We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK CITATION INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Pesticide Biodegradation: Mechanisms, Genetics and Strategies to Enhance the Process

Ma. Laura Ortiz-Hernández,
Enrique Sánchez-Salinas,
Edgar Dantán-González and
María Luisa Castrejón-Godínez

Additional information is available at the end of the chapter

1. Introduction

As a result of human activities, currently a large number of pollutants and waste are eliminated to the environment. Worldwide, more than one billion pounds of toxins are released into the air and water. Approximately 6×10^6 chemical compounds have been produced; annually 1,000 new products are synthetized and between 60,000 and 95,000 chemicals are commercially used [1]. Among these substances are chemical pesticides, which are used extensively in most areas of crop production in order to minimize pest infestations, to protect the crop yield losses and to avoid reducing the product quality.

The pesticides belong to a category of chemicals used worldwide as herbicides, insecticides, fungicides, rodenticides, molluscicides, nematicides, and plant growth regulators in order to control weeds, pests and diseases in crops as well as for health care of humans and animals. The positive aspect of application of pesticides renders enhanced crop/food productivity and drastic reduction of vector-borne diseases [2,3]. A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mites, nematodes, weeds, rats, etc.), including insecticide, herbicide, fungicide, and various other substances used to control pests [4]. The definition of pesticide varies with times and countries. However, the essence of pesticide remains basically constant: it is a (mixed) substance that is poisonous and efficient to target organisms and is safe to non-target organisms and environments [5].

Pesticides are by no means a new invention. In fact, intentional pesticide use goes back thousand years when Sumerians, Greeks, and Romans killed pests using various compounds such as sulphur, mercury, arsenic, copper or plant extracts. However, results were frequently poor because of the primitive chemistry and the insufficient application methods. A rapid emergence in pesticide use began mainly after World War II with the introduction of DDT (dichlorodiphenyltrichloroethane), BHC (benzene hexachloride), aldrin, dieldrin, endrin, and 2,4-D (2,4-dichlorophenoxyacetic acid). These new chemicals were effective, easy to use, inexpensive, and thus enormously popular. However, under constant chemical pressure, some pests became genetically resistant to pesticides, non-target organisms were harmed, and pesticide residues often appeared in unexpected places [3].

Chemical pesticides can be classified in different ways, but one of the most used is according to their chemical composition, which allows to group pesticides in a uniform and scientific way and to establish a correlation between structure, activity, toxicity and degradation mechanisms, among others. Table 1 shows the most important pesticides according to their chemical composition. Some general characteristics of pesticides are shown in Table 2.

Group	Main 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. The organophosphate pesticides can be aliphatic, cyclic and heterocyclic.
Carbamates	Chemical structure based on a plant alkaloid *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.

Table 1. Classification of pesticides according to their chemical composition [6].

Pesticides	Characteristics	Examples
Organochlorines	Soluble in lipids, they accumulate in fatty tissue of animals, are transferred through the food chain; toxic to a variety of animals, long-term persistent.	DDT, aldrin, lindane, chlordane, mirex.
Organophosphates	Soluble in organic solvents but also in water. They infiltrate reaching groundwater, less persistent than chlorinated hydrocarbons; some affect the central nervous system. They are absorbed by plants, transferred to leaves and stems, which are the supply of leaf-eating insects or feed on wise.	Malathion, methyl parathion, diazinon
Carbamates	Carbamate acid derivatives; kill a limited spectrum of insects, but are highly toxic to vertebrates. Relatively low persistence	Sevin, carbaryl
Pyrethroids	Affect the nervous system; are less persistent than other pesticides; are the safest in terms of their use, some are used as household insecticides.	Pyrethrins
Biological	Only the *Bacillus thuringiensis* (Bt) and its subspecies are used with some frequency; are applied against forest pests and crops, particularly against butterflies. Also affect other caterpillars.	Dispel, foray, thuricide

Table 2. General characteristics of pesticides [7].

Worldwide approximately 9,000 species of insects and mites; 50,000 species of plant pathogens, and 8,000 species of weeds damage crops. Different pests such as insects and plants causing losses estimated in 14% and 13% respectively. Pesticides are indispensable in agricultural production. About one-third of the agricultural products are produced by using pesticides. Without pesticide application the loss of fruits, vegetables and cereals from pest injury would reach 78%, 54% and 32% respectively. Crop loss from pests declines to 35% to 42% when pesticides are used [8].

Over 1990s, the global pesticide sales remained relatively constant, between 270 to 300 billion dollars, of which 47% were herbicides, 79% were insecticides, 19% were fungicides/bactericides, and 5% the others. Over the period 2007 to 2008, herbicides ranked the first in three major categories of pesticides (insecticides, fungicides/bactericides, herbicides). Fungicides/bactericides increased rapidly and ranked the second. Europe is now the largest pesticide consumer in the world, followed by Asia. As for countries, China, the United States, France, Brazil and Japan are the largest pesticide producers, consumers or traders in the world. Most of the pesticides worldwide are used to fruit and vegetable crops. In the developed countries pesticides, mainly herbicides are mostly used to maize. Since the 1980s hundreds of thousands of pesticides have been developed, including various biopesticides [5].

The global agricultural sector is the primary user of pesticides, consuming over 4 million tons of pesticides annually. Pesticides have been extensively used for decades and have substantially increased the food production [9]. However a large amount of applied pesticides often never reach their intended target due to their degradation, volatilization and leaching, leading

to serious ecological problems [9-10]. Under actual agricultural practices, different groups of pesticides are often simultaneously or consecutively applied interacting with each other [11].

Although pesticides are beneficial in controlling the proliferation of pests, their unregulated and indiscriminate applications for the application of pesticides can cause adverse effects to human health, to different life forms and to the ecosystems, which depend on the degree of sensitivity of organisms and toxicity of pesticides. The continued application of pesticides has increased its concentration in soils and waters, besides; they enter to the food chains. Dispersion mechanisms also have increased the level of environmental risk for the occupationally exposed population and the inhabitants of surrounding villages. Despite ban on application of some of the environmentally persistent and least biodegradable pesticides (like organochlorines), in many countries their use is ever on rise. Pesticides cause serious health hazards to living systems because of their rapid fat solubility and bioaccumulation in non-target organisms [2]. The main forms of pollution are direct applications to agricultural crops, accidental spills during transport and manufacturing, as well as waste from tanks where cattle are treated to ectoparasites control [4].

The effects of the impacts of pesticides can be analyzed from two different points of view: environmental and public health. The first occurs when pesticides are introduced to food chains, for example: a) producing a change in the decline of populations of phytoplankton and zooplankton (indicators of water pollution); b) producing carcinogenic, neurotoxic, and on fertility and viability (in invertebrates, fish, amphibians, insects and mammals) of their descendants; c) the presence of pesticides in the environment have caused the resistance of organisms considered as pests and disease vectors (for example malaria, dengue and Chagas disease), and instead other beneficial insect populations are diminished (like pollinators); d) alter biogeochemical cycles by decreasing the macro and microbiota, e) leaching of pesticides pollute water bodies, f) can be adsorbed pesticides when soil particles interact with positively or negatively charged, thus increasing their persistence in the environment (4-26 weeks). From the point of view of public health impact of pesticides is mainly acute intoxications (especially in occupationally exposed populations) or indirect exposure of the general population (through air, water and food contaminated with pesticide residues) [12].

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 1).

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 [13]. Contamination of soil from pesticides 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 [14]. The behavior of pesticides in soils, the efficiency, persistence and potential as environmental contaminants, depend on their retention and degradation on soil constituents [15]. 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
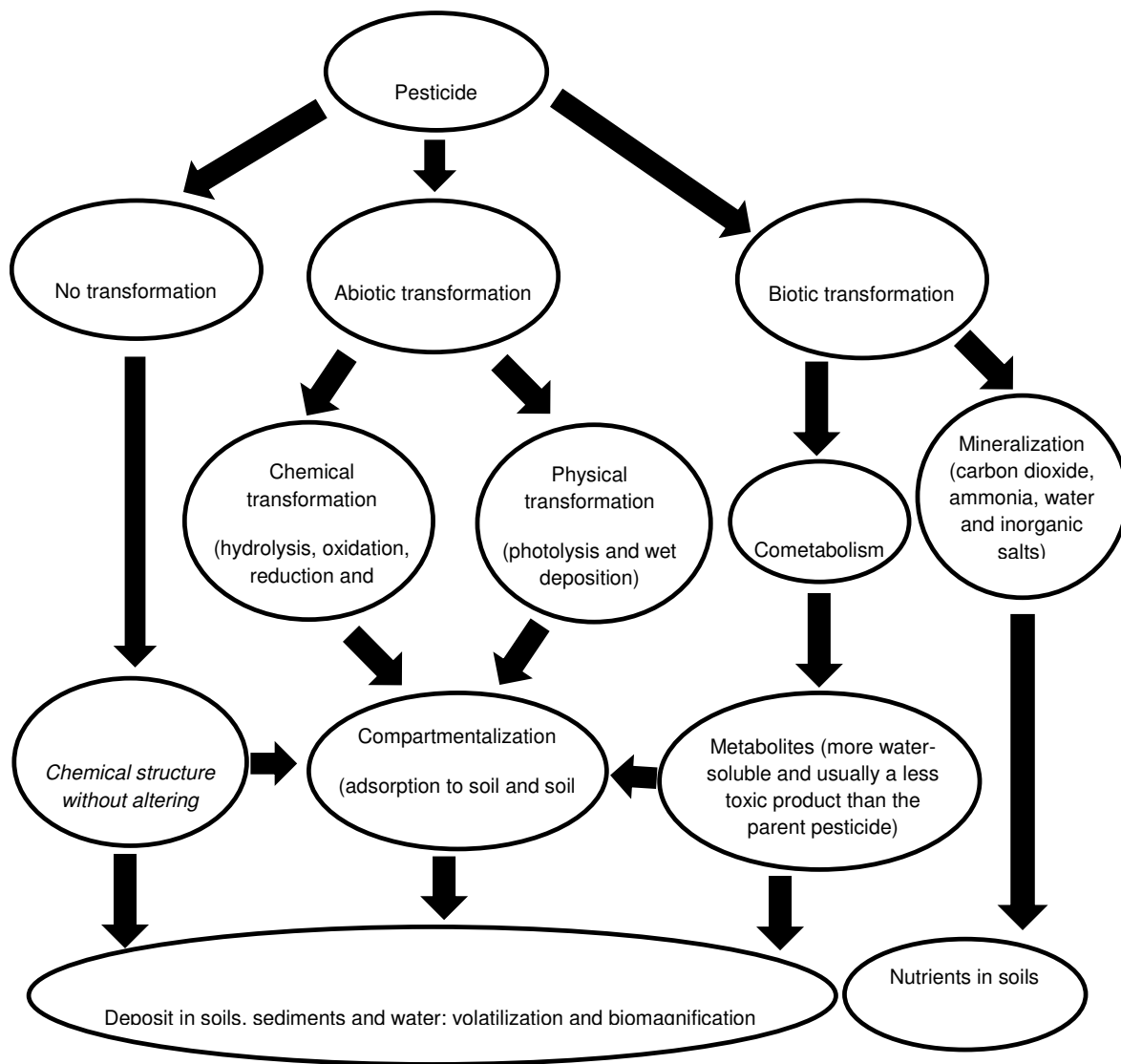
Figure 1. Fate of pesticides in the environment.

nitrogen sources or carbon, etc. can completely modify the microbial population and therefore the microbial activity [13].

On the other hand, liquid and solid wastes and obsolete products are stored or disposed in an inappropriate manner, which has favored the appearance of significant amounts of environmental liabilities, which in most cases are not reported to the appropriate authority. There are more than half a million tons of obsolete, unused, forbidden or outdated pesticides, in several developing and transitional countries, which endanger the environment and health of millions of people [16] In the absence of a clear obsolete pesticides management strategy, over the years, significant amounts of obsolete pesticides have been stockpiled in developing countries [17]. An obsolete pesticide may be recognized as one that is undesirable or impossible to use and has to be eliminated [17-20]. Because of their characteristics, obsolete pesticides are hazardous wastes that should be managed as such. Obsolete pesticides have accumulated in almost every

developing country or economy in transition over the past several decades [17]. It is estimated that in Africa and Middle East there are more than 100,000 tons of these products, in Asia almost 200,000 and a similar quantity in East Europe and the old Soviet Union. Nowadays the FAO is elaborating the inventories of Latin America [19,21-22]. In Mexico, there is knowledge of the existence of obsolete pesticide products, both liquid and solid. A total of 551 records of obsolete pesticide products have been registered, distributed in 29 of 33 states of Mexico, achieving a total of 26,725.02 liters, 147,274 kg and 500 m^3 of highly polluted soils. In addition there are 28 reports of pesticide-contaminated sites in 15 states of the Mexican Republic [23]. Besides, some data indicate that the total of empty pesticide containers can be about 7,000 tons annually [24].

