Revista Internacional de Contaminación Ambiental
ISSN: 0188-4999
rvp@atmosfera.unam.mx
Universidad Nacional Autónoma de México
México

ORTIZ-HERNÁNDEZ, Ma. Laura; SÁNCHEZ-SALINAS, Enrique; CASTREJÓN GODÍNEZ, María Luisa; DANTAN GONZÁLEZ, Edgar; POPOCA URSINO, Elida Carolina

MECHANISMS AND STRATEGIES FOR PESTICIDE BIODEGRADATION: OPPORTUNITY FOR WASTE, SOILS AND WATER CLEANING

Revista Internacional de Contaminación Ambiental, vol. 29, septiembre, 2013, pp. 85-104
Universidad Nacional Autónoma de México
Distrito Federal, México

Available in: http://www.redalyc.org/articulo.oa?id=37028958005

How to cite
Complete issue
More information about this article
Journal's homepage in redalyc.org

Scientific Information System
Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal
Non-profit academic project, developed under the open access initiative

Rev. Int. Contam. Ambie. 29 (Número especial sobre plaguicidas) 85-104
Septiembre 2013

MECHANISMS AND STRATEGIES FOR PESTICIDE BIODEGRADATION: OPPORTUNITY FOR WASTE, SOILS AND WATER CLEANING

Ma. Laura ORTIZ-HERNÁNDEZ[1*], Enrique SÁNCHEZ-SALINAS[1], María Luisa CASTREJÓN GODÍNEZ[2], Edgar DANTAN GONZÁLEZ[3] y Elida Carolina POPOCA URSINO[3]

[1] Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos
[2] Programa de Gestión Ambiental Universitario, Universidad Autónoma del Estado de Morelos
[3] Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos
*Autora responsable: ortizhl@uaem.mx

(Recibido julio 2013, aceptado agosto 2013)

Key words: pesticide, biodegradation, biobeds, cells immobilization

ABSTRACT

Pesticides are substances or mixtures of substances intended to prevent, destroy or control any pest, and they are widely used mainly in agriculture, industry and the domestic sector. These compounds have been extensively used for decades and have significantly increased food production. However, a large amount of applied pesticides often never reach their intended target due to their degradation, volatilization and leaching, resulting in serious environmental problems. This article reviews the main problems that the use of these compounds causes to the environment and health and discusses the basis for biodegradation that can be used for remediation of contaminated sites. It also provides information about the cell immobilization of specific microorganisms on different types of supports, as a strategy to increase the efficiency of pesticide degradation. We also review and discuss the use of biobeds as an economic, clean and efficient strategy to provide a tool for the *in situ* degradation of pesticide residues.

Palabras clave: plaguicidas, biodegradación, biocamas, inmovilización celular

RESUMEN

Los plaguicidas son sustancias o mezclas de sustancias que se destinan a prevenir, destruir o controlar cualquier plaga y son ampliamente utilizados en el sector agrícola, industrial y doméstico, principalmente. Estos compuestos se han usado por décadas y por ello se ha incrementado significativamente la producción de alimentos. Sin embargo, de la cantidad total de plaguicidas aplicados, un gran porcentaje no alcanza el sitio blanco, ya que pueden degradarse, volatilizarse y/o lixiviarse, dando como resultado serios problemas ambientales. Este artículo revisa los principales problemas que se causan al ambiente y a la salud por la utilización de estos compuestos y discute las bases para la biodegradación para que sus principios puedan ser utilizados para la remediación de sitios contaminados. También se proporciona información acerca de la inmovilización de células de microorganismos específicos sobre diferentes soportes, como una estrategia para incrementar la eficiencia de degradación de los plaguicidas.

Por otro lado, se revisa y discute acerca del empleo de las biobeds, como una estrategia económica, limpia y eficiente para proveer una herramienta in situ para la degradación de residuos de plaguicidas.

INTRODUCTION

Because of human activities, a large number of pollutants and waste are currently dispersed within the environment. Approximately 6×10^6 chemical compounds have been produced, 1000 new products are synthesized annually, and between 60 000 and 95 000 chemicals are commercially used (Shukla *et al.* 2010). Among these substances are chemical pesticides, which are used extensively in most areas of crop production to minimize pest infestations, to protect the crop yield losses and to avoid reducing the product quality. 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 insecticides, herbicides, fungicides and various other substances used to control pests (EPA 2012).

Pesticides belong to a category of chemicals used worldwide to prevent or control pests, diseases, weeds and other plant pathogens in an effort to reduce or eliminate yield losses and maintain high product quality (Damalas and Eleftherohorinos 2011). The positive aspect of the application of pesticides has resulted in enhanced crop/food productivity and a drastic reduction of vector-borne diseases (Damalas 2009, Agrawal *et al.* 2010). Chemical pesticides can be classified in different ways, but they are most commonly classified according to their chemical composition. This method allows the uniform and scientific grouping of pesticides to establish a correlation between structure, activity, toxicity and degradation mechanisms, among other characteristics. **Table I** shows the most important pesticides and their general characteristics, and **figure 1** shows examples of some chemical structures of pesticides.

Global insecticide use in 2007 has been estimated at 404 000 metric tons of active ingredient (Grube *et al.* 2011). The agricultural sector is the primary user of pesticides, consuming over four million tons of pesticides annually; 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 (Chen *et al.* 2009, Chevillard *et al.* 2012). Under actual agricultural practices, different groups of pesticides are often simultaneously or consecutively applied and consequently interact with each other (Myresiotis *et al.* 2012). A population inhabiting a contaminated site may be subjected to selective pressure from the contamination, which may result in an elevated

TABLE I. GENERAL CHARACTERISTICS OF SOME PESTICIDES (Badii and Landeros 2007)

Pesticides	Characteristics	Main composition
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.	Carbon atoms, chlorine, hydrogen and occasionally oxygen. They are nonpolar and lipophilic
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 and then transferred to leaves and stems, which are the supply of leaf-eating insects or feed on wise.	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	Carbamate acid derivatives; kill a limited spectrum of insects, but are highly toxic to vertebrates. Relatively low persistence	Chemical structure based on a plant alkaloid *Physostigma venenosum*
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.	Compounds similar to the synthetic pyrethrins (alkaloids obtained from petals of *Chysanthemun cinerariefolium*).
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.	Viruses, microorganisms or their metabolic products

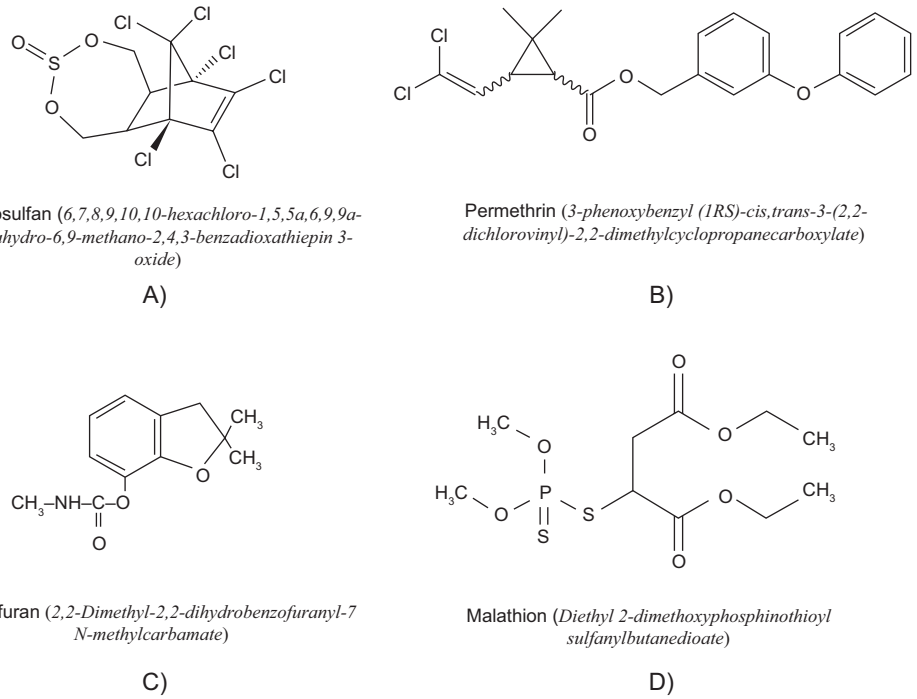

Fig. 1. Examples of chemical structure of pesticides A) Organochloride, B) Pyrethroid, C) Carbamate, and D) Organophosphate

resistance in this population compared to resistance in a population of conspecifics living at a clean site (Klerks *et al.* 2011).

The unregulated and indiscriminate application of pesticides can cause adverse effects to human health, to different life forms and to the ecosystems. The extent of these effects depends on the degree of sensitivity of the organisms and the toxicity of the pesticides. The continued application of pesticides has increased its concentration in soils and waters and their effects can also be magnified through the food chain. Dispersion mechanisms have also increased the level of environmental risk for the occupationally exposed population and the inhabitants of surrounding villages. Pesticides cause serious health hazards to living systems because of their rapid fat solubility and bioaccumulation in non-target organisms (Agrawal *et al.* 2010). The main forms of pollution are the direct application of pesticides to agricultural crops, accidental spills during transport and manufacturing, as well as waste from tanks where cattle are treated for ectoparasite control (EPA 2012).

In addition, liquid and solid wastes and obsolete products are stored or disposed in an inappropriate manner, which has favored the appearance of significant environmental liabilities. An obsolete pesticide may be recognized as one that is undesirable or impossible to use and must be eliminated (Martinez 2004, Karstensen *et al.* 2006, Shah and Devkota 2009, Dasgupta *et al.* 2010). In the absence of a clear obsolete pesticide management strategy, over the years, significant amounts of obsolete pesticides have been stockpiled in developing countries (Dasgupta *et al.* 2010). 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 (Ortiz-Hernández *et al.* 2011). Obsolete pesticides have accumulated in almost every developing country or economy in transition over the past several decades (Dasgupta *et al.* 2010). It is estimated that there are more than 100,000 tons of these products in Africa and the Middle East, almost 200 000 tons in Asia and a similar quantity in Eastern Europe and the former Soviet Union. Currently, the FAO is recording the inventories of Latin America (Farrera 2004, Karstensen *et al.* 2006, Ortiz-Hernández and Sánchez-Salinas 2010). However, it is difficult to estimate the exact quantities of obsolete pesticides because many of the products are very old and documentation is often lacking (Vijgen and Egenhofer 2009).

For the total destruction of these obsolete pesticides, the results of projects undertaken by IHPA (International HCH & Pesticides Association) suggest that the cost for cleaning up, repackaging,

transporting and final ultimate destroying of obsolete pesticide to be at 4000 USD per ton. The FAO assumes roughly similar figures. For Africa the costs are estimated to be in the order of 4000-7000 USD per ton (Vijgen and Egenhofer 2009).

BIODEGRADATION AS A STRATEGY TO REDUCE THE NEGATIVE IMPACT OF PESTICIDES

Due to the problems mentioned above, the development of technologies for environmental remediation or waste destruction that guarantees their elimination in a safe, efficient and economical way is important. The mechanisms for the cleanup of pesticides in soil such as chemical treatment, volatilization and incineration have met public opposition because of problems such as the production of large volumes of acids and alkalis that must subsequently be disposed. The potentially toxic emissions and the elevated economic costs are also significant concerns. Overall, most of these physical-chemical cleaning technologies are expensive and inefficient (Nyakundi *et al.* 2011). A methodology for degradation that has gained acceptance is the bioremediation, which is conducted through the biodegradation of these chemical compounds. According to the definition by the International Union of Pure and Applied Chemistry, the term biodegradation is defined as the breakdown of a substance catalyzed by enzymes *in vitro* or *in vivo*. Biodegradation may be defined for the purpose of hazard assessment into the following categories (Meleiro-Porto *et al.* 2011):

1. Primary. Alteration of the chemical structure of a substance resulting in loss of a specific property of that substance.
2. Environmentally acceptable. Biodegradation to such an extent as to remove undesirable properties of the compound. This change often corresponds to primary biodegradation but it depends on the circumstances under which the products are discharged into the environment.
3. Ultimate. Complete breakdown of a compound to either fully oxidized or reduced simple molecules (such as carbon dioxide/methane, nitrate/ammonium and water). It should be noted that the biodegradation products can be more harmful than the substance degraded.

The microbial degradation of pesticides in the environment is an important route for the removal of these compounds. The biodegradation of these compounds is often complex and involves biochemical reactions. Although many enzymes efficiently catalyze the biodegradation of pesticides, the full understanding of the biodegradation pathway often requires new investigations. Several pesticide biodegradation studies have shown only the total of degraded pesticide, but have not investigated in depth the new biotransformed products and their fate in the environment (Meleiro-Porto *et al.* 2011).

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 (Sun *et al.* 2010). With knowledge of the biodegradation processes, is possible to apply it to improve the bioremediation of sites contaminated with pesticides. Bioremediation enables the destruction of many organic contaminants at a reduced cost, and in recent years, bioremediation technology has progressed for the degradation of a wide range of pollutant compounds. Bioremediation can offer an efficient and cheap option for the decontamination of polluted ecosystems and the destruction of pesticides (Blackburn and Hafker 1993, Vidali 2001, Dua *et al.* 2002, Singleton 2004, Singh and Walker 2006).

MICROORGANISMS INVOLVED IN THE BIODEGRADATION OF PESTICIDES

Different microorganisms have been used to biotransform pesticides. 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 an adequate carbon source and electron donors for certain soil microorganisms (Torres 2003), thereby generating a method for the treatment of pesticide-contaminated sites (Araya and Lakhi 2004, Qiu *et al.* 2007). However, the transformation of such compounds depends not only on the presence of microorganisms with appropriate degrading enzymes but also on a wide range of environmental parameters (Aislabie and Lloyd-Jones 1995, Alves *et al.* 2010). Additionally, some physiological, ecological, biochemical and molecular aspects play an important role in the microbial transformation of pollutants (Iranzo *et al.* 2001, Vischetti *et al.* 2002, Becker and Seagren 2010, Megharaj *et al.* 2011).

There are different sources of microorganisms with the ability to degrade pesticides. Because pesticides are mainly applied to agricultural crops,

soil is most affected by these chemicals. Industry's effluent-sediment, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of pesticides, and even some live organisms are also affected. In general, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of sites contaminated with some type 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 makes it possible to utilize new tools to restore polluted environments or to treat wastes before their final disposition (Ortiz-Hernández *et al.* 2011).

PRINCIPLES OF PESTICIDE BIODEGRADATION

Biodegradation is a process that involves the complete breakdown 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 it may be fortuitous in nature (co-metabolism) (Becker and Seagren 2010). 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 (Megharaj *et al.* 2011). The ubiquitous nature of microorganisms, their numbers and large biomass relative to other living organisms on earth, their more diverse catalytic mechanisms (Paul *et al.* 2005), and their ability to function even in the absence of oxygen and other extreme conditions are greatly important in the use of microorganisms for the degradation of pesticides.

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 the transfer of substrates and products within a well-coordinated microbial community, a process referred to as metabolic cooperation (Abraham *et al.* 2002). Microorganisms have the ability to interact both chemically and physically with substances, leading to structural changes or the complete degradation of the target molecule. Pesticides interact with soil organisms and their metabolic activities and may alter the physiological and biochemical behavior of soil microbes. Many recent studies have revealed the adverse impacts of pesticides on soil microbial biomass and soil respiration; generally, a decrease in soil respiration reflects the reduction in microbial biomass. Some microbial groups are capable of using applied pesticides as a source of energy and nutrients for their multiplication, whereas the pesticide may be toxic to other organisms. Likewise, sometimes the application of pesticides reduces microbial diversity but increases the functional diversity of microbial communities. Pesticide application may also inhibit or kill certain groups of microorganisms and outnumber other groups by reducing competition (Hussain *et al.* 2009). Among the microbial communities, bacteria, fungi and actinomycetes are the main transformers and pesticide degraders (Briceño *et al.* 2007). 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 (Diez 2010).

Fungi and bacteria are considered excellent extracellular enzyme-producing microorganisms. Moreover, the ability of fungi to form extended mycelial networks, the low specificity of their catabolic enzymes and their independence from organic chemicals as a growth substrate make fungi well suited for bioremediation processes (Harms *et al.* 2012). Fungi are critical to the biogeochemical cycles and are responsible for the bulk of the degradation of environmental xenobiotics in the biosphere (Liang *et al.* 2005). White rot fungi have been proposed as promising bioremediation agents, especially for compounds that are 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 species that degrade pesticides 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 (Bass and Field 2011).

Enzymes are central to the biology of many pesticides (Riya and Jagatpati 2012). Applying enzymes to transform or degrade pesticides is an innovative treatment technique for the 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 (Scott *et al.* 2008). Trigo *et al.* (2009) 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 the intermediate compounds (Ramakrishnan *et al.* 2011).

The 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 the 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. Fungi and bacteria are involved in these processes and produce intracellular or extracellular enzymes including hydrolytic enzymes, peroxidases, oxygenases, etc. (Van Eerd *et al.* 2003, Ortiz-Hernández *et al.* 2011).

Due to the diversity of chemicals 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 can be found in **table II**.

Among the enzymes that degrade pesticides, the 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 (Scott *et al.* 2008). In this group, we can find the phosphotriesterases (PTEs), which are one of the most important classes (Chino-Flores *et al.* 2012). These enzymes have been isolated from different microorganisms that hydrolyze and detoxify organophosphate pesticides (OP). This reaction reduces OP toxicity by decreasing the ability of OP to inactivate AchE (Ghanem and Raushel 2005, Singh and Walker 2006, Porzio *et al.* 2007, Shen *et al.* 2010, Theriot and Grunden 2010). The first isolated phosphotriesterase belongs to *Pseudomonas diminuta* MG; this enzyme shows a highly catalytic activity towards organophosphate pesticides. The PTEs are encoded by a gene called *opd* (organophosphate-degrading). *Flavobacterium* ATCC 27551 contains the *opd* gene that encode a PTE (Latifi *et al.* 2012). 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. This enzyme has a potential use for the cleaning of organophosphorus pesticide-contaminated environments (Ortiz-Hernández *et al.* 2003). There are other enzymes involved in the overall degradation of a pesticide. The parathion degradation pathway is an example of this process (Abo-Amer 2012) (**Fig. 2**).

Esterases are enzymes that catalyze the hydrolysis of carboxylic esters (carboxyesterases), amides (amidases), phosphate esters (phosphatases), etc. (Bansal 2012). In the reaction catalyzed by esterases, a wide range of ester substrates can be hydrolyzed into their alcohol and acid components as follows:

$$R = O\text{-}OCH_3 + H_2O \longleftrightarrow R = O\text{-}OH + CH_3OH$$

Many insecticides (organophosphates, carbamates and pyrethroids) have a carboxylic ester component, and the enzymes capable of hydrolyzing this type of ester bond are known as carboxylesterases.

Oxidoreductases are a broad group of enzymes that catalyzes 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 electron donors, electron acceptors or both. These enzymes have applications in bioremediation, during which they catalyze an oxidation/reduction reaction by including molecular oxygen (O_2) as the 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 (Scott *et al.* 2008).

As an example of the many functions of these enzymes in the degradation of pesticides, we present the malathion degradation pathway. This process involves esterases and oxidoreductase enzymes, and different

TABLE II. RELEVANT MICROBIAL ENZYMES IN PESTICIDE BIODEGRADATION (Singh and Walker 2006, Scott *et al.* 2008, Riya and Jagatpati 2012)

Enzyme		Organism	Pesticide	Bioremediation strategy
Oxidoreductases	Gox	*Pseudomonas* sp. LBr *Agrobacterium* strain T10	Glyphosate	Plant
Monooxygenases	ESd	*Mycobacterium* sp.	Endosulphan and endosulphato	-
	Ese	*Arthrobacter* sp.	Endosulphan, aldrin, malation, DDDT and endosulphate	-
	Cyp1A1/1A2	Rats	Atrazine, norflurazon and isoproturon	Plant
	Cyp76B1	*Helianthus tuberosus*	Linuron, chlortoluron and isoproturon	Plant
	P450	*Pseudomonas putida*	Hexachlorobenzene and pentachlorobenzene	-
Dioxygenases	TOD	*Pseudomonas putida*	Herbicides trifluralin	-
	E3	*Lucilia cuprina*	Synthetic pyrethroids and insecticides phosphotriester	-
Phosphodiesterases	PdeA	*Delftia acidovorans*	Organophosphorus compounds	-
Phosphotriesterases	OPH OpdA	*Agrobacterium radiobacter* *Pseudomonas diminuta* *Flavobacterium* sp.	Insecticides phosphotriester: Parathion, methyl parathion, malathion, coumaphos, others.	Bioremediation and free enzymes
Phosphonatase	Phn	*Escherichia coli* *Sinorhizobium meliloti*	Organophosphorus compounds	-
Haloalkane dehalogenases	LinB	*Sphingobium* sp. *Shingomonas* sp.	Hexachlorocyclohexane (β and δ isomers)	Bioaugmentation
	AtzA	*Pseudomonas* sp. ADP	Herbicides chloro-s-trazine	Plants and bacteria
	TrzN	*Nocardioides* sp.	Herbicides chloro-s-trazine	-
	LinA	*Sphingobium* sp. Shingomonas sp.	Hexachlorocyclohexane (γ isomers)	Bioaugmentation
	TfdA	*Ralstonia eutropha*	2,4-dichlorophenoxyacetic acid and pyridyl-oxyacetic	Plant
	DMO	*Pseudomonas maltophilia*	Dicamba	Plant
C-P-lyase	Glp A&B	*Pseudomonas pseudomallei*	Organophosphorus compounds	-
ND	hocA	*Pseudomonas monteilli*	Organophosphorus compounds	-
	mpd	*Pleisomonas* sp.	Organophosphorus compounds	-

ND= not determined

microorganisms and catalytic activities can lead to the complete mineralization of a pesticide (**Fig. 3**).

A fungus capable of using chlorpyrifos as the sole carbon source was isolated from organophosphate-contaminated soil and was characterized as *Cladosporium cladosporioides* (collection number CCTCC M 20711) (Gao *et al.* 2012). Based on the metabolic products formed, the degradation pathway for chlorpyrifos by the strain was proposed (**Fig. 4**). Specifically, the parent chlorpyrifos was first metabolized by hydrolysis to produce 3,5,6-trichloro-2-pyridinol (TCP) and diethylthiophosphoric acid (DETP). Subsequently, the hydrolysis product TCP was further transformed by ring breakage, resulting in its complete detoxification (Chen *et al.* 2012). A novel chlorpyrifos hydrolase from cell extract was purified 35.6-fold to apparent homogeneity with 38.5 % overall recovery by ammonium sulfate precipitation, gel filtration chromatography and anion-exchange chromatography. The enzyme is a monomeric structure with a molecular mass of 38.3 kDa. The pI value was estimated to be 5.2. The optimal pH and temperature of the purified enzyme were 6.5 and 40 ºC, respectively. No cofactors were required for the hydrolysis of chlorpyrifos (Gao *et al.* 2012).

Lu *et al.* (2013), reported a bacterial strain, *Cupriavidus* sp. DT-1, capable of degrading chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) and using these compounds as sole carbon source was isolated and characterized. Investigation of the degradation pathway showed that chlorpyrifos was first hydrolyzed to TCP, successively dechlorinated to 2-pyridinol, and then subjected to the cleavage of the pyridine ring

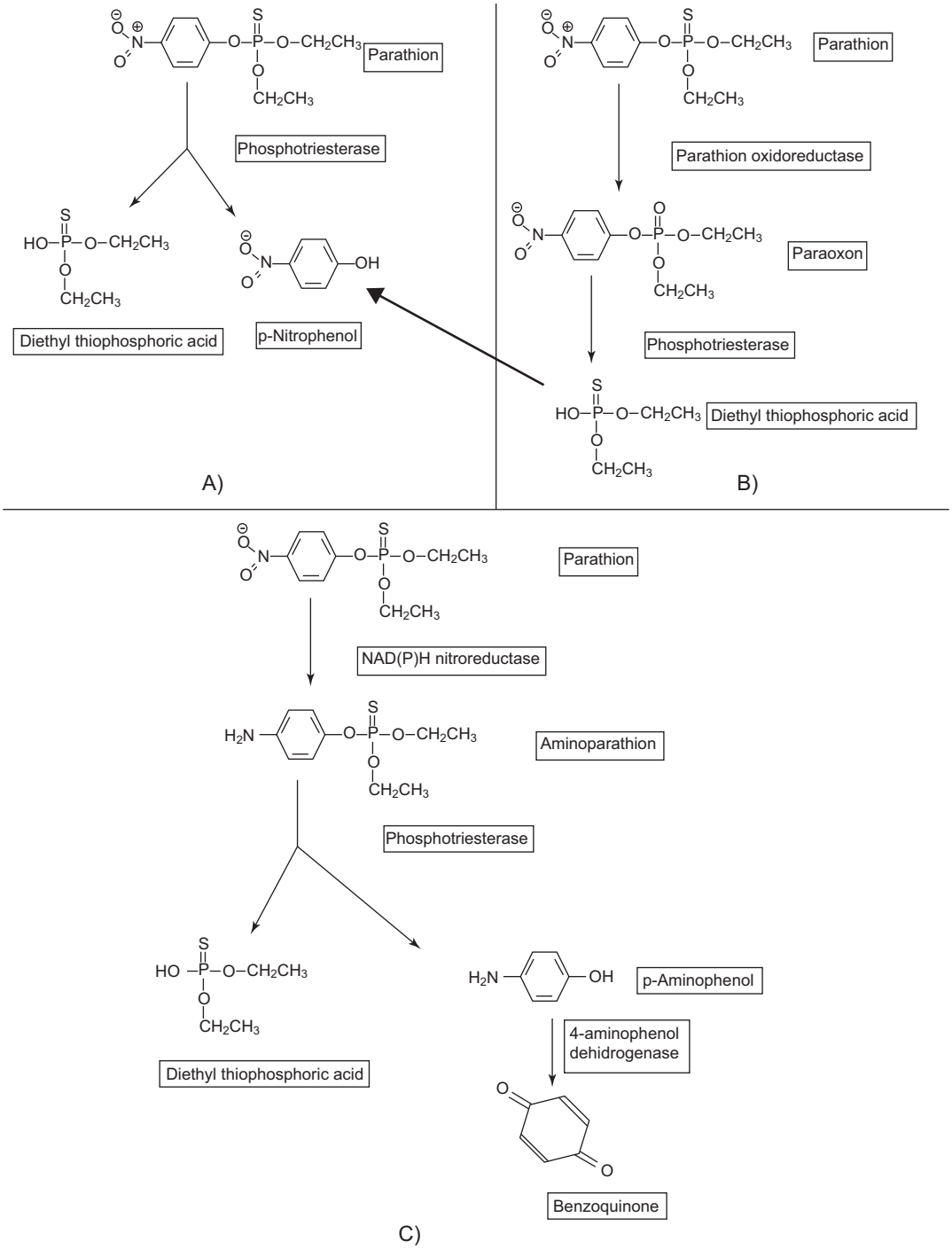

Fig. 2. Parathion degradation pathway. A) Aerobic pathway involves initial hydrolysis of parathion to p-nitrophenol and diethylthiophosphoric acid. B) Other aerobic reaction involves the oxidation of parathion to paraoxon and then it follows the same way as A). C) Under anerobic conditions, parathion is reduced to aminoparathion, which is hydrolyzed to p-aminophenol and diethylthiophosphoric acid (modified from University of Minnesota. Biocatalysis/Biodegradation Database, http://umbbd.ethz.ch/pthn/pthn_map.html)

and further degradation. The *mpd* gene, encoding the enzyme responsible for chlorpyrifos hydrolysis to TCP, was cloned and expressed in *Escherichia coli* BL21. Inoculation of chlorpyrifos-contaminated soil with strain DT-1 resulted in a degradation rate of chlorpyrifos and TCP of 100 % and 94.3 %, respectively as compared to a rate of 28.2 % and 19.9 % in uninoculated soil.

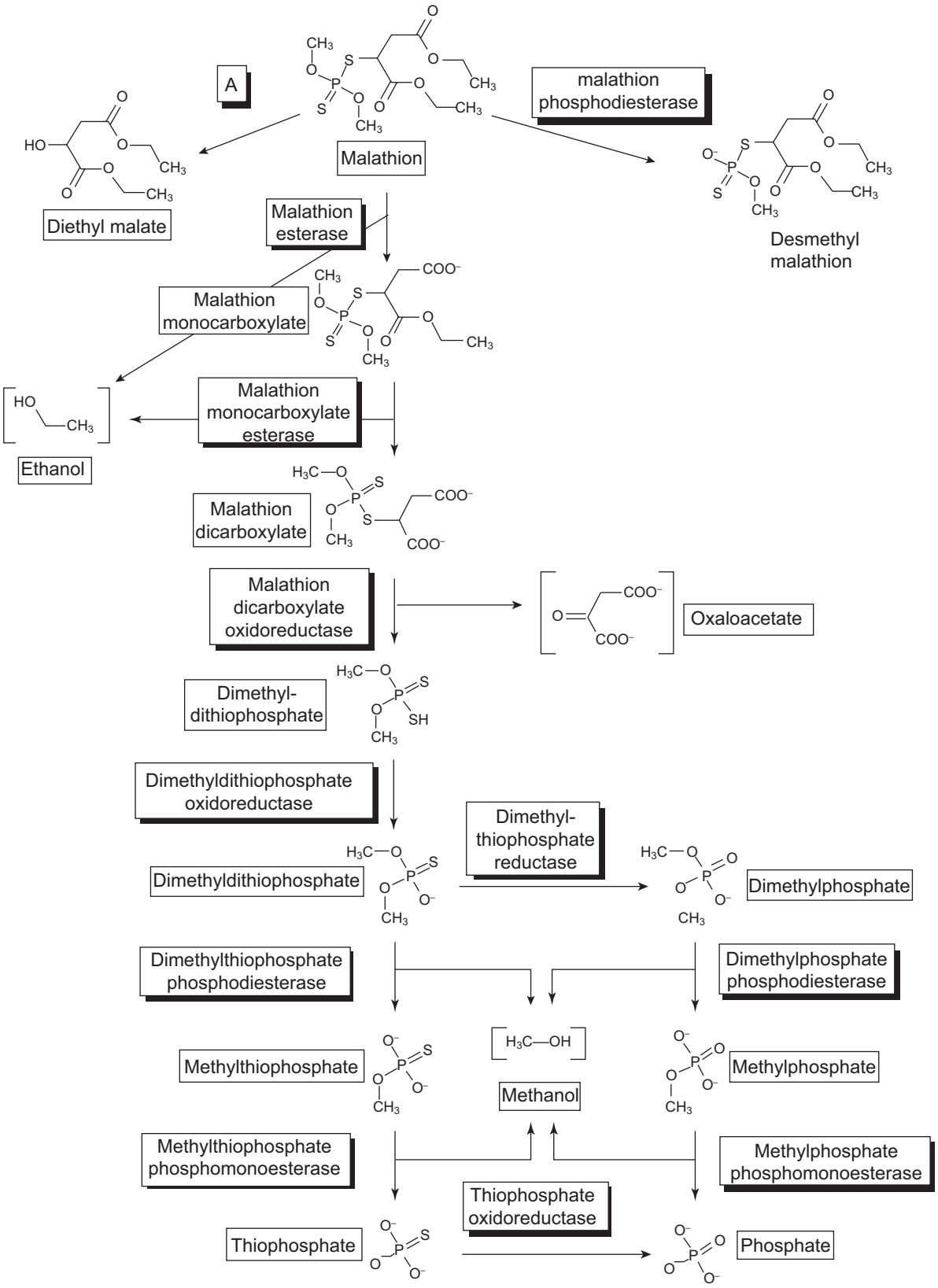

Fig. 3. Malathion degradation pathway (University of Minnesota. Biocatalysis/Biodegradation Database, http://www.umbbd.ethz.ch/end/end_map.html).

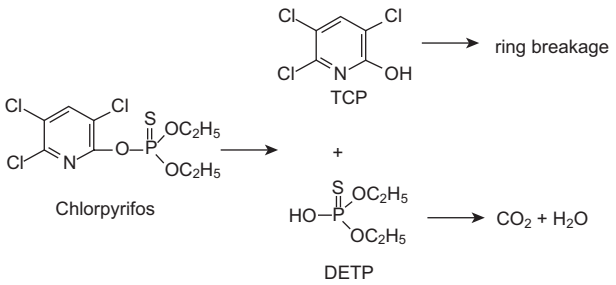

Fig. 4. Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by a new fungal strain *Cladosporium cladosporioides* (Chen *et al.* 2012).

Lu *et al.* (2013) reported a bacterial strain, *Cupriavidus* sp. DT-1, that is capable of degrading chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP). The strain was isolated and characterized by using these compounds as a sole carbon source. Investigation of the degradation pathway showed that chlorpyrifos was first hydrolyzed to TCP, successively dechlorinated to 2-pyridinol, and then subjected to the cleavage of the pyridine ring and further degradation. The *mpd* gene that encodes the enzyme responsible for chlorpyrifos hydrolysis to TCP was cloned and expressed in *Escherichia coli* BL21. Inoculation of chlorpyrifos-contaminated soil with strain DT-1 resulted in chlorpyrifos and TCP degradation at rates of 100 % and 94.3 %, respectively, compared to rates of 28.2 % and 19.9 % in uninoculated soil.

CELLS IMMOBILIZATION TO IMPROVE THE EFFICIENCY OF PESTICIDE DEGRADATION

An immobilized cell is defined as a living cell that, by natural or artificial means, is prevented from moving independently from its original location to all parts of an aqueous phase of a system. The underlying concept is that immobilized microorganisms in matrices, either biological or inert, may enhance the required biotechnological benefits from the mass culture of the microorganism by degrading a specific metabolite or removing pollutants (de-Bashan and Bashan 2010).

Microorganisms do not live as pure cultures of dispersed single cells but instead accumulate at interfaces to form polymicrobial aggregates such as films, mats, flocs (floating biofilms), sludge or biofilms. Multispecies aggregates can form stable microconsortia, develop physiochemical gradients, and undergo horizontal gene transfer and intense cell–cell communication. These consortia therefore represent highly competitive environments (Flemming and Wingender 2010). Immobilization of microorganisms on inert supports has generated an increasing interest because of the benefits that can be obtained from the process (Jo *et al.* 2010). An immobilized cell is defined as a living cell that, by natural or artificial means, is prevented from moving independently from its original location to all parts of an aqueous phase of a system (Tampion and Tampion, 1987).

Cell immobilization has been employed for the biological removal of pesticides because it confers the possibility of maintaining catalytic activity over long periods of time (Martin *et al.* 2000, Richins *et al.* 2000, Chen and Georgiou 2002). 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 the toxic effects of hazardous compounds and harsh environments. Immobilization can increase the cells' survival and metabolic activity in bioremediation systems (Tao *et al.* 2009, Moslemy *et al.* 2002). 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 such as pesticides (Ha *et al.* 2008). 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 (Sun *et al.* 2010). 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 (Ha *et al.* 2009).

There are two types of processes for cell immobilization: those based on physical retention (entrapment and inclusion membranes) and those based on chemical bonds, such as biofilm formation (Kennedy and Cabral 1983). Cell immobilization methods may use various materials or substrates both inorganic (clays, silicates, glass and ceramics) and organic (cellulose, starch, dextran, agarose, alginate, chitin, collagen, keratin, polyacylamide hydrazide, activated pumice and activated carbon) (Arroyo 1998, Jo *et al.* 2010). The applicability of several natural or synthetic polymers as matrices for immobilization of viable cells motivated the study of the use of different gels such as alginate, agar-agar and agarose (Taha *et al.* 2013).

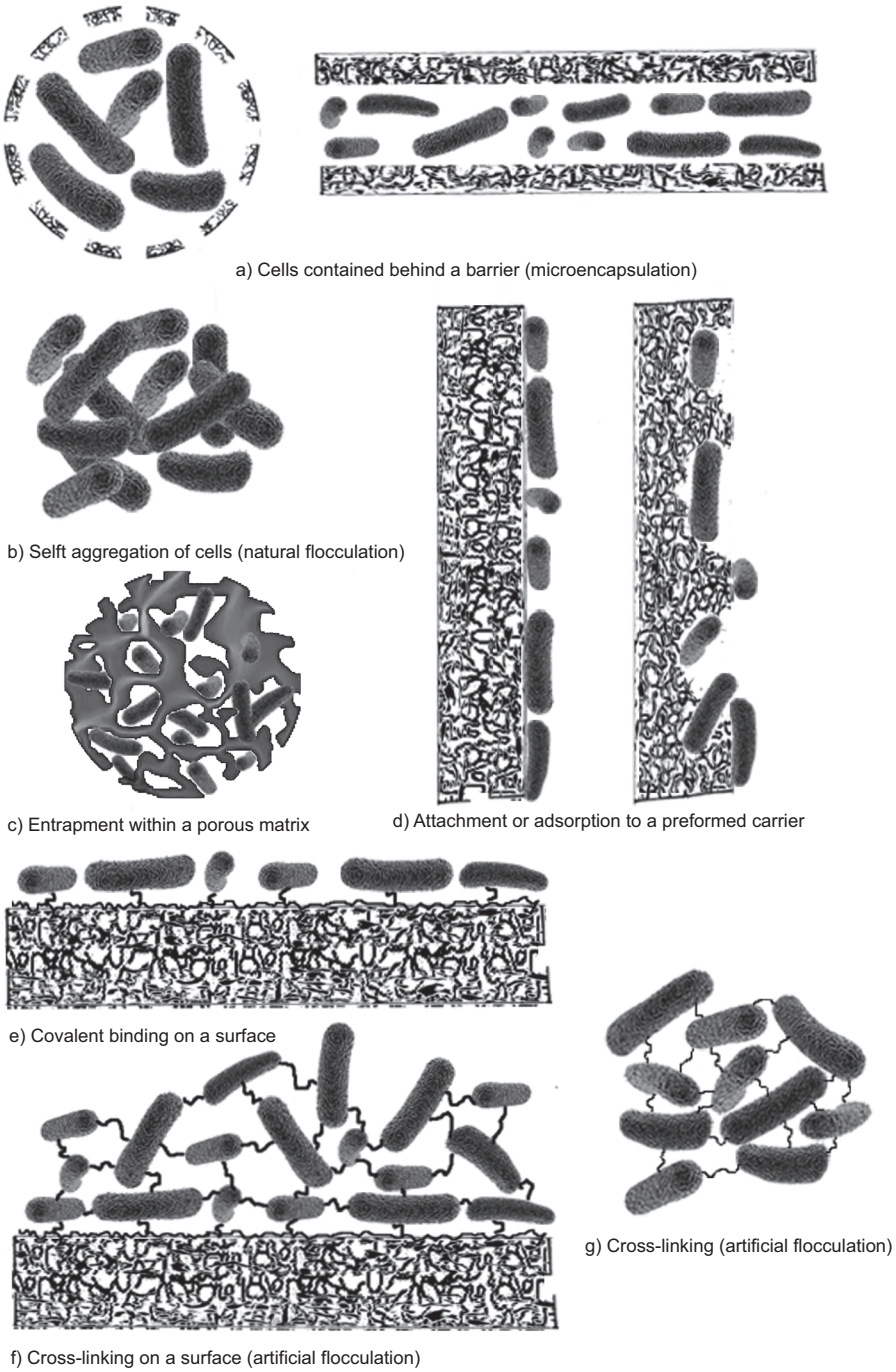

Fig. 5. Cell immobilization methods

Entrapment in natural polymeric gels has become the preferred technique for the immobilization of cells; however, immobilized cells on supports have been used more frequently in xenobiotics biodegradation than for pesticides (Lusta *et al.* 1990).

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 and the possibility of using it repeatedly. Above all, the support must be affordable enough to allow its future use for pesticide degradation. **Figure 5** describes the main methods of immobilization (Kennedy and Cabral 1983, Heitkamp *et al.* 1990, Wang *et al.* 1997,

TABLE III. SUPPORTS FOR IMMOBILIZATION OF MICROORGANISMS IN XENOBIOTICS REMOTION

Support	Microorganism	Xenobiotic	Reference
Glass beads	*Escherichia coli* (transformed)	Coumaphos	Mansee *et al.* 2005
Ceramic	*Pseudomonas GCH1*	Propachlor	Martín *et al.* 2000
Polyurethane, alginate, alginate poly vinyl alcohol	*Pseudomonas spp.*	Phenol	Chivita and Dussán 2003
Coffee beans	*Pseudomonas aeruginosa* *Flavimonas oryzihabitans*	Dichlorodiphenyltrichloroethane Endosulfan	Barragán *et al.* 2007
Ca Alginate beads	*Escherichia coli (OPH)*	Coumaphos, diethylphosphate and chlorferon	Ha *et al.* 2009
Tezontle	*Pseudomonas fluorescens*	2,4-dichlorophenoxiacetic acid Dichlorodiphenyltrichloroethane	Santacruz *et al.* 2005
Ca Alginate beads, Tezontle	Bacterial consortia	Methyl parathion, tetrachlorvinphos	Yáñez-Ocampo *et al.* 2009, 2011
Tezontle	*Flavobacterium* sp. ATCC 27551	Methyl parathion	Abdel-Razek *et al.* 2013
Corncob	Rhodococcus sp. Pseudomonas sp.	n-Hexadecane n-heptadecane	Rivelli *et al.* 2013
Montmorillonite	*Arthrobacter chlorophenolicus* A6	4-chlorophenol (4-CP)	Lee *et al.* 2013a
Alginate	*Bacillus sphaericus* strain CT7 *Pseudomonas* sp. strain W4	Nonylphenol (NP),	Hsu *et al.* 2013
Coir, banana stem, bulrush, water hyacinth stem	*Burkholderia cepacia* PCL3	Carbofuran	Laocharoen *et al.* 2013
Luffa aegyptiaca Mill.	*Metarhizium anisopliae* and bacterial consortium	Parathion methyl and coumaphos	Moreno-Medina 2011
Alginate	*Dermocarpella* sp.	Ammonium	Lee *et al.* 2013b
Alginate, silica gel, agarose	*Arthrospira platensis* (SAG257.80)	Plumb	Duda-Chodak *et al.* 2013
Ca Alginate beads	*Candida tropicalis YMEC14*	Poliphenols	Ettayebi *et al.* 2003
Alginate	*Candida xylopsoci*	Mercury	Amin and Latif 2013
Agave tequiliana Webber (blue)	*Trametes versicolor* *Pleurotus ostreatus* *Klebsiella sp.*	Acid blue 113 Disperse blue 3 Basic green 4	Garzón-Jiménez 2009
Polyurethane foam	*Phanerochaete chrysosporium* strain 1198	Bagasse	Shararia *et al.* 2013
Alginate beads	*Streptomyces* spp. (A2, A5, A11, and M7)	Chlorpyrifos and pentachlorophenol	Fuentes *et al.* 2013

Karamanev *et al.* 1998, Pedersen and Christensen 2000). The methods can be grouped into two types: the active that induce the capture of microorganisms in a matrix, and the passive use the tendency of microorganisms to attack either natural or synthetic surfaces, which enables them to form biofilms. The supports used for immobilization may be of synthetic or natural origin (**Table III**).

Bacterial biofilms are defined as sessile communities characterized by cells that are attached to a substratum, to an interface or to each other. Large amounts of extracellular matrix material are often produced during biofilm formation. This matrix holds the cells in association with each other and with the surface, and it commonly contains exopolysaccharides (EPS), proteins, DNA, surfactants, lipids, glycolipids, and ions such as Ca^{2+}, which form dense granules, grow attached to a static solid surface (static biofilm) or in a suspension bracket (Davey and O'Toole 2000, Nicolella *et al.* 2000, Flemming and Wingender 2010, Prigent-Combaret *et al.* 2012). Biofilms form in several steps starting with the attack or recognition of the surface, followed by growth and the 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 takes place which varies depending on the presence of nutrients from the medium or friction with the surrounding water flow. Finally, a period of biofilm aging may occur during which cells detach and colonize other surfaces (Yañez-Ocampo *et al.* 2009).

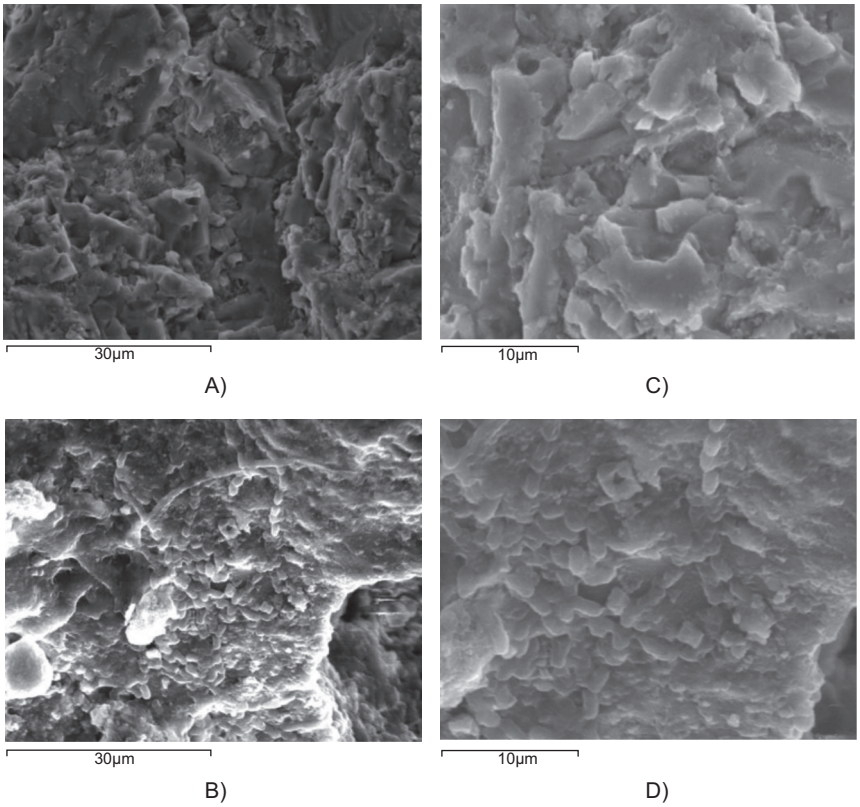

Fig. 6. Scanning electron micrographs showing tezontle and immobilized cells on tezontle. A) Tezontle (2000 X); B) Tezontle with immobilized cells (2000 X); C) Tezontle (4000 X) and D) Tezontle with immobilized cells (4000 X)

Tezontle is a native volcanic rock of Morelos state (central Mexico) and has yielded good results in the degradation of mixtures of pesticides (in Nahuatl, "tezt" means "rock" and "zontli" means "hair"). 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 (**Fig. 6**).

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 contact with the immobilized microorganisms so that biodegradation can be executed. This strategy has been very efficient and can be used for the degradation of pesticide wastes. Yáñez-Ocampo *et al.* (2011) and el Razek *et al.* (2013) immobilized a bacterial consortium in a biofilm on tezontle. This system 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 and immobilized cells were packaged in an upflow reactor, which resulted in a greater viability of the bacteria and more efficient removal of pesticides.

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

The loofa sponge (*Luffa cylindrica*) has been used as a carrier material for immobilizing various microorganisms for the purpose of either the adsorption or degradation of various xenobiotics. This sponge has been used as a natural support to immobilize various organisms such as *Chlorella sorokiniana, Porphyridium cruentum, Penicillium cyclopium* and *Funalia trogii* for nickel II, cadmium II and dyes

and chlorinated substances treatment. Loofa grows well in both tropical and subtropical climates and the sponges are produced in large quantities in México 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. Because of their random lattice of small cross sections coupled with very high porosity, their potentiality as carriers for cell immobilization is very high (Akhtar *et al.* 2004, Iqbal and Edyvean 2004, Mazmanci and Unyayara 2005). Moreno-Medina (2011) used this sponge and reported methyl parathion removal at efficiencies of 75%.

BIOBEDS: A STRATEGY FOR PESTICIDE BIODEGRADATION *IN SITU*

In response to the environmental and health problems related to pesticides, the BioBed (BB) was developed in the early 1990s. Biobeds are a simple and inexpensive construction designed to collect and degrade pesticide spills (Torstensson 2000, Juwarkar *et al.* 2010).

The original design consists of a hole in the ground in which a layer of waterproof clay is placed on the bottom (10 cm). A mixture of straw, peat and soil in proportions of 50-25-25 respectively and 50 cm in thickness is then added, followed by a layer of grass on the surface. Straw is the main component for ligninolytic fungi growth, the soil is used for adsorption and promotes microbial activity and the peat contributes to moisture control (Torstensson and Castillo 1997, Castillo *et al.* 2008).

Due to the low maintenance of the work, the short time required and low costs, the BB has generated great interest in many countries such as France, Italy, the United Kingdom and Chile. Its introduction has led to adaptations of the design according to the climatic conditions of the location and the available organic materials such as olive branches, citrus peels, cotton waste, garden compost and bagasse. Similarly, the name has been adapted in different ways to include terms such as biofilter, biomassbed, Phytobac®, Biobac and Biotable (Fogg *et al.* 2004, Vischetti *et al.* 2004, Coppola *et al.* 2007, De Roffignac *et al.* 2008, Karanasios *et al.* 2012a, Tortella *et al.* 2012).

An efficient mix of materials for a BB must include wide surfaces for the retention of pesticides, which will reduce leaching, while providing a robust and active microbial community (Vischetti *et al.* 2008). However, strong adsorption reduces the bioavailability of the pesticide and limits its biodegradation. When measuring the adsorption of a mixture of pesticides in the soil and in a variety of biomixes, we observed that these biobeds had greater adsorption compared soil pesticides (Karanasios *et al.* 2010), whereby care must be taken in choosing the biomix components. Castillo and Torstensson (2007) have evaluated the effects of the mixture composition, as well as various other factors and found that the original configuration at acidic pH (5.9), 60% humidity and 20ºC, is the optimal condition for degradation in Sweden. Another key parameter is the flow of water. Different studies have shown that under high volumes of water applied at low frequencies (600 mL per week) results in high levels of leaching compared to systems with a low volume applied to high frequencies (100-200 mL per day) (Karanasios *et al.* 2012b).

Under laboratory conