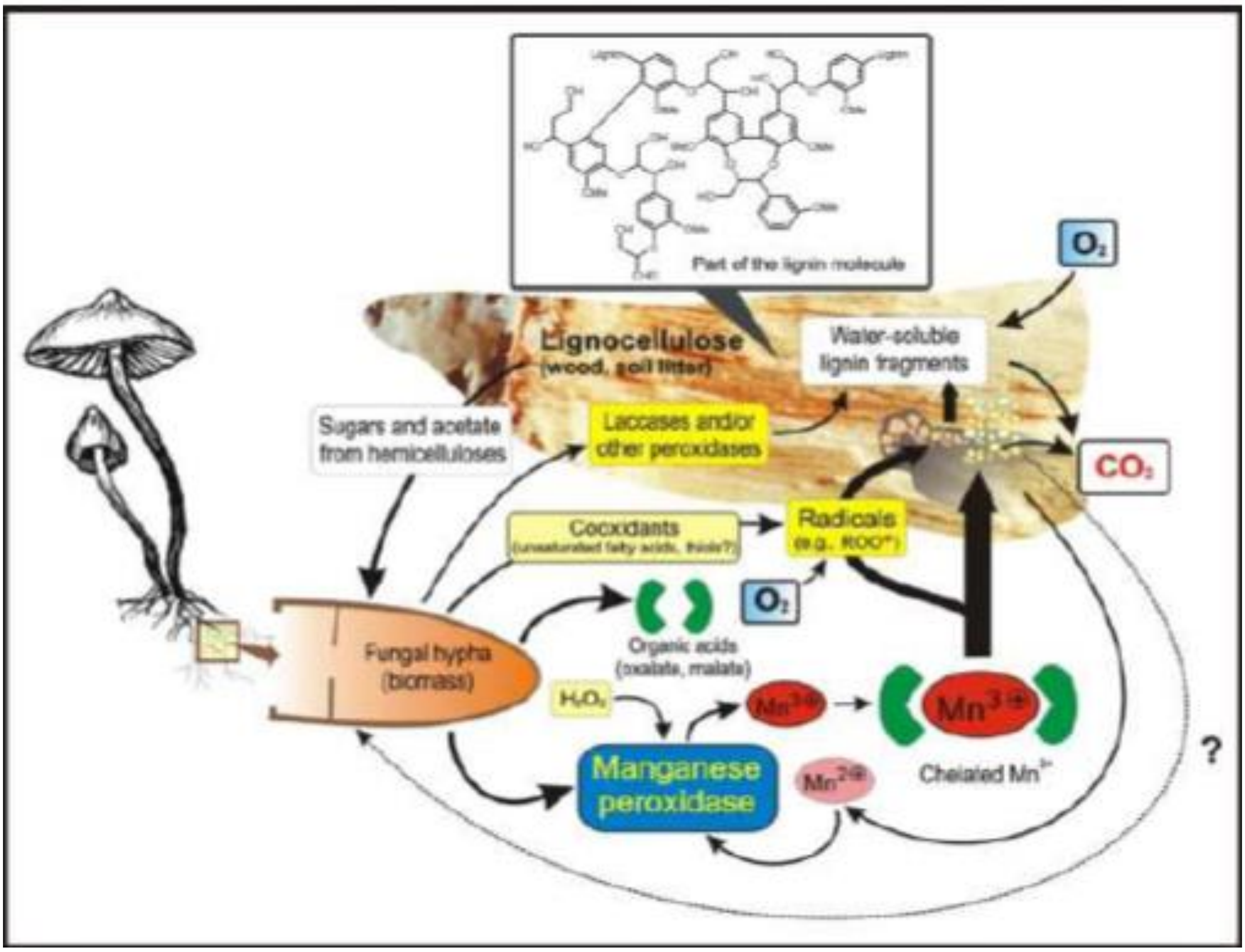


Fig 2: Mechanism of enzyme action

Enzymes used in biodegradation

- Microbial peroxidases
- Microbial oxidoreductases
- Microbial Laccases
- Microbial Oxygenases
- Monooxygenases
- Microbial dioxygenases

E.g. Manganese Peroxidase - MnP is a hydrogen peroxide dependent enzyme, but it can only oxidize organics when in the presence of Mn (Il). MnP oxidizes Mn (II) to Mn(III), which acts as an obligatory oxidation intermediate for the oxidation of of various compounds. The Mn (IlI) ions migrate away from the enzyme and start the oxidation of the lignin and other compounds.



Mechanism of Manganese Peroxidase

Biodegradation of Insecticide

For example, the fate of pesticides in the environment is strongly related to the soil sorption processes that control not only their transfer but also their bioavailability (Besse-Hoggan *et al.* 2009) [3]. Contamination of soil from pesticides (insecticide) as a result of their bulk handling at the farmyard or following application in the field or accidental release may lead occasionally to contamination of surface and ground water (Singh and Walker 2006) [9]. In soils, several parameters influence the rate of biodegradation processes: environmental factors such as moisture and temperature, physicochemical properties of the soil, presence of other nitrogen sources or carbon, etc.can completely modify the microbial population and therefore the microbial activity (Besse-Hoggan *et al.* 2009) [3].

In natural environments, pesticides or their degradation products may be further transformed or degraded by other microorganisms or eventually leading to complete degradation by the microbial consortium. However, persistent xenobiotics like pesticides and metabolic dead-end products will accumulate in the environment, become part of the soil humus, or enter the food chain leading to biomagnification (Figure 2).

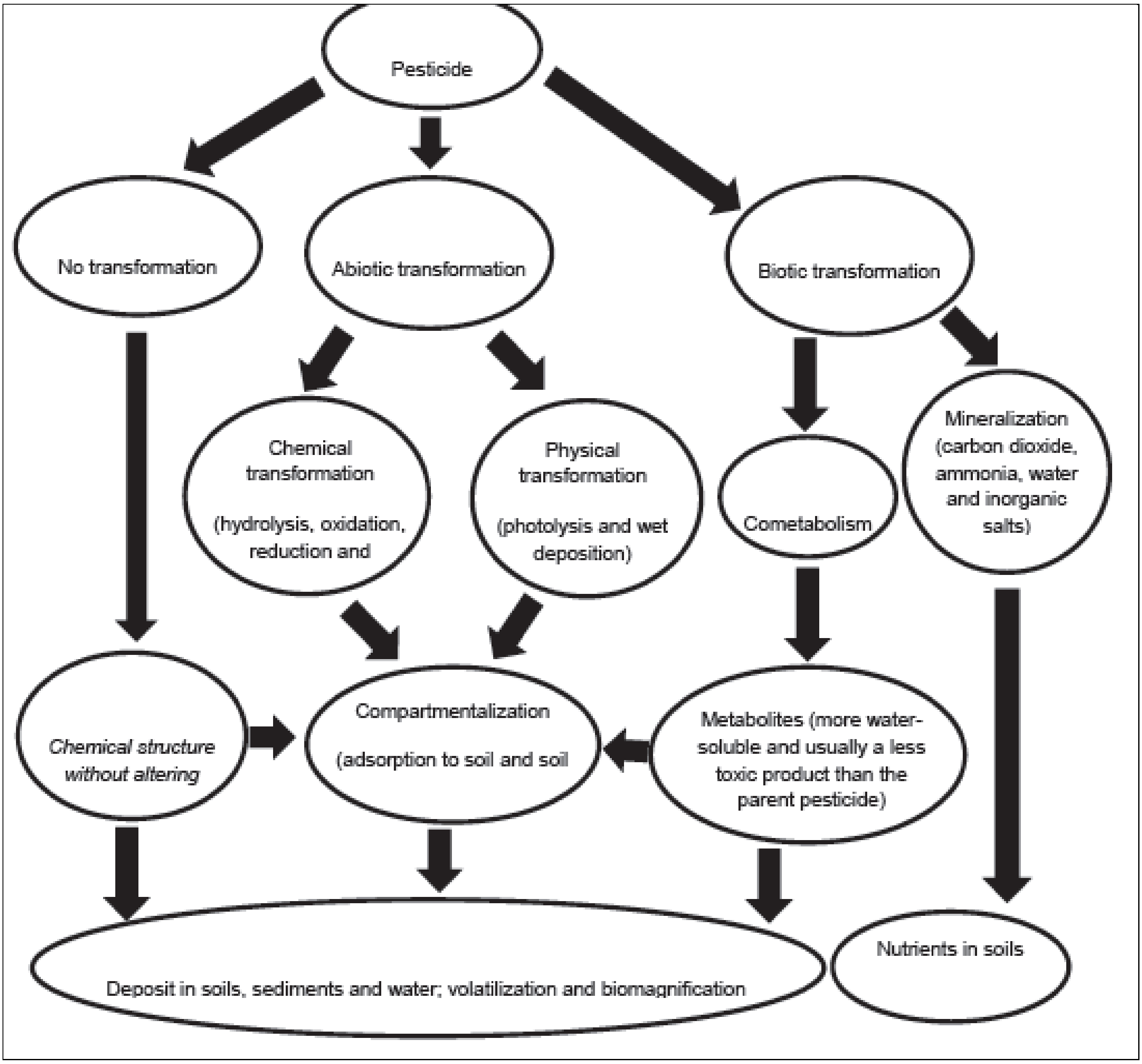


Fig 2: Fate of pesticides/insecticide in the environment

There are different sources of microorganisms with the ability to degrade insecticide. Because pesticides are mainly applied to agricultural crops, soil is the medium that mostly gets these chemicals, besides insecticide industry's effluent, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of insecticide, and even some live organisms. In general, microorganisms that have been identified as insecticide degraders have been isolated from a wide variety of sites contaminated with some kind of insecticide. At present, in different laboratories around the world there are collections of microorganisms characterized by their identification, growth and degradation of insecticide.

Microbial processes that eliminate organic environmental contamination are important. Progress in the biotechnology of biodegradation relies upon the underlying sciences of environmental microbiology and analytical geochemistry. Recent key discoveries advancing knowledge of biodegradation (in general) and the aromatic-hydrocarbon biodegradation (in particular) have relied upon characterization of microorganisms: pure-culture isolates, laboratory enrichment cultures, and in contaminated field sites. New analytical and molecular tools (ranging from sequencing the DNA of biodegrading microorganisms) have deepened our insights into the mechanisms (how), the occurrence (what), and the identity (who) of active players that effect biodegradation of organic environmental pollutants (Jeon and Madsen 2012) [5] (Figure 3).

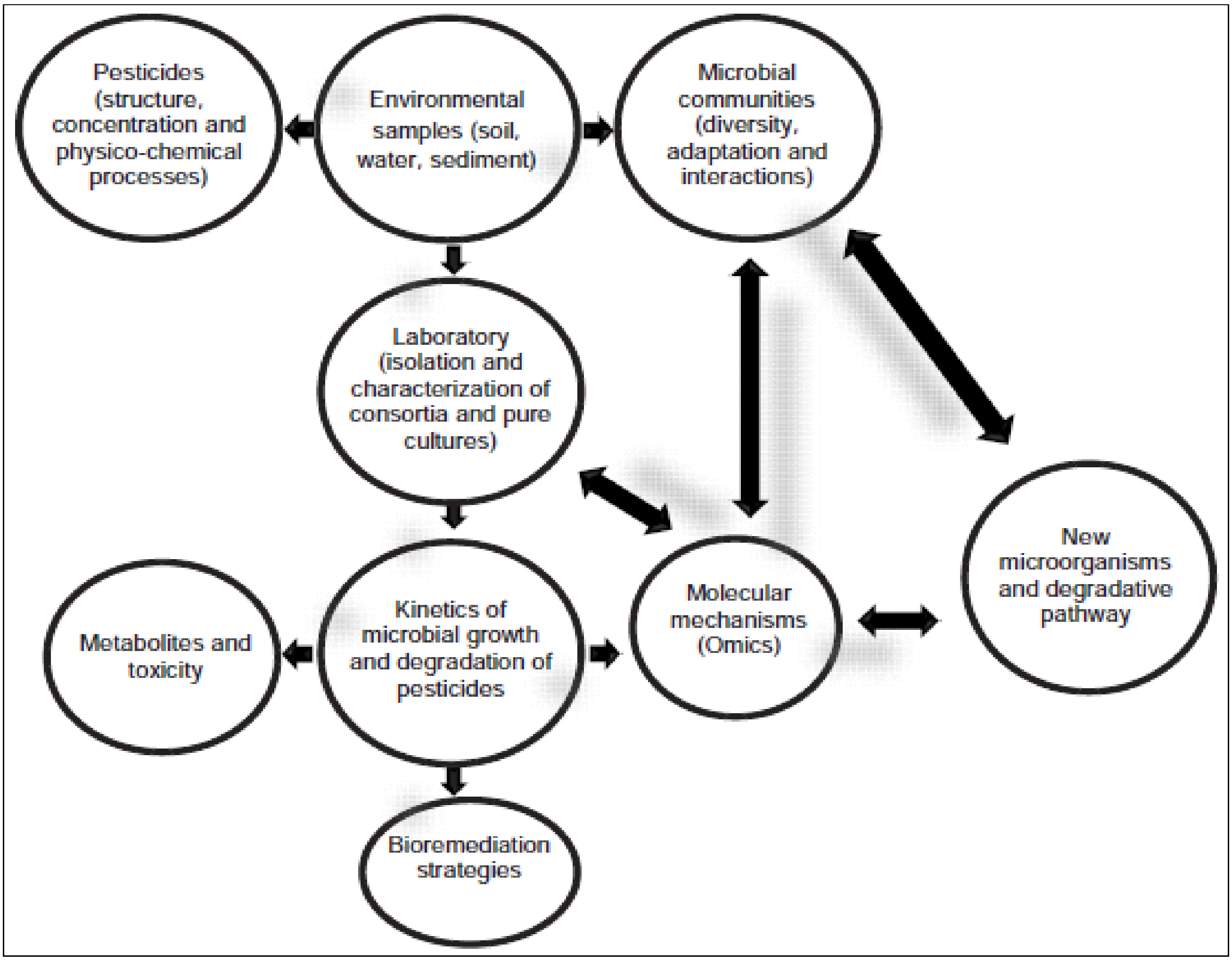


Fig 3: Representation of the relationships between pesticide (insecticide), microbial communities, and the discovery of new biodegradation processes, Omics = high throughput-based characterization of biomolecules characteristic of bioprocesses; DNA, genomics; mRNA, transcriptomics; protein, proteomics; metabolites, metabolomics

Biochemical Mechanisms Involved in Microbial Degradation of Insecticide

The biodegradation of pesticides, is often complex and involvesa series of biochemical reactions:

1. Detoxification: Conversion of the pesticide molecule to a nontoxic compound. A single chance in the side chain of a complex molecule may render the chemical non-toxic.

2. Degradation: The breaking down / transformation of a complex substrate into simpler products leading finally to mineralization. e.g. Thirum (fungicide) is degraded by a strain of *Pseudomonas* and the degradation products are dimethlamine, proteins,sulpholipaids, etc.

3. Conjugation: In which an organism make the substrate more complex or combines the pesticide with cell metabolites. Conjugation is accomplished by those organisms catalyzing the reaction of addition of an amino acid, organic acid or methyl crown to the substrate, for e.g., in the microbial metabolism of sodium dimethlydithiocarbamate, the organism combines the fungicide with an amino acid molecule normally present in the cell and thereby inactivate the pesticides/chemical.

4. Activation: It is the conversion of non-toxic substrate intoa toxic molecule, for eg. Herbicide, 4-butyric acid (2, 4-DB) and the insecticide Phorate are transformed and activated microbiologically in soil to give metabolites that are toxic to weeds and insects.

5. Changing the spectrum of toxicity: Some fungicides/pesticides are designed to control one particular group of organisms / pests, but they are metabolized to yield products inhibitory to entirely dissimilar groups of organisms, for e.g. the fungicide PCNB fungicide is converted in soil to chlorinated benzoic acids that kill pests.

6. Leaching: Since many of the pesticides can be solublized, they are removed by leaching.
Example: DDT

Biodegradation of DDT involves co- metabolism (Bollag & Liu, 1990).

- Dechlorination is the major mechanism for the microbial conversion of both the *o,p'*-DDTand*p,p'*-DDTisomers of DDT to DDD (Fries *et al.*,1969).
- The reaction involves the substraction of an aliphatic chlorine for a hydrogen atom.
- Degradation proceeds by successive reductive dechlorination reactions of DDT to yield 2,2-bis (*p*-chlorophenyl) ethylene (DDNU), which is then oxidised to 2,2- bis (*p*-chlorophenyl) ethanol (DDOH)
- Further oxidation of DDOH yields bis (*p*-chlorophenyl)

acetic acid (DDA) which is decarboxylated to bis (*p*-chlorophenyl) methane (DDM).

- DDM is metabolized to 4,4'dichloro benzophenone (DBP) or, alternatively, may undergo cleavage of one of the aromatic rings to form *p*-chlorophenyl acetic acid (PCPA).

Under anaerobic conditions DBP was not further metabolized.

Conclusion

- ✓ Recovery of a contaminated medium by using living organisms.
- ✓ Approach to enhance the degrading capability.
- ✓ Application in all types of contaminated fields.
- ✓ Effective process.
- ✓ Genetic engineered micro-organisms.
- ✓ Eco-friendly technology

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