2. Strategies to reduce the impact to the environment and health

Due to the problems mentioned above, development of technologies that guarantee their elimination in a safe, efficient and economical way is important. In order to reduce the effects of pesticides on the environment and health, for remediation of contaminated sites and for the treatment of pesticide residues and/or obsolete pesticides, different methods have been developed. Among the existent technologies there are those that apply physical treatments, such as adsorption and percolator filters; chemical treatments such as the advanced oxidation which involve the use of powerful transient species, mainly the hydroxyl radical. Other technique used for the degradation of pesticides the heterogeneous photocatalysis with TiO_2 is a method for producing the radical mentioned [25]. A method currently used is high temperature incineration in special furnaces: pesticides are packaged in the places where they were abandoned, then transported to a country that has special facilities to dispose of hazardous wastes. FAO estimates that the cost of these operations varies between 3,000 and 4,000 USD/ton [6]. Other strategies that have been studied for the degradation of these compounds include the photodegradation [26]. However all these methods have several disadvantages such as the use of chemical catalysts, as titanium dioxide, and the use of expensive technology in the case of ozone. For some pesticides alkaline hydrolysis is used, such in the case of organophosphates, which must include a rigorous control of the conditions under which the experiments performed, such as maintenance of alkaline pH, as well as the presence of complexes formed with metal ions, which involves the formation of secondary pollutants.

These conventional physicochemical approaches are generally expensive and remediation process is often incomplete due to the conversion of the parent compound to metabolites which are more persistent and equally or more toxic to non-target organisms [14].

An alternative pesticides treatment with important global boom is bioremediation, which is conducted through the biodegradation of these chemical compounds. This technique relies on the ability of microorganisms to convert organic contaminants in simple and harmless compounds to the environment. Bioremediation overcomes the limitations of traditional methods for the disposal of hazardous compounds, so it has allowed the destruction of many organic contaminants at a reduced cost. Consequently, in the last years, bioremediation

technology has progressed to an unknown virtual technology considered for the degradation of a wide range of pollutant compounds. Bioremediation can offer an efficient and cheap option for decontamination of polluted ecosystems and destruction of pesticides [14, 27-30]. As an efficient, economical and environmentally friendly technique, biodegradation has emerged as a potential alternative to the conventional techniques. However, the biodegradation process of many pesticides has not been fully investigated [31].

3. Microorganisms involved in the biodegradation of pesticides

Different biological systems, as microorganisms, have been used to biotransform pesticides. It has been reported that a fraction of the soil biota can quickly develop the ability to degrade certain pesticides, when they are continuously applied to the soil. These chemicals provide adequate carbon source and electron donors for certain soil microorganisms [32], establishing a way for the treatment of pesticide-contaminated sites [33-34].

Furthermore, the isolated microorganisms capable of degrading pesticides can be used for bioremediation of other chemical compounds to whom any microbial degradation system is known [14]. However, the transformation of such compounds depends not only on the presence of microorganisms with appropriate degrading enzymes, but also a wide range of environmental parameters [35]. Additionally, some physiological, ecological, biochemical and molecular aspects play an important role in the microbial transformation of pollutants [36-37].

There are different sources of microorganisms with the ability to degrade pesticides. Because pesticides are mainly applied to agricultural crops, soil is the medium that mostly gets these chemicals, besides pesticide industry's effluent, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of pesticides, and even some live organisms. In general, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of sites contaminated with some kind of pesticide. At present, in different laboratories around the world there are collections of microorganisms characterized by their identification, growth and degradation of pesticides. The isolation and characterization of microorganisms that are able to degrade pesticides give the possibility to count with new tools to restore polluted environments or to treat wastes before the final disposition [16].

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 [38], (Figure 2).
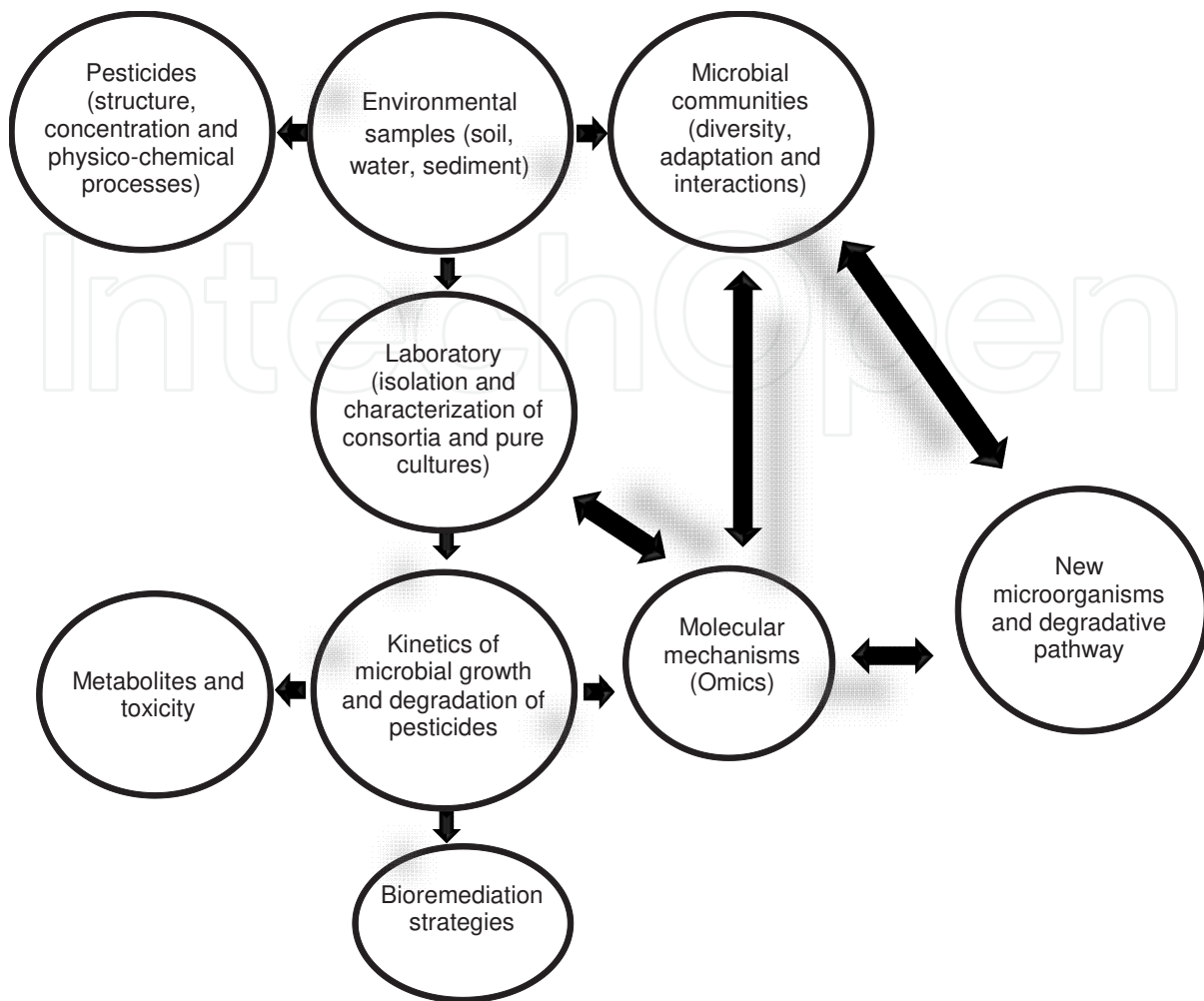
Figure 2. Representation of the relationships between pesticides, microbial communities, and the discovery of new biodegradation processes, Omics = high throughput-based characterization of biomolecules characteristic of bioprocesses; DNA, genomics; mRNA, transcriptomics; protein, proteomics; metabolites, metabolomics.

In the literature there are some examples of microbial pesticide degradation, among them, the following reports deserve mention:

According to [39], *Pseudomonas*, is the most efficient bacterial genus for the degradation of toxic compounds. The ability of these bacteria to degrade these compounds, is related to the contact time with the compound, the environmental conditions in which they develop and their physiological versatility. In other report [40], evaluated three *Pseudomonas* species for the biodegradation of the herbicide aroclor 1242, showing that these bacteria have a great ability to degrade it, according to their degradation percentage, 99.8, 89.4 and 98.4 respectively.

[41] isolated various fungi species from Algerian pesticide contaminated soils. Observing that the most frequent species isolated were *Aspergillus fumigatus, A. niger, A. terreus, Absidia and Rhizopus microsporus var corymberifera microsporis*. In this report, 53 of the isolated species were noted for their ability to degrade the herbicide metribuzin in liquid medium. It was demonstrated, at the same time, that the herbicide promoted the *Absidia* and *Fusarium* genera growth;

these genera were capable to eliminate the 50% of the compound after 5 days. Moreover, the species *Botrytis cinerea* could eliminate the linuron and metroburon herbicides almost completely, and other 31 isolated species also could eliminate metroburon. [42] reports the fungi *Trichoderma viridae* ability in the endosulfan and methyl parathion pesticides degradation.

Another experiments have been demonstrated the efficiency of the bacterium *Rhodococcus sp.* to degrade triazines to nitrate. [43] conducted a test to study the atrazine herbicide transformations resulting from microbial decomposition. After microbial action this compound was transformed into nitrite (30%), nitrous oxide (3.2%), ammonia (10%) and formaldehyde (27%).

Several bacterial genera are adapted to grow in pesticide contaminated soils. These microorganisms have enzymes involved in the hydrolysis of P-O, P-F, P-S and P-C bonds, which are found in a wide variety of organophosphorus pesticides [14]. Some bacteria isolated from the soil are capable of degrading pesticides as ethyl-parathion and methyl-parathion.

4. Biodegradation mechanisms

Biodegradation that involves the capabilities of microorganisms in the removal of pollutants is the most promising, relatively efficient and cost-effective technology. Biodegradation is a process that involves the complete rupture of an organic compound in its inorganic constituents. The microbial transformation may be driven by energy needs, or a need to detoxify the pollutants, or may be fortuitous in nature (cometabolism). Because of the ubiquitous nature of microorganisms, their numbers and large biomass relative to other living organisms in the earth, wider diversity and capabilities in their catalytic mechanisms [44], and their ability to function even in the absence of oxygen and other extreme conditions the search for pollutant-degrading microorganisms, understanding their genetics and biochemistry, and developing methods for their application in the field have become an important human endeavor [45].

As much as the diversity in sources and chemical complexities in organic pollutants exists, there is probably more diversity in microbial members and their capabilities to synthesize or degrade organic compounds [46-47]. The microbial populations of soil or aquatic environments are composed of diverse, synergistic or antagonistic communities rather than a single strain. In natural environments, biodegradation involves transferring the substrates and products within a well-coordinated microbial community, a process referred to as metabolic cooperation [48]. Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule. Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders [49]. Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it nontoxic. The biotransformed pesticide is released into the environment, where it is susceptible to further degradation by bacteria [50].

Fungi and bacteria are considered as the extracellular enzyme-producing microorganisms for excellence. White rot fungi have been proposed as promising bioremediation agents, especially

for compounds not readily degraded by bacteria. This ability arises from the production of extracellular enzymes that act on a broad array of organic compounds. Some of these extracellular enzymes are involved in lignin degradation, such as lignin peroxidase, manganese peroxidase, laccase and oxidases. Several bacterial that degrade pesticide have been isolated and the list is expanding rapidly. The three main enzyme families implicated in degradation are esterases, glutathione S-transferases (GSTs) and cytochrome P450 [51].

Enzymes are central to the biology of many pesticides [52]. Applying enzymes to transform or degrade pesticides is an innovative treatment technique for removal of these chemicals from polluted environments. Enzyme-catalyzed degradation of a pesticide may be more effective than existing chemical methods. Enzymes are central to the mode of action of many pesticides: some pesticides are activated *in situ* by enzymatic action, and many pesticides function by targeting particular enzymes with essential physiological roles. Enzymes are also involved in the degradation of pesticide compounds, both in the target organism, through intrinsic detoxification mechanisms and evolved metabolic resistance, and in the wider environment, via biodegradation by soil and water microorganisms [53]. [54] suggested that (i) the central metabolism of the global biodegradation networks involves transferases, isomerases, hydrolases and ligases, (ii) linear pathways converging on particular intermediates form a funnel topology, (iii) the novel reactions exist in the exterior part of the network, and (iv) the possible pathway between compounds and the central metabolism can be arrived at by considering all the required enzymes in a given organism and intermediate compounds [47].

For pesticides degradation, three are mainly enzyme systems involved: hydrolases, esterases (also hydrolases), the mixed function oxidases (MFO), these systems in the first metabolism stage, and the glutathione S-transferases (GST) system, in the second phase [55]. Several enzymes catalyze metabolic reactions including hydrolysis, oxidation, addition of an oxygen to a double bound, oxidation of an amino group (NH_2) to a nitro group, addition of a hydroxyl group to a benzene ring, dehalogenation, reduction of a nitro group (NO_2) to an amino group, replacement of a sulfur with an oxygen, metabolism of side chains, ring cleavage. The process of biodegradation depends on the metabolic potential of microorganisms to detoxify or transform the pollutant molecule, which is dependent on both accessibility and bioavailability [47].

Metabolism of pesticides may involve a three-phase process. In Phase I metabolism, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar or amino acid, which increases the water solubility and reduces toxicity compared with the parent pesticide. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also non-toxic. In these processes fungi and bacteria are involved producing intracellular or extra cellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc [16, 56].

Due to the diversity of chemistries used in pesticides, the biochemistry of pesticide bioremediation requires a wide range of catalytic mechanisms, and therefore a wide range of enzyme classes. Information for some pesticide degrading enzymes could be founded in Table 3.

Enzyme	Organism	Pesticide
Oxidoreductases (Gox)	*Pseudomonas* sp. LBr	Glyphosate
	Agrobacterium strain T10	
Monooxygenases:		
ESd	*Mycobacterium* sp.	Endosulphan and Endosulphato
Ese	*Arthrobacter* sp.	Endosulphan, Aldrin, Malation, DDDT and Endosulphato
Cyp1A1/1ª2	Rats	Atrazine, Norflurazon and Isoproturon
Cyp76B1	*Helianthus tuberosus*	Linuron, Chlortoluron and Isoproturon
P450	*Pseudomonas putida*	Hexachlorobenzene and Pentachlorobenzene
Dioxygenases (TOD)	*Pseudomonas putida*	Herbicides Trifluralin
E3	*Lucilia cuprina*	Synthetic pyrethroids and insecticides phosphotriester
Phosphotriesterases:	Agrobacterium radiobacter	Insecticides phosphotriester
OPH/OpdA	Pseudomonas diminuta	
	Flavobacterium sp.	
Haloalkane Dehalogenases:	*Sphingobium* sp.	Hexachlorocyclohexane (β and δ isomers)
LinB	*Shingomonas* sp.	
AtzA	*Pseudomonas* sp. ADP	Herbicides chloro-s-trazina
TrzN	*Nocardioides* sp.	Herbicides chloro-s-trazina
LinA	*Sphingobium* sp.	Hexachlorocyclohexane (γ isomers)
	Shingomonas sp.	
TfdA	*Ralstonia eutropha*	2,4 - dichlorophenoxyacetic acid and pyridyl-oxyacetic
DMO	*Pseudomonas maltophilia*	Dicamba

Table 3. Relevant enzymes in the bioremediation of pesticides [52-53].

5. Generalities of the major enzymatic activities applied for pesticide biodegradation

5.1. Hydrolases

Hydrolases are a broad group of enzymes involved in pesticide biodegradation. Hydrolases catalyze the hydrolysis of several major biochemical classes of pesticide (esters, peptide bonds, carbon-halide bonds, ureas, thioesters, etc.) and generally operate in the absence of redox cofactors, making them ideal candidates for all of the current bioremediation strategies [53].

As an example of the catalytic activity of enzymes hydrolases, the degradation pathway of carbofuran, a pesticide the group of carbamates is presented (Figure 3). This pesticide can be transformed in the environment and different metabolites are generated and accumulated in potentially contaminated sites (soil, water and sediments, mainly). Different organisms isolated from contaminated sites that have been identified and characterized as transformers of carbofuran, resulting in different metabolites [57].

Among the hydrolases involved in the degradation of pesticides are including different types such as:

5.2. Phosphotriesterases (PTEs)

Among the most studied pesticide degrading enzymes, the PTEs are one of the most important groups [58]. These enzymes have been isolated from different microorganisms that hydrolyze and detoxify organophosphate pesticides (OPs). This reduces OP toxicity by decreasing the ability of OPs to inactivate AchE [14, 59-62]. The first isolated phosphotriesterase belongs to the *Pseudomonas diminuta* MG species; this enzyme shows a highly catalytic activity towards organophosphate pesticides. The phosphotriesterases are encoded by a gene called *opd* (organophosphate-degrading). *Flavobacterium* ATCC 27551 presents the *opd* gene encoding to a PTE [63]. The gene was cloned and sequenced by [64]. These enzymes specifically hydrolyze phosphoester bonds, such as P–O, P–F, P–NC, and P–S, and the hydrolysis mechanism involves a water molecule at the phosphorus center [65]. Different microbial enzymes with the capacity to hydrolyze MP have been identified, such as organophosphorus hydrolase (OPH; encoded by the *opd* gene), methyl-parathion hydrolase (MPH; encoded by the *mpd* gene), and hydrolysis of coroxon (HOCA; encoded by the *hocA* gene), which were isolated from *Flavobacterium* sp. [66], *Plesimonas* sp. strain M6 [67] and *Pseudomonas moteilli* [68], respectively.
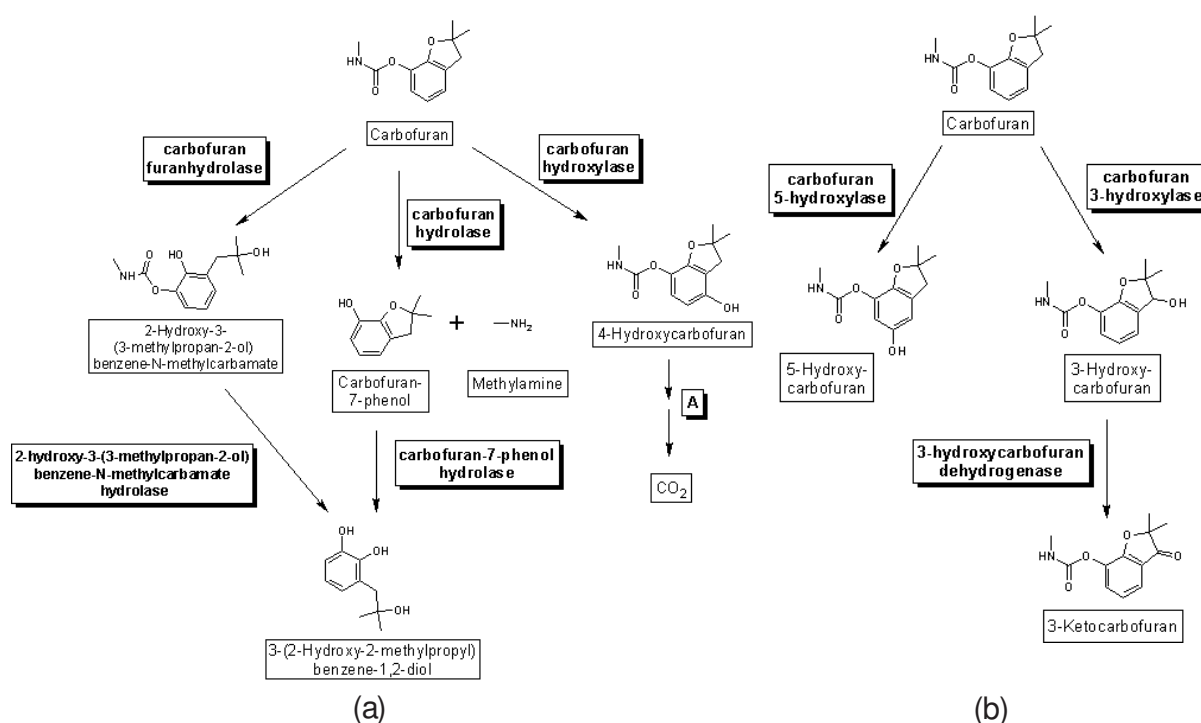

Figure 3. Degradation pathway of carbofuran. In a) several bacteria are involved in the hydrolysis of metabolites and b) fungal degradation of carbofuran may occur via hydroxylation at the three position and oxidation to 3-ketocarbofuran (University of Minnesota. Biocatalysis/Biodegradation Database, http://www.umbbd.ethz.ch/cbf/cbf_image_map1.html).

The phosphotriesterase enzyme is a homo-dimeric protein with a monomeric molecular weight of 36 Kda. As a first step in the PTE organophosphorous pesticide hydrolysis mechanism, the enzymatic active site removes a proton from water, activating this molecule, them, the activated water directly attacks the central phosphorus of the pesticide molecule producing an inversion in its configuration The oxygen is polarized by the active site, with the participation of a zinc atom [6, 69], (Figure 4). This enzyme has potential use for the cleaning of organophosphorus pesticides contaminated environments [65].
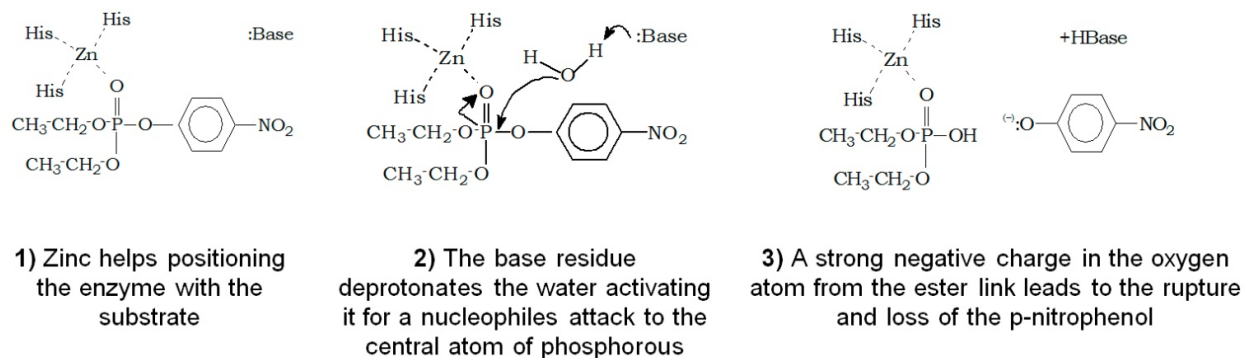
1) Zinc helps positioning the enzyme with the substrate

2) The base residue deprotonates the water activating it for a nucleophiles attack to the central atom of phosphorous

3) A strong negative charge in the oxygen atom from the ester link leads to the rupture and loss of the p-nitrophenol

Figure 4. Proposed mechanism for PTE activity. Zinc's active site functions in phosphate polarization, making phosphor more susceptible to the attack. 1) A base subtracts a proton from a water molecule with the subsequent attack of the hydroxyl to the central phosphorous. 2) The intermediary complex originates the products 3) p-nitrophenol and diethyl thiophosphate [6].

5.3. Esterases

Esterases are enzymes that catalyze hydrolysis reactions over carboxylic esters (carboxiesterases), amides (amidases), phosphate esters (phosphatases), etc. [70]. In the reaction catalyzed by esterases, hydrolysis of a wide range of ester substrates occurs in their alcohol and acid components as following:

$$R = O\text{-}OCH_3 + H_2O \leftrightarrow R = O\text{-}OH + CH_3OH$$
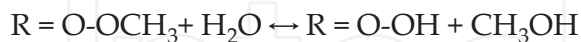
Many insecticides (organophosphates, carbamates and pyrethroids) have associated a carboxylic ester, and the enzymes capable of hydrolyze such ester bond are known with the name of carboxylesterases.

At present, multiple classification nomenclature systems are used for these enzymes. According to the International Union of Biochemistry and Molecular Biology (IUBMB) nomenclature, carboxylesterases are located in the group of hydrolases (3), subgroup 1, and within it, in subtype 1 (Enzyme Commission 3.1.1.1, EC 3.1.1.1). Another common classification is the nomenclature divides the esterases into three groups according to the nature of their interactions with organophosphorus insecticides. Carboxylesterases belong, according to this classification, the group of ali-esterases and B-esterases. Esterases are a large family of enzymes in arthropods [71].

The esterases are a group of enzymes highly variable, which has been recognized as one of the most important in the metabolism of xenobiotics and its mechanism is associated with the mass production of multifunctional hydrolytic enzymes Organophosphate pesticides can be hydrolyzed by such enzymes [72-74]. There are different types of esterases and with very different distribution in tissues and organisms. The Carboxiesterases (type B esterases) are a group that hydrolyze, additionally to endogenous compounds, xenobiotics with ester, amide, thioester, phosphate esters (parathion, paraoxon) and acid anhydrides (DIPFP=DFP) in mammals.

Esterases A, contain a Cys residue in the active center and esterases B contain a Ser residue. In esterases A, the organophosphates interact with the functional group-SH forming a bond between P=S, which is easily hydrolyzed by H_2O. In the esterase B, organophosphates interaction with the SER-OH forming a P=O bond that is not hydrolyzed by H_2O. Organophosphates that bind to the esterase B stoichiometrically inhibit its enzymatic activity.

Esterases are a diverse group that protects the target site (acetylcholinesterase) by catalyzing the hydrolysis of insecticides, or acting as an alternative blank [75]. Esterases in general have a wide range of substrate specificities; they are capable of binding to phosphate triesters, esters, thioesters, amides and peptides [76].

5.4. Oxidoreductases

Oxidoreductases are a broad group of enzymes that catalyze the transfer of electrons from one molecule (the reductant or electron donor) to another (the oxidant, or electron acceptor). Many of these enzymes require additional cofactors, to act as either electron donors, electron acceptors or both. Oxidoreductases have been further sub classified into 22 subclasses (EC 1.1-1.21 and 1.97). Several of these have applications in bioremediation, albeit their need for cofactors complicates their use in some applications. There are enzymes that catalyze an oxidation/reduction reaction by including the molecular oxygen (O_2) as electron acceptor. In these reactions, oxygen is reduced to water (H_2O) or hydrogen peroxide (H_2O_2). The oxidases are a subclass of the oxidoreductases [53].

As an example of the many functions of these enzymes in the degradation of pesticides, as an example we present the ensodulfan degradation pathway. In this process not only oxidoreductase enzymes are involved, but different microorganisms and catalytic activities, in combination, can lead to complete mineralization of a pesticide (Figure 5). Endosulfan (*1,2,5,6,7,7-hexachloro-5-norbornene-2,3- dimethanolcyclic sulfite*) is an organochlorine insecticide of the cyclodiene family of pesticides. It is highly toxic and endocrine disruptor, and it is banned in European Union and several countries. Because it has been extensively applied directly to fields, it can be detected a considerable distance away from the original site of application. Contamination of drinking water and food, as well as detrimental effects to wildlife are important concerns [77]. The molecular structure has two stereochemical isomers α and β endosulfan. The end-use product of endosulfan is a mixture of two isomers, typically in a 2:1 ratio.

Microorganisms play a key role in removal of xenobiotics like endosulfan from the contaminated sites because of their dynamic, complex and complicated enzymatic systems which degrade these chemicals by eliminating their functional groups of the parent compound. This pesticide can undergo either oxidation or hydrolysis reactions. Several intensive studies on the degradation of endosulfan have been conducted showing the primary metabolites to normally be endosulfan sulfate and endosulfan diol (endodiol). Endosulfan sulphate will be present in the environment as a result of the use of endosulfan as an insecticide. If endosulfan sulphate is released to water, it is expected to absorb to the sediment and may bioconcentrate in aquatic organism. This metabolite has a similar toxicity as endosulfan and has a much longer half-life in the soil compared to endosulfan. Therefore, production of endosulfan sulfate by biological systems possesses an ecological hazard in that it contributes to long persistence of endosulfan in soil. Endodiol is much less toxic to fish and other organisms than the parent compound.

Thus, it is important to note that some microbial enzymes are specific to one isomer, or catalyze at different rates for each isomer [78]. For example, a *Mycobacterium tuberculosis* ESD enzyme degrades beta-endosulfan to the monoaldehyde and hydroxyether (depending on the reducing equivalent stoichiometry), but transforms alpha-endosulfan to the more toxic endosulfan sulfate. However, oxidation of endosulfan or endosulfan sulfate by the monooxygenase encoded by *ese* in *Arthrobacter* sp. KW yields endosulfan monoalcohol [79]. Both *ese* and *esd* proteins are part of the unique Two Component Flavin Dependent Monooxygenase Family, which require reduced flavin. They are conditionally expressed when no or very little sulfate or sulfite is available, and endosulfan is available to provide sulfur in these starved conditions.

Alternatively, hydrolysis of endosulfan in some bacteria (*Pseudomonas aeruginosa*, *Burkholderia cepaeia*) yields the less toxic metabolite endosulfan diol [80]. Endosulfan can spontanteously hydrolyze to the diol in alkaline conditions, so it is difficult to separate bacterial from abiotic hydrolysis. The diol can be converted to endosulfan ether or endosulfan hydroxyether and then endosulfan lactone [81]. Hydrolysis of endosulfan lactone yields endosulfan hydroxy-carboxylate. These various branches of endosulfan degradation all result in desulfurization while leaving the chlorines intact, exhibiting the recalcitrance to bioremediation found in many organohalogen aromatics.

5.5. Mixed Function Oxidases (MFO)

In the reaction catalyzed by the MFO (EC 1.14.14.1), an atom of one molecule of oxygen is incorporated into the substrate, while the other is reduced to water. For this reason the MFO requires Nicotiamide-adenine dinucleotide phosphate (NADPH) and O_2 for its operation.

It is an enzyme system comprising two enzymes, cytochrome P450 and NADPH-cytochrome P450 reductase, both membrane proteins. They are also known as dependent cytochrome P-450 monooxygenases or P450 system. The genes encoding the different isozymes comprise a superfamily of over 200 genes grouped into 36 families based on their sequence similarity. Cytochrome P450 enzymes are active in the metabolism of wide variety of xenobiotics [82].

The cytochrome P450 family is a large, well characterized group of monooxygenase enzymes that have long been recognized for their potential in many industrial processes, particularly due to their ability to oxidize or hydroxylate substrates in an enantiospecific manner using molecular oxygen [83]. Many cytochrome P450 enzymes have a broad substrate range and have been shown to catalyse biochemically recalcitrant reactions such as the oxidation or hydroxylation of non-activated carbon atoms. These properties are ideal for the remediation of environmentally persistent pesticide residues. Over 200 subfamilies of P450 enzymes have been found across various prokaryotes and eukaryotes. All contain a catalytic iron-containing porphyrin group that absorbs at 450 nm upon binding of carbon monoxide. In common with many of the other oxidoreductases described before, P450 enzymes require a non-covalently bound cofactor to recycle their redox center (most frequently NAD(P)H is used), which limits their potential for pesticide bioremediation to strategies that employ live organisms.

In insects, MFOs are found in the endoplasmic reticulum and mitochondria, are involved in a large number of processes such as growth, development, reproduction, detoxification, etc. MFO are involved in the metabolism of both endogenous and exogenous substances, for this reason these compounds promote their induction Due to its high inspecificity, the MFOs metabolize a wide range of compounds such as organophosphates, carbamates, pyrethroids, DDT, inhibitors of the chitin synthesis, juvenile hormone mimics, etc. [84].

5.6. Glutathione S-Transferase (GST)

The GSTs (EC 2.5.1.18) are a group of enzymes that catalyze the conjugation of hydrophobic components with the tripeptide glutathione (Figure 6). In this reaction, the thiol group of glutathione reacts with an electrophilic place in the target compound to form a conjugate which can be metabolized or excreted, and they are involved in many cellular physiological activities, such as detoxification of endogenous and xenobiotic compounds, intracellular transport, biosynthesis of hormones and protection against oxidative stress [85].
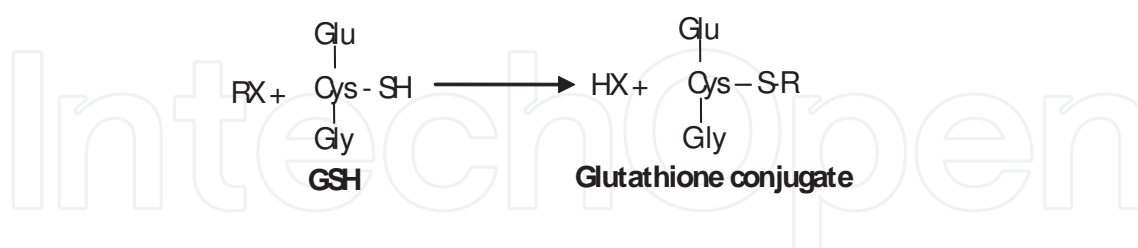
Figure 6. Representation of the conjugation reaction catalyzed by glutathione S-transferase (GST).

6. Genetics for pesticide degradation

In order to investigate genetic basis of pesticides biodegradation, several works with special emphasis on the role of catabolic genes and the application of recombinant DNA technology, had been reported. Pesticide-degrading genes of only a few microorganisms have been
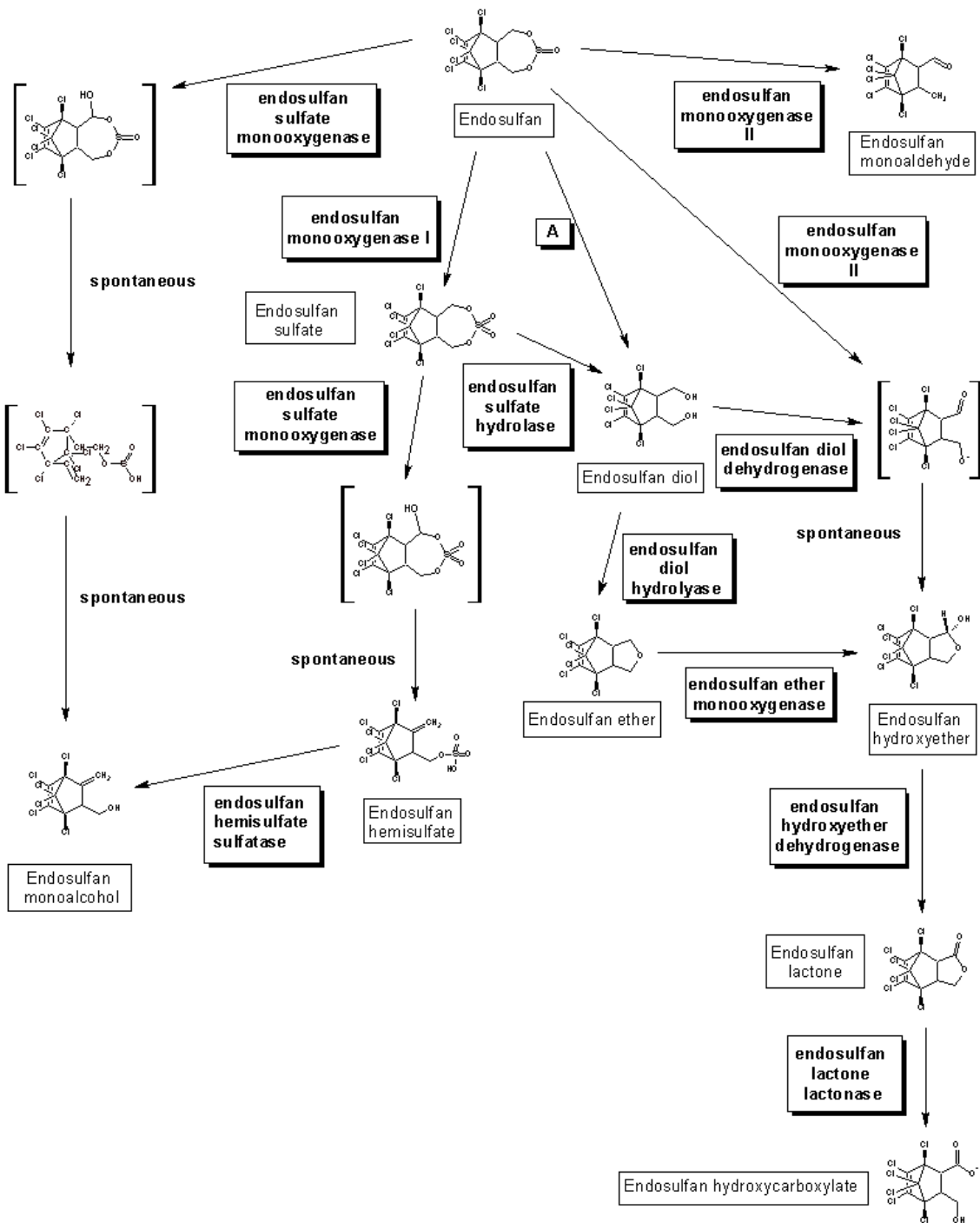
Figure 5. Degradation pathway of endosulfan (University of Minnesota. Biocatalysis/Biodegradation Database, http://www.umbbd.ethz.ch/end/end_map.html).

characterized. Most of genes responsible for catabolic degradation are located on the chromosomes, but in a few cases these genes are found in plasmids or transposons. The recent advances in metagenomics and whole genome sequencing have opened up new avenues for searching the novel pollutant degradative genes and their regulatory elements from both culturable and nonculturable microorganisms from the environment. Mobile genetic elements

such as plasmids and transposons have been shown to encode enzymes responsible for the degradation of several pesticides. The isolation of pesticide degrading microorganisms and the characterization of genes encoding pesticide degradation enzymes, combined with new techniques for isolating and examining nucleic acids from soil microorganisms, will yield unique insights into the molecular events that lead to the development of enhanced pesticide degradation phenomenon.

An understanding of the genetic basis of the mechanisms of how microorganisms biodegrade pollutants and how they interact with the environment is important for successful implementation of the technology for in situ remediation [86].

Different microbial enzymes with the capacity to hydrolyze pesticides have been identified [57], such as **organophosphorus hydrolase** (OPH; encoded by the *opd* gene). This gene has been found in bacterial strains that can use organophosphate pesticides as carbon source; these have been isolated in different geographic regions. These plasmids show considerable genetic diversity, but the region containing the *opd* gene is highly conserved. **Methyl-parathion hydrolase** (MPH; encoded by the *mpd* gene), Are *Pseudaminobacter*, *Achrobacter*, *Brucella* and *Ochrobactrum* genes, they were identified by comparison with the gene *mpd* from *Pleisomonas* sp. M6 strain [87], the gene for the organophosphorus hydrolase has 996 nucleotides, a typical promoter sequence of the promoter TTGCAA N17 TATACT from *E. coli* [88].

In the various isolates of microorganisms capable of degrading pesticide, several genes have been described, in the table 4 shown the most studied.

7. Genetic engineering

Microorganisms respond differently to various kinds of stresses and gain fitness in the polluted environment. This process can be accelerated by applying genetic engineering techniques. The recombinant DNA and other molecular biological techniques have enabled (i) amplification, disruption, and/or modification of the targeted genes that encode the enzymes in the metabolic pathways, (ii) minimization of pathway bottlenecks, (iii) enhancement of redox and energy generation, and (iv) recruiting heterologous genes to give new characteristics [45,89]. Various genetic approaches have been developed and used to optimize the enzymes, metabolic pathways and organisms relevant for biodegradation [90]. New information on the metabolic routes and bottlenecks of degradation is still accumulating, requiring the need to reinforce the available molecular toolbox. Nevertheless, the introduced genes or enzymes, even in a single modified organism, need to be integrated within the regulatory and metabolic network for proper expression [89-91].

Detoxification of organophosphate pesticides was the first demonstrated by genetically engineered microorganisms and the genes encoding these hydrolases have been cloned and expressed in *P. pseudoalcaligenes*, *Escherichia coli*, *Streptomyces lividans*, *Yarrowia lipolytica* and *Pichia pastoris* [92-96].

Gene	Organism
Bacteria	
Opd	*Pseudomonas diminuta*
opaA	*Alteromonas* spp.
opdA	*A. radiobacter*
adpB	*Nocardia* sp.
pepA	*Escherichia coli*
hocA	*Pseudomonas monteilli*
pehA	*Burkholderia caryophilli*
Phn	*Bacillus cereus*
ophB	*Burkholderia* sp. JBA3.
ophC2	*Stenotrophomonas* sp. SMSP-1.
OpdB	*Lactobacillus brevis.*
Imh	*Arthrobacter* sp. scl-2.
Mpd	*Ochrobactrum* sp. Yw28, *Rhizobium radiobacter*
Oph	*Arthrobacter* sp
Mph	*Arthrobacter* sp. L1 (2006).
MpdB	*Burkholderia cepacia*
opdE	*Enterobacter* sp.
Fungi	
A-opd	*Aspargillus niger*
P-opd	*Penicillium lilacinum*

Table 4. Genes with the ability to degrade pesticides (Modified from 14).

Another strategy that has been used is phytoremediation, the use of plants to clean-up polluted soil and water resources is recognized as an economically cheaper, aesthetically pleasing, and environmentally friendly 'Green technology [97,98]. However, the limitation with plants is that they lack the catabolic pathways for complete degradation/mineralization of externally added organic compounds. The potential of plants to degrade organic pollutants can be further enhanced by engineering plants by introduction of efficient heterologous genes that are involved in the degradation of organic pollutants [98].

Unfortunately, the rates of hydrolysis several enzymes differ dramatically for members of the family of OP compounds, ranging from hydrolysis at the diffusion-controlled limit for paraoxon to several orders of magnitude slower for malathion, chlorpyrifos, and others pesticides [99]. Although site-directed mutagenesis has been used to improve the substrate

specificity and stereoselectivity of OPH [99-100], the ability to deduce substitutions that are important for substrate specificity is still limited to the active-site residues.

Two interesting papers have shown that an biological solution for efficient decontamination might be to direct evolution. Directed evolution has recently been used to generate OPH variants with up to 25-fold improvements in hydrolysis of methyl parathion [101], a substrate that is hydrolyzed 30-fold less efficiently than paraoxon, and other report the directed evolution of OPH to improve the hydrolysis of a poorly hydrolyzable substrate, chlorpyrifos (1,200-fold less efficient than paraoxon). Up to 700-fold improvement was obtained, and the best variant hydrolyzes chlorpyrifos at a rate similar to that of the hydrolysis of paraoxon by wild-type OPH [102].

8. The application of genomics and functional genomics

8.1. Metagenomics

The complexity of microbial diversity results from multiple interacting parameters, which include pH, water content, soil structure, climatic variations and biotic activity. Current estimates indicate that more than 99% of the microorganisms present in many natural environments are not readily culturable and therefore not accessible for biotechnology or basic research [103]. During the last two decades, development of methods to isolate nucleic acids from environmental sources has opened a window to a previously unknown diversity of microorganisms. Analysis of nucleic acids directly extracted from environmental samples allows researchers to study natural microbial communities without the need for cultivation [103-104].

Each organism in an environment has a unique set of genes in its genome; the combined genomes of all the community members make up the "metagenome". Metagenome technology (metagenomics) has led to the accumulation of DNA sequences and these sequences are exploited for novel biotechnological applications [105,106]. Due to the overwhelming majority of non-culturable microbes in any environment, metagenome searches will always result in identification of hitherto unknown genes and proteins [105-106].

8.2. Functional genomics

In its broadest definition, functional genomics encompasses many traditional molecular genetics and biological approaches, such as the analysis of phenotypic changes resulting from mutagenesis and gene disruption [107]. Functional genomics has emerged recently as a new discipline employing major innovative technologies for genome-wide analysis supported by bioinformatics. These new techniques include proteomics for protein identification, characterization, expression, interactions and transcriptomic profiling by microarrays and metabolic engineering [107]. The application of proteomics in environmental bioremediation research provides a global view of the protein compositions of the microbial cells and offers a promising approach to address the molecular mechanisms of bioremediation. With the combination of

proteomics, functional genomics provide an insight into global metabolic and regulatory networks that can enhance the understanding of gene functions.

The fundamental strategy in a functional genomics approach is to expand the scope of biological investigations from studying a single gene or protein to studying all the genes or proteins simultaneously in a systematic fashion. The classic approach to assess gene function is to identify which gene is required for a certain biological function at a given condition through gene disruption or complementation. With the combination of technologies, such as transcriptomics and proteomics complementing traditional genetic approaches, the detailed understanding of gene functions becomes feasible [105-107].

Metabolic engineering combines systematic analysis of metabolic and other pathways with molecular biological techniques to improve cellular properties by designing and implementing rational genetic modifications [108]. Understanding microbial physiology, will adapt to the host cells to support changes and become more efficient bioremediation processes, events that would be difficult to acquire during evolution [105].

With these new genomics tools scientists are in a better position to answer questions such as how oxygen stress, nutrient availability, or high contaminant concentrations along differing geochemical gradients or at transitional interfaces impact the organohalide respiring community structure and function. Ultimately, by tracking the overall microbial community structure and function in addition to key functional players, informed decisions can then be made regarding how to best manipulate the field conditions to achieve effective bioremediation of, e.g., pesticides.

9. Strategies to enhance the efficiency of pesticide degradation: Case cells immobilization

Cell immobilization has been employed for biological removal of pesticides because it confers the possibility of maintaining catalytic activity over long periods of time [109-111]. Whole-cell immobilization has been shown to have remarkable advantages over conventional biological systems using free cells, such as the possibility of employing a high cell density, the avoidance of cell washout, even at high dilution rates, easy separation of cells from the reaction system, repeated use of cells, and better protection of cells from harsh environments. Previous reports have suggested that this higher productivity results from cellular or genetic modifications induced by immobilization. There is evidence indicating that immobilized cells are much more tolerant to perturbations in the reaction environment and less susceptible to toxic substances, which makes immobilized cell systems particularly attractive for the treatment of toxic substances like pesticides [112]. In addition, the enhanced degradation capacity of immobilized cells is due primarily to the protection of the cells from inhibitory substances present in the environment. The degradation rates for repeated operations were observed to increase for successive batches, indicating that cells became better adapted to the reaction conditions over time [113].

There are two types of processes for cell immobilization: those based on physical retention (entrapment and inclusion membrane) and those based on chemical bonds, such as biofilm formation [114]. In cell immobilization methods may be used various materials or substrates inorganic (clays, silicates, glass and ceramics) and organic (cellulose, starch, dextran, agarose, alginate, chitin, collagen, keratin, etc.) [115]. Entrapment in polymeric gels natural has become the preferred technique for the immobilization of cells, however, immobilized cell on supports have been used more frequently in xenobiotics biodegradation as pesticides [116].

In order to degrade pesticides, is important to search for materials with favorable characteristics for the immobilization of cells, including aspects such physical structure, ease of sterilization, the possibility of using it repeatedly, but above all, the support must be cheap than allow in the future apply it for pesticide degradation. Table 5 describes the main methods of immobilization [115,117-120]. Thus, the methods can be grouped in two ways: the active that induce the capture of microorganisms in a matrix, and the passive that uses the tendency of microorganisms to attack surfaces either natural or synthetic, which form biofilms.

TYPE	METHOD	DESCRIPTION	MATERIAL / MATRIX
Chemical bond	Carrier union	Based on the union of biocatalyzers to insoluble carriers through covalent or ionic liknks, physical adsorption and biospecific union. The carrier materials must have enough mechanical strength, physical, chemical and biological stability. They must be economic and malleable but not toxic. This method does not apply with cells, for it is difficult to find the immobilization conditions.	• Water insoluble polysaccharides: dextran, cellulose, agarose • Proteins: albumin • Synthetic polymers: polystyrene deriver, ionic exchange resins • Organic materials: ceramics, magnetite and glass
	Cross-linked	It uses multifunctional reactive. Cells are linked to a matrix in such way that they form concentrate pellets	Dialdehydes, glutaraldehydes and diisocyanates are used.
Physical Retention	Entrapment	This method consists on the retention in inner cavities of a porous matrix	It uses porous matrix: alginate, agar, *k*-carrageenan, polyacrylamide, chitosan, collagen, polystyrene, cellulose triacetate, activated charcoal, porous ceramic and diatomaceous earth
	Inclusion in membrane	The enzymes or cells are surrounded by semipermeable membranes. This method allows multiple steps. Reactions to take place in reactors	Materials that form surfactant micelles

Table 5. Classification and description of methods of immobilization.

By the other hand, a biofilm can be defined as a coherent complex structure of microorganism organized in colony and cell products such as extracellular polymers (exopolymer), which either spontaneously or in forming dense granules, grow attached to a solid surface static (static biofilm) or in a suspension bracket [121,122]. The biofilm formation process is performed in several steps starting with the attack or recognition to the surface, followed by growth and utilization of various carbon and nitrogen sources for the formation of products with adhesive properties. In parallel a stratified organization dependent on oxygen gradients and other abiotic conditions takes place. This process is known as colonization. Then an intermediate period of maturation of the biofilm is carried out which varies depending on the presence of nutrients from the medium or friction with the surrounding water flow. Finally, a period of aging biofilm where a detachment of cells may occur and colonize other surfaces [123].

The hydrodynamic plays an important role in the development of biofilm as these organizations develop in a solid-liquid interface, where the flow rate passing through it influences the physical detachment of microorganisms. They possess a system of channels that allow the transport of nutrients and waste; this is vital when modify the environment that deprives microorganisms of molecules necessary for their development. Other biofilm characteristic is its resistance to host defenses and antimicrobial agents. While the microorganism are susceptible to different control factors, the colonies organized and included in a exopolymer form an impermeable layer where only the most superficial microorganisms are affected. Also when released biofilm cells, they can travel and to be deposited on new niche maintaining the same characteristics of a biofilm adhered to a surface. Microorganisms are communicated with each other. This is what has been called quorum sensing and involves regulation and expression of specific genes through signaling molecules that mediate intercellular communication [14, 124]. This characteristic is dependent on cell density; for example, biofilm with a high cell density, it induces expression of resistance genes that provide protection and survival [125]. Similarly, microorganisms can produce substances to promote the propagation of colonies and inhibit the growth of other leaving pathogens microorganisms in a more favorable position within the biofilm [126]. The supports may be of synthetic or natural origin (Table 6).

A material that has yielded good results in the degradation of mixtures of pesticides is the tezontle (in Nahuatl, tezt means rock and zontli means hair), that is a native volcanic rock of Morelos state (central Mexico). This rock is highly porous, provides a large contact surface, and can also be sterilized and reused. The presence of micropores allows the establishment of bacterial microcolonies. The immobilization method with this material is based on the colonization of the tezontle micropores through the formation of a biofilm. Subsequently, a current with the pesticides wastes is passed through to allow the contact with the immobilized microorganisms, so this way the biodegradation can be executed. This strategy has been really efficient and is a tool that can be used for the degradation of pesticides wastes [123]. In our work group, a bacterial consortium was immobilized in a biofilm on tezontle and exhibited a considerable capacity for the removal of a mixture of organophosphate pesticides, which are the pesticides widely used in agriculture and stockbreeding in Mexico. In addition, this material with immobilized cells was packaged in an up-flow reactor, which was obtained the greater viability of the bacteria as more efficient removal of pesticides [123].

Support	Xenobiotic	Microorganism	Efficiency	Reference
Glass beads	Coumaphos	*E. coli (transformed)*	Removal: 80%	[127]
Ceramic	Propachlor	*Pseudomonas GCH1*	Removal: 98% (39% absorption to the material)	[128]
Ca Alginate beads	Poliphenols	*Candida tropicalis YMEC14*	After 24 h of fermentation, removal of 69.2% and 55.3% of monophenols and polyphenols respectively,	[129]
Polyurethane Alginate Alginate poly vinyl alcohol	Phenol	*Pseudomonas spp.*	Removal time: 23 days (in suspension culture), 15 days (in polyurethane) and 7 days (in alginate)	[130]
Coffe beans	DDT Endosulfan	*P. aeruginosa* and *F. oryzihabitans,*	Removal: 68% DDT	[131]
Agave tequiliana Webber (blue)	Blue ácido113 Disperse Blue 3 Basic Green 4	*Trametes versicolor P. ostreatus Klebsiella sp.*	79.85% (9.98 mg/L/day) 62.39% (7.8 mg/L/day) 94.7% (11.84 mg/L/day) Mineralization	[132]
Ca Alginate beads	Coumaphos, diethylphosphate and chlorferon	*Escherichia coli (OPH)*	Degradation: 163 mg/g biomass/h (immobilized) 0.034 mg/g biomass/h (suspended)	[113]
Tezontle	2,4-D DDT	*Pseudomonas fluorescens*	Removal: 99%	[133]
Ca Alginate beads Tezontle	Methyl parathion/ tetrachlorvinphos	Bacterial consortia	Removal of 78% of methyl parathion Removal of 49 % of tetracholvinphos	[123]

Table 6. Different materials used as supports for immobilization of microorganisms in bioremediation.

Furthermore, there are several reports that indicate a variety of materials that provide the features necessary to immobilize microorganisms. For example, the use of various plant fibers as support for immobilizing bacterial consortium to degrade xenobiotics has important advantages. The use of the natural structural materials such as petiolar felt-sheath of palm for the cell entrapment has added another dimension to a variety of immobilization matrices. The advantages accruable from such biostructures are reusability, freedom from toxicity problems, mechanical strength for necessary support, and open spaces within the matrix for growing cells thus avoiding rupture and diffusion problems. These have suggested the need to search for other types of biomaterials from diverse plant sources that may be used for cell entrapment.

The loofa sponge (*Luffa cylindrica*) was used as carrier material for immobilizing various microorganisms for the purpose of either adsorption or degradation of various xenobiotics as

shown in Table 7. This sponge have been used as natural support to immobilize various organisms such as *Chlorella sorokiniana, Porphyridium cruentrum, Penicillium cyrlopium, Funalia trogii* for nickel and cadmium II treatment, besides dyes and chlorinated substances. Loofa grows well in both tropical and subtropical climates and the sponges are produced in large quantities in Mexico where they are currently used for bathing and dish washing. They are light, cylindrical in shape and made up of an interconnecting void within an open network of matrix support materials. As a result of their random lattice of small cross sections coupled with very high porosity, their potentiality as carriers for cell immobilization is very high. The sponges are strong, chemically stable, and composed of interconnecting voids within an open network of fibers. Because of the random lattices of small cross sections of the sponges coupled with high porosity, the sponges are suitable for cell adhesion [134-136]. This sponge was used by our work group and we found methyl parathion removal efficiencies of 75%.

Xenobiotic	Immobilized microorganism	Efficiency	Reference
Nickel II, Chrome	*Chlorella sorokiniana:*	ND	[135].
Lead II ions, copper II and zinc II	*Phanerochaete chrysosporium*	Adsorption: 135.3, 102.8, 50.9 mg/g of Pb(II), Cu(II) y Zn(II) respectively.	[136]
Pb II, Hg II and Cd II ions	*Aspergillus terreus*	Adsorption: 247.2, 37.7 y 23.8 mg/g for Pb II, Hg II y Cd II respectively	[31]
Black 5 (RB5) reactive	*Funalia trogii*	ND	[134]
Blue 172 reactive	*Proteus vulgaris NCIM-2027*	Total discoloration at 37 ° C and pH 8.0 to 5-h in static incubation	[137]
Carbendazim and 2,4-diclorofexiacetic acid (2,4-D)	Bacterial consortium	Complete degradation to 5.5 and 1.5 days respectively	[138]
Carbendazim and 2,4-D	Bacterial consortium	Removal: 20 and, 50% respectively	[139]
Removal of organic matter and ammonium from wastewater	Aerobic bacteria	Chemical oxigen demand removal: 80% Nitrogen removal: 85.6%	[140]
Methylene blue, Crude oil Malachite green dye	--	Adsorption of 49 mg/g Adsorption of 4.6 g oil/g sorbent (in 24 hours.). Adsorption capacity of 29.4 mg / g	[141-143]

Table 7. Loofa use (*L. cylindrica*) as supports for immobilization of microorganisms in bioremediation. ND = Not detected.

10. Final considerations

For the biological degradation of pesticides, it is important to understand the molecular mechanisms involved in enzymatic catalysis, which will be possible to design new alternatives and/or efficient tools for the treatment of pesticide residues or for the bioremediation of contaminated sites. This information could be used in the future to treat pesticide residues in the field (such as waste resulting after washing pesticide containers), or the obsolete pesticides. Moreover, in implementing strategies to increase the efficiency of degradation, such as cell immobilization (bacteria or fungi), we may have tools to abate the existence of obsolete pesticides and waste generated, it will reduce the danger of pesticides on the environment and health.

Author details

Ma. Laura Ortiz-Hernández*, Enrique Sánchez-Salinas, Edgar Dantán-González and María Luisa Castrejón-Godínez

*Address all correspondence to: ortizhl@uaem.mx.

Biotechnology Research Center, Autonomous University of State of Morelos, Col. Chamilpa, Cuernavaca, Morelos, C.P., Mexico

References

[1] Shukla KP, Singh NK, Sharma S. Bioremediation: developments, current practices and perspectives. Genet. Eng. Biotechnol. J, 2010; 3: 1-20.

[2] Agrawal A, Pandey RS, Sharma B. Water Pollution with Special Reference to Pesticide Contamination in India. J. Water Resource and Protection 2010; 2: 432-448.

[3] Damalas CA. Understanding benefits and risks of pesticide use. Scientific Research and Essay. 2009; 4(10): 945-949.

[4] EPA. What is a Pesticide? http://www.epa.gov/opp00001/about/. (accessed 16 July 2012).

[5] Zhang W, Jiang F, Feng Ou J. Global pesticide consumption and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences, 2011; 1(2): 125-144

[6] Ortiz-Hernández ML. Biodegradación de plaguicidas organofosforados por nuevas bacterias aisladas del suelo. Thesis. Biotechnology PhD. Universidad Autónoma del Estado de Morelos. México. 2002.

[7]   Badii M, Landeros J. Plaguicidas que afectan la salud humana y la sustentabilidad. CULCYT/ Toxicología De Plaguicidas. 2007: 4-19.

[8]   Pimentel D. Environmental and economic costs of the application of pesticides primarily in the United States. In: Food, Energy, and Society, Third Edition Edited by. Pimentel M. and Pimentel D. CRC Press, 2007.

[9]   Chen H, He X, Rong X, Chen W, Cai P, Liang W, Li S. Huang Q. Adsorption and biodegradation of carbaryl on montmorillonite, kaolinite and goethite. Applied Clay Science 2009; 46(1): 102–108.

[10] Chevillard A, Coussy HA, Guillard V, Gontard N, Gastaldi E. Investigating the biodegradation pattern of an ecofriendly pesticide delivery system based on wheat gluten and organically modified montmorillonites. *Polymer Degradation and Stability* 2012; 97(10): 2060–2068.

[11] Myresiotis CK, Vryzas Z, Papadopoulou-Mourkidou E. Biodegradation of soil-applied pesticides by selected strains of plant growth-promoting rhizobacteria (PGPR) and their effects on bacterial growth. *Biodegradation* 2012; 23: 297–310.

[12] Sanchez SE, Ortiz HL. Riesgos y estrategias en el uso de plaguicidas. INVENTIO 2011; 7(14): 21-27.

[13] Besse-Hoggan P, Alekseeva T, Sancelme M, Delort A, Forano C. Atrazine biodegradation modulated by clays and clay/humic acid complexes. *Environmental Pollution* 2009; 157(10): 2837–2844.

[14] Singh BK, Walker A. Microbial degradation of organophosphorus compounds. *FEMS Microbiol. Rev.* 2006; 30 (3): 428–471.

[15] Worrall F, Fernandez-Perez M, Johnson A, Flores-Cesperedes F, Gonzalez-Pradas E. Limitations on the role of incorporated organic matter in reducing pesticide leaching. *J Contain. Hydrol.* 2001. 49: 241-262.

[16] Ortiz-Hernández ML, Sánchez-Salinas E, Olvera-Velona A, Folch-Mallol JL. Pesticides in the Environment: Impacts and its Biodegradation as a Strategy for Residues Treatment, Pesticides - Formulations, Effects, Fate, Margarita Stoytcheva (Ed.), InTech, DOI: 10.5772/13534. Available from: http://www.intechopen.com/books/pesticides-formulations-effects-fate/pesticides-in-the-environment-impacts-and-its-biodegradation-as-a-strategy-for-residues-treatment. 2011.

[17] Dasgupta S, Meisner C, Wheeler D. Stockpiles of obsolete pesticides and cleanup priorities: A methodology and application for Tunisia. *Journal of Environmental Management.* 2010; 91(4): 824-830.

[18] Martínez J. *Practical Guideline on Environmentally Sound Management of Obsolete Pesticides in the Latin America and Caribbean Countries*. Basel Convention Coordinating Centre for Latin America and the Caribbean, Montevideo, Uruguay. 2004.

[19] Karstensen KH, Nguyen KK, Le BT, Pham HV, Nguyen DT, Doan TT, Nguyen HH, Tao MQ, Luong DH, Doan HT. Environmentally sound destruction of obsolete pesticides in developing countries using cement kilns. *Environmental Science & Policy*. 2006; 9(6): 577-586.

[20] Shah BP, Devkota B. Obsolete Pesticides: Their Environmental and Human Health Hazards. *The Journal of Agriculture and Environment*. 2009; 10: 51-56.

[21] Farrera PR. *Acerca de los plaguicidas y su uso en la agricultura*. Revista Digital Ceniap Hoy, 2004; 6.

[22] Ortiz-Hernández ML, Sánchez-Salinas E. Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México. Rev. Int. Contam. Ambient. 2010; 26(1): 27-38.

[23] Giner de los Ríos DCF. *Estudio: Precisión del Inventario de Plaguicidas Obsoletos y Sitios Contaminados con Éstos*. Donative TF-053710. Actividades de habilitación para ayudar a México a cumplir con el Convenio de Estocolmo sobre Contaminantes Orgánicos Persistentes. 2007.

[24] Albert LA. Panorama de los plaguicidas en México. *Revista de Toxicología en Línea*. 2005.

[25] Ferrusquía Ch, Roa G, García M, Amaya A, Pavón T. Evaluación de la degradación de metil paratión en solución usando fotocatálisis heterogénea. *Rev. Latinoamericana de Recursos Naturales* 2008; 4 (2): 285-290.

[26] Torres-Duarte C, Roman R, Tinoco R, Vazquez-Duhalt R. Halogenated pesticide transformation by a laccase mediator system. *Chemosphere* 2009; 77 (5): 687–692.

[27] Vidali M. Bioremediation. An overview. *Pure Appl. Chem.* 2001; 73(7): 1163–1172.

[28] Singleton I. Microbial metabolism of xenobiotics: fundamental and applied research. Journal of Chemical Technology and Biotechnology, 2004. DOI:10.1002/jctb.280590104.

[29] Blackburn JW, Hafker WR. The impact of biochemistry, bioavilability, and bioactivity on the selection of bioremediation technologies. Trends in Biotechnology. 1993. 11 (8): 328-333.

[30] Dua M, Singh A, Sethunathan N, Johri AK. Biotechnology and bioremediation: successes and limitations. Appl Microbiol Biotechnol. 2002; 59:143–152. DOI 10.1007/s00253-002-1024-6.

[31] Sun,W, Chen Y, Liu L, Tang J, Chen J, Liu P. Conidia immobilization of T-DNA inserted *Trichoderma atroviride* mutant AMT-28 with dichlorvos degradation ability and exploration of biodegradation mechanism. *Bioresource Technology* 2010; 101 (23): 9197-9203.

[32] Galli C. *Degradación por medios bacterianos de compuestos químicos tóxicos*. Comisión Técnica Asesora en: Ambiente y desarrollo sostenible, Buenos Aires, Argentina. 2002.

[33] Qiu X, Zhong Q, Li M, Bai W, Li, B. Biodegradation of p-nitrophenol by methyl para-thion-degrading *Ochrobactrum* sp. B2. International Biodeterioration and Biodegrada-tion 2007; 59: 297-301.

[34] Araya M, Lakhi A. Response to consecutive nematicide applications using the same product in mussa AAAcv. Grande naine originated from in vitro propagative materi-al and cultivated in virgin soil. Nematologia Brasileira 2004; 28(1): 55-61.

[35] Aislabie J, Lloyd-Jones G. A review of bacterial-degradation of pesticides. *Australian Journal of Soil Research* 1995; 33(6); 925-942.

[36] Iranzo M, Sain-Pardo I, Boluda R, Sanchez J, Mormeneo S. The use of microorgan-isms in environmental remediation. *Annals Microbiol* 2001; 51: 135-143.

[37] Vischetti C, Casucci C, Perucci P. Relationship between changes of soil microbial bio-mass content and imazamox and benfluralin degradation. Springer Verlag, 2002.

[38] Jeon CO, Madsen EL. *In situ* microbial metabolism of aromatic-hydrocarbon environ-mental pollutants. Current Opinion in Biotechnology. 2012; Available online 19 Sep-tember 2012.

[39] Abo-Amer AE. Characterization of a strain of Pseudomonas putida isolated from ag-ricultural soil that degrades cadusafos (an organophosphorus pesticide). World J Mi-crobiol Biotechnol 2012; 28: 805-814.

[40] Vásquez M, Reyes W. *Degradación de Aroclor 1242 por Pseudomas sp*. Biblioteca Nacio-nal del Perú, Perú. 2002.

[41] Bordjiba O, Steiman R, Kadri M, Semadi A, Guiraud P. Removal of herbicides from liquid media by fungi isolated from a contaminated soil. Journal of Environmental Quality, 2001; *30(2): 418-426*. DOI: 10.2134/jeq2001.302418x*Vol.*

[42] Senthilkumar S, Anthonisamy A, Arunkumar S, Sivakumari V. 2011. Biodegradation of methyl parathion and endosulfan using *Pseudomonas aeruginosa* and *Trichoderma viridae*. J Environ Sci Eng. 53(1):115-122.

[43] Fournier D, Halasz A, Spain J, Fiurasek P, Hawari J. Determination of key metabo-lites during biodegradation of hexahidro 1,3,5 Trinitro 1,3,5 Triazine with Rhodococ-cus sp Strain DN 22. Applied and Enviromental Microbiology 2002; 68: 166-172.

[44] Paul D, Pandey G, Pandey J, Jain RK. Accessing microbial diversity for bioremedia-tion and environmental restoration. Trends Biotechnol 2005; 23:135–142.

[45] Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R. Bioreme-diation approaches for organic pollutants: A critical perspective. *Environment Interna-tional* 2011; 37: 1362–1375.

[46] Ramakrishnan B, Megharaj M, Venkateswarlu K, Naidu R, Sethunathan N. The impacts of environmental pollutants on microalgae and cyanobacteria. Crit Rev Environ Sci Tech. 2010; 40: 699–821.

[47] Ramakrishnan B, Megharaj M, Venkateswarlu K, Sethunathan N, Naidu R. Mixtures of Environmental Pollutants: Effects on Microorganisms and Their Activities in Soils Reviews of Environmental Contamination and Toxicology, 2011; 211: 63-120.

[48] Abraham WR, Nogales B, Golyshin PN, Pieper DH, Timmis KN. Polychlorinated biphenyl-degrading microbial communities and sediments. Curr Opin Microbiol 2002; 5:246–253.

[49] Briceño G, Palma G, Duran N. Influence of Organic Amendment on the Biodegradation and Movement of Pesticides. Critical Reviews in Environmental Science and Technology 2007; 37: 233-271, DOI: 10.1080/10643380600987406.

[50] Diez MC. Biological aspects involved in the degradation of organic pollutants *J. Soil. Sci. Plant. Nutr.* 2010,10(3): 244–267.

[51] Bass C, Field LM. Gene amplification and insecticide resistance. Pest Management Science. 2011; 67 (8): 886–890.

[52] Riya P, Jagatpati T. Biodegradation and bioremediation of pesticides in Soil: Its Objectives, Classification of Pesticides, Factors and Recent Developments. World Journal of Science and Technology 2012; 2(7): 36-41.

[53] Scott C, Pandey G, Hartley CJ, Jackson CJ, Cheesman MJ, Taylor MC., Pandey R, Khurana JL., Teese M, Coppin CW, Weir KM, Jain RK., Lal R, Russell RJ, Oakeshott JG. The enzymatic basis for pesticide bioremediation. Indian J. Microbiol 2008; 48: 65-79.

[54] Trigo A, Valencia A, Cases I Systemic approaches to biodegradation. FEMS Microbiol Rev, 2009; 33: 98-108.

[55] Li X, Schuler MA, Berenbaum MR. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Ann Rev Entomol* 2007; 52: 231-253.

[56] Van Eerd LL, Hoagland R E, Zablotowicz RM, Hall JC. Pesticide metabolism in plants and microorganisms. *Weed Science.* 2003; 51(4): 472–495.

[57] Yan Q-X, Hong Q, Han P, Dong X-J, Shen YJ. Li SP. Isolation and characterization of a carbofuran-degrading strain *Novosphingobium* sp. FND-3. FEMS Microbiol Lett. 2007; 271: 207-213.

[58] Chino-Flores C, Dantán-González E, Vázquez-Ramos A, Tinoco-Valencia R, Díaz-Méndez R, Sánchez-Salinas E, Castrejón-Godínez ML, Ramos-Quintana F, Ortiz-Hernández ML. Isolation of the *opdE* gene that encodes for a new hydrolase of *Enterobacter* sp. capable of degrading organophosphorus pesticides. Biodegradation 2012; 23: 387-397.

[59] Ghanem E, Raushel FM. Detoxification of organophosphate nerve agents by bacterial phosphotriesterase. Toxicol Appl Pharmacol 2005; 207: 459-470. DOI: 10.1016/j.taap. 2005.02.025.

[60] Porzio E, Merone L, Mandricha L, Rossia M, Manco G. A new phosphotriesterase from *Sulfolobus acidocaldarius* and its comparison with the homologue from *Sulfolobus solfataricus.* Biochemie, 2007; 89: 625-636. DOI:10.1016/j.biochi.2007.01.007.

[61] Theriot CM, Grunden AM. Hydrolysis of organophosphorus compounds by microbial enzymes. Appl Microbiol Biotechnol, 2010; 89: 35-43. DOI:10.1007/s00253-010- 589 2807-9.

[62] Shen YJ, Lu P, Mei H, Yu HJ, Hong Q, Li SP Isolation of a methyl-parathion degrading strain *Stenotrophomonas* sp. SMSP-1 and cloning of the *ophc2* gene. Biodegradation, 2010; 21: 785-792. DOI:10.1007/s10532-010-9343-2.

[63] Latifi AM, Khodi S, Mirzaei M, Miresmaeili M, Babavalian H. Isolation and characterization of five chlorpyrifos degrading bacteria. African Journal of Biotechnology 2012; 11(13): 3140-3146.

[64] Mulbry WW, Karns JS. Parathion hydrolase specified by the *Flavobacterium* opd gene: Relationship between the gene and protein. J. Bacteriol 1989;171: 6740-6746.

[65] Ortiz-Hernández ML, Quintero-Ramírez R, Nava-Ocampo AA, Bello-Ramírez AM. Study of the mechanism of *Flavobacterium* sp. for hydrolyzing organophosphate pesticides. Fundam Clin Pharmacol, 2003; 17(6): 717-23.

[66] Sethunathan N, Yoshida T. A *Flavobacterium* sp. that degrades diazinon and parathion. Can J Microbiol, 1973; 19: 873-875. DOI:10.1139/m73-138.

[67] Cui Z, Li S, Fu G. Isolation of methyl-parathion-degrading strain M6 and cloning of the methyl-parathion hydrolase gene. Appl Environ Microbiol, 2001; 67: 4922-4925. DOI:10.1128/AEM.67.10.4922-4925.2001.

[68] Horne J, Sutherland TD, Harcourt RL, Russell RJ, Oakesthott JG. Identification of an opd (organophosphate degradation) gene in an Agrobacterium isolate. Appl. Environ. Microbiol 2002; 68: 3371-3376.

[69] Kapoor M, Rajagopal R. Enzymatic bioremediation of organophosphorus insecticides by recombinant organophosphorous hydrolase. International Biodeterioration & Biodegradation 2011; 65: 896-901.

[70] Bansal OP. Degradation of pesticides Editorial CRC Press. 2012.

[71] López SN. Evaluación de mecanismos de resistencia a insecticidas en *Frankliniella occidentales* (Pergande): implicación de carboxilesterasas y acetilcolinesterasas. PhD Thesis, Universidad de Valencia, España; 2008.

[72]   Rosario-Cruz R, García-Vázquez Z, George EJ. Deteccion inmunoquimica de esterasas en dos cepas de la garrapata *Boophilus microplus* (Acarii: Ixodidae) resistentes a Ixodicidas. Téc. Pecu Méx 2000; 38(2): 203-210.

[73]   Galego LG, Ceron CR, Carareto MA. Characterization of esterases in a Brazilian population of *Zaprionus indianus* (Diptera: Drosophilidae). Genetica 2006; 126:89-99.

[74]   Baffi MA, Rocha LG, Soares de Sousa C, Ceron CR, Bonetti AM. Esterase enzymes involved in pyrethroid and organophosphate resistance in a Brazilian population of *Riphicephallus* (*Boophilus*) *microplus* (Acari, Ixodidae). Molecular & Biochemical Parasitology 2008; 160: 70-73.

[75]   Reiner E, Aldridge WN, Hoskin CG. Enzymes Hydrolyzing Organophosphorus Compounds John Wiley & Sons, Inc, New York. 1989.

[76]   Dary O, Georghiou GP, Parsons E, Pasteur N. Microplate adaptation of Gomori´s assay for quantitative determination of general esterase activity in single insects. Departament of Entomology, University of California. Riverside, California 92521. Journal Economic Entomology 1990; 53(6): 2187-2192.

[77]   Siddique T, Okeke BC, Arshad M, Frankenberger WT Jr. Enrichment and isolation of endosulfan-degrading microorganisms. J Environ Qual. 2003; 32(1): 47-54.

[78]   Kwon GS, Sohn HY, Shin KS, Kim E. Seo BI. Biodegradation of the organochlorine insecticide, endosulfan, and the toxic metabolite, endosulfan sulfate, by *Klebsiella oxytoca* KE-8. Appl Envirnon Microbiol. 2005; 67(6): 845-50.

[79]   Weir KM. Sutherland TD, Horne I, Russell RJ, Oakeshott JG. A single monooxygenase, *ese*, is involved in the metabolism of the organochlorides endosulfan and endosulfate in an *Arthrobacter* sp. Appl Envirnon Microbiol. 2006; 72(5): 3524-30.

[80]   Kumar K, Devi SS, Krishnamurthi K, Kanade GS, Chakrabarti T. Enrichment and isolation of endosulfan degrading and detoxifying bacteria. Chemosfere. 2007; 68(2): 317-22. Epub 2007 Feb 7.

[81]   Hussain S. Arshad M, Saleem M, Khalid A. Biodegradation of alpha- and beta-endosulfan by soil bacteria. Biodegradation. 2007; 18(6): 731-40.

[82]   Khaled A, Miia T, Arja R, Jukka H, Olavi P. Metabolism of Pesticides by Human Cytochrome P450 Enzymes In Vitro-A Survey, Insecticides-Advances in Integrated Pest Management. InTech. 2012.

[83]   Urlacher VB, Lutz-Wahl S, Schmid RD. Microbial P450 enzymes in biotechnology. Appl Microbiol Biotechnol 2004; 64: 317–325.

[84]   Alzahrani AM. Insects Cytochrome P450 Enzymes: Evolution, Functions and Methods of Analysis. Global Journal of Molecular Sciences 2009; 4(2): 167-179.

[85] Shi H, Pei L, Gu S, Zhu S, Wang Y, Zhang Y, Li B. Glutathione S-transferase (GST) genes in the red flour beetle, *Tribolium castaneum*, and comparative analysis with five additional insects. Genomics 2012; 100(5): 327-335.

[86] Hussain S, Siddique T, Arshad M, Saleem M. Bioremediation and Phytoremediation of Pesticides: Recent Advances. *Critical Reviews in Environmental Science and Technology* 2009; 39(10): 843-907.

[87] Zhongli C, Shunpeng L, Guoping F. Isolation of methyl parathion-degrading strain m6 and cloning of the methyl parathion hydrolase gene. Apple. and Environ. Microbiol. 2001; 67(10): 4922-4925.

[88] Zhang R, Cui Z, Jiang J, Gu X, Li S. Diversity of organophosphorus pesticides degrading bacteria in a polluted soil and conservation of their organophosphorus hydrolase genes. Can. J. Microbiol. 2005; 5: 337-343.

[89] Shimizu H. Metabolic engineering–integrating methodologies of molecular breeding and bioprocess systems engineering. *J Biosci Bioeng* 2002; 94: 563–73.

[90] Pieper DH, Reineke W. Engineering bacteria for bioremediation. *Curr Opin Biotechnol* 2000; 11: 262–70.

[91] Cases I, de Lorenzo V. Promoters in the environment: transcriptional regulation in its natural context. *Nat Rev Microbiol* 2005; 3: 105–18.

[92] Wu NF, Deng MJ, Liang GY, Chu XY, Yao B, Fan YL. Cloning and expression of ophc2, a new organophosphorus hydrolase gene. *Chin Sci Bull* 2004; 49: 1245–1249.

[93] Fu GP, Cui Z, Huang T, Li SP. Expression, purification, and characterization of a novel methyl parathion hydrolase. *Protein Expr Purif* 2004; 36: 170–176.

[94] Yu H, Yan X, Shen W, Hong Q, Zhang J, Shen Y, Li S. Expression of methyl parathion hydrolase in Pichia pastoris. *Curr Microbiol* 2009; 59: 573–578.

[95] Wang XX, Chi Z, Ru SG, Chi ZM. Genetic surface-display of methyl parathion hydrolase on Yarrowia lipolytica for removal of methyl parathion in water. *Biodegradation* 2012; 23: 763–774 DOI: 10.1007/s10532-012-9551-z.

[96] Shen YJ, Lu P, Mei H, Yu HJ, Hong Q, Li SP. Isolation of a methyl parathion-degrading strain Stenotrophomonas sp. SMSP-1 and cloning of the ophc2 gene. *Biodegradation* 2009; DOI: 10.1007/s10532-010-9343-2.

[97] Eapen S, Singh S, D'Souza S F. Advances in development of transgenic plants for remediation of xenobiotic pollutants. *Biotechnol. Adv.* 2007; 25: 442-451.

[98] Singh S, Sherkhane PD, Kale SP, Eapen S. Expression of a human cytochrome P4502E1 in Nicotiana tabacum enhances tolerance and remediation of g-hexachlorocyclohexane. *New Biotechnology*, 2011; 28: 423-429.

[99] Casey MT, Grunden AM. Hydrolysis of organophosphorus compounds by microbial enzymes. *Appl Microbiol Biotechnol* 2011; 89:35–43 DOI: 10.1007/s00253-010-2807-9.

[100] Van Dyk JS, Brett P. Review on the use of enzymes for the detection of organochlorine, organophosphate and carbamate pesticides in the environment. *Chemosphere* 2011; 82: 291-307.

[101] Cho MH, Mulchandani CA., Chen W. Bacterial cell surface display of organophosphorus hydrolase for selective screening of improved hydrolysis of organophosphate nerve agents. *Appl. Environ. Microbiol* 2002; 68: 2026–2030.

[102] Cho MH, Mulchandani CA, Chen W. Altering the Substrate Specificity of Organophosphorus Hydrolase for Enhanced Hydrolysis of Chlorpyrifos. *Applied and Environmental Microbiology* 2004; 70: 4681–4685 DOI: 10.1128/AEM.70.8.4681-4685.2004.

[103] Zhou J, He Z, Van Nostrand JD, Wu L, Deng Y. Apply-ing Geo Chip analysis to disparate microbial communities. *Microbe* 2010; 5: 60–65.

[104] Maphosa F, Lieten SH, Dinkla I, Stams AJ, Smidt H, Fennell DE. Ecogenomics of microbial communities in bioremediation of chlorinated contaminated sites. *Frontiers in Microbiology* 2012; 3: 1-14 DOI: 10.3389/fmicb.2012.00351.

[105] Rayu S, Karpouzas DG, Singh BK. Emerging technologies in bioremediation: constraints and opportunities. *Biodegradation* 2012; 23: 917–926 DOI: 10.1007/s10532-012-9576-3.

[106] Rajendhran J, Gunasekaran P. Strategies for accessing soil metagenome for desired applications. *Biotechnology Advances* 2008; 26: 576-590 DOI: 10.1016/j.biotechadv.2008.08.002.

[107] Zhao B, Poh LC. Insights into environmental bioremediation by microorganisms through functional genomics and proteomics. *Proteomics* 2008; 8: 874-881 DOI: 10.1002/pmic.200701005.

[108] Koffas M, Roberge C, Lee K, Stephanopoulos G. Metabolic engineering. *Annu Rev Biomed Eng* 1999; 1: 535–557.

[109] Richins R, Mulchandani A, Chen W. Expression, immobilization, and enzymatic characterization of cellulose-binding domain-organophosphorus hydrolase fusion enzymes, Biotechnol. Bioeng. 2000; 69: 591-596.

[110] Chen W, Georgiou G Cell-surface display of heterologousproteins: from high throughput screening to environmental applications. Biotechnol Bioeng. 2002; 5:496-503. DOI:10.1002/bit.10407.

[111] Martin M, Mengs G, Plaza E, Garbi C, Sánchez A, Gibello A, Gutierrez F, Ferrer E. Propachlor removal by Pseudomonas strain GCH1 in a immobilized-cell system. Appl Environ Microbiol. 2000; 66(3):1190-1194.

[112] Ha J, Engler CR, Lee SJ. Determination of diffusion coefficients and diffusion charac-
teristics for chlorferon and diethylthiophosphate in Ca-alginate gel beads. Biotechnol
Bioeng. 2008; 100(4): 698-706. DOI:10.1002/bit.21761.

[113] Ha J, Engler CR, Wild J. Biodegradation of coumaphos, chlorferon, and diethylthio-
phosphate using bacteria immobilized in Ca-alginate gel beads. Bioresource Technol-
ogy 2009; 100: 1138-1142.

[114] Kennedy JF, Cabral JMS. Solid Phase Biochemistry. Schouten, W.H. (ed.) Wiley Pub.,
New York. 1983.

[115] Arroyo M. Inmovilización de enzimas. Fundamentos, métodos y aplicaciones. *Ars.
Pharmaceutica.* 1998; 39(2): 23-39.

[116] Lusta KA, Starostina NG, Fikhte BA. Immobilization of microorganisms: cytophysio-
logical aspects. In: De Bont, JAM, Visser J, Mattiasson B, Tramper J. (Ed). Proceedings
of an International Symposium: Physiology of Immmobilized Cells. Amsterdam,
Netherlands: Elsevier. 1990.

[117] Heitkamp M, Camel V, Reuter T, Adams W. Biodegradation of p-nitrophenol inan
aqueous waste stream by immobilized bacteria. *Appl. Environ. Microbiol.* 1990; 56(10):
2967-73.

[118] Wang P, Sergeeva M, Lim L, Dordick J. Biocatalytic Plastics as active and stable ma-
terials for biotransformations. *Nature Biotechnol.* 1997; 15: 789-793.

[119] Karamanev G, Chavarie C, Samson R. Soil immobilization: new concept for biotreat-
ment of soil contaminants. Biotechnol. Bioeng. 1998; 4 (57): 471-476.

[120] Pedersen S, Christensen M. Immobilized biocatalysts. En: Straathof, A. y Adler-
creutz, P. (edts) Applied Biocatalysis. Harwood Academic Publishers. Netherlands.
2000.

[121] Davey ME, O'Toole GA. Microbial Biofilms: from Ecology to Molecular Genetics. Mi-
crobiology and Molecular Biology Reviews. 2000; 64(4): 847-867.

[122] Nicolella CM, van Loosdrecht, Heijnen JJ. Review article Wastewater treatment with
particulate biofilm reactors. Journal of Biotechnology. 2000; 80: 1-33

[123] Yañez-Ocampo G., Penninckx M., Jiménez-Tobon G.A, Sánchez-Salinas E, Ortiz-Her-
nández ML. Removal of two organophosphate pesticides employing a bacteria con-
sortium immobilized in either alginate or tezontle. *Journal of Hazardous Materials*
2009; 168: 1554-1561.

[124] Betancourth M, Botero JE, Rivera SP. Biopelículas: una comunidad microscópica en
desarrollo. Colombia Médica. 2004; 35(3-1)

[125] Dorigo U, Leboulanger C, Bérard A, Bouchez A, Humbert J F, Montuelle B. Lotic bio-
film community structure and pesticide tolerance along a contamination gradient in
a vineyard área. Aquat Microb Ecol. 2007; 50: 91-102.

[126] Villena GK, Gutiérrez-Correa M. Biopelículas de *Aspergullus niger* para la producción de celulasas algunos aspectos estructurales y fisiológicos. Rev. Peru. Biol. 2003; 10(1): 78-87.

[127] Mansee AH, Chen W, Mulchandani A. Detoxification of the organophosphate nerve agent coumaphos using organophosphorus hydrolase immobilized on cellulose materials. J Ind Microbiol Biotechnol. 2005; 32: 554–560.

[128] Martín M, Mengs G, Plaza E, Garbi C, Sánchez M, Gibello A, Gutiérrez F, Ferrer E. Propachlor removal by Pseudomonas strain GCH1 in a immobilized-cell system. Appl. Environ. Microbiol. 2000; 66(3): 1190-1194.

[129] Ettayebi K, Errachidi F, Jamai L, Tahri-Jouti MA, Sendide K, Ettayebi M. Biodegradation of polyphenols with immobilized *Candida tropicalis* under metabolic induction. FEMS Microbiology Letters 2003; 223: 215-219.

[130] Chivita L, Dussán J. Evaluación de matrices para la inmovilización de *Pseudomonas spp.* En biorremediación de fenol. Rev. Colombiana de Biotecnología 2003. V(2); 5-10.

[131] Barragán HB, Costa C, Peralta J, Barrera J, Esparza F, Rodríguez R. Biodegradation of organochlorine pesticides by bacteria grown in microniches of the porous structure of green vean coffee. International Biodeterioration and Biodegradation. 2007; 59: 239-244.

[132] Garzón-Jiménez RC. Cinética de degradación de colorantes textiles de diferentes clases químicas por hongos y bacterias inmovilizados sobre fibra de *Agave tequilana* Webber var. Azul. Thesis. Pontificia Universidad Javeriana. 2009.

[133] Santacruz G, Bandala E, Torres LG. Chlorinated pesticidas (2,4 –D and DDT) biodegradation at high concentrations using immobilized *Pseudomonas fluorescens*. J. Environ. Sc. Heal. 2005; 40(4):571-583.

[134] Mazmanci M, Unyayara. Decolourisation of reactive black 5 by *Funalia trogii* immobilized on *Loofa cylindrical* sponge. Process Biochemistry. 2005; 40: 337-342.

[135] Akhtar N, Iqbal J, Iqbal M. Removal and recovery of nickel (II) from aqueous solution by Loofa sponge immobilized biomass of *Chlorella sorokiniana* characterization studies. J. Hazard. Mater. 2004; 108(1-2): 85-94.

[136] Iqbal M, Edyvean RGJ. Biosorption of lead, copper and zinc ions on loofa immobilized biomass of *Phanerochaete chrysosporium*. Mineral Engineering 2004; 17: 217-223.

[137] Saratale RG, Saratale GD, Chang JS. Govindwar SP. Decolorization and biodegradation of reactive dyes and dye wastewater by a developed bacterial consortium. Biodegradation. 2010; 21 (6): 999-1015. DOI: 10.1007/s10532-010-9360-1.

[138] Pattanasupong A, Naimogase H, Sugimoto E, Hori Y, Hirata K, Tani K, Nasu M, Miyamoto K. Degradation of carbendazim and 2,4-dichlorophenoxyacetic acid by

immobilized consortium on Loofa sponge. J. of Bioscience and Bioengineering. 2004; 98 (1): 28-33.

[139] Nagase H, Pattanasupong A, Sugimoto E, tani K, Nasu M, Hirata K, Miyamoto K. Effect of environmental factors on performance of immobilized consortium system for degradation of carbendazim and 2,4-dichlorophenoxyacetic acid in continuous culture. Biochemical Engineering Journal 2006; 29: 163-168.

[140] Nabizadeh R, Naddafi K, Mesdaghinia A, Nafez AH. Feasibility study of organic matter and Ammonium removal using loofa sponge as a supporting medium in an aerated submerged fixed-film reactor (ASFFR). Electronic Journal of Biotechnology 2008; 11(4). DOI: 10.2225/vol11-issue4-fulltext-8.

[141] Demir H; Top A; Balkose D, Ulku S. Dye adsorption behavior of Luffa cylindrica fibers. Journal of Hazardous Material, 2008; 153(1-2): 389-394.

[142] Annunciado TR, Sydenstricker THD, Amico SC. Experimental investigation of various vegetable fibers as sorbent materials for oil spills. Marine Pollution Bulletin. 2005; 50:1340-1346.

[143] Altinisik A, Gur E, Seki Y. A natural sorbent, *Luffa cylindrica* for removal of a model basic dye. Journal of hazardous materials, 2010; 179: 658-664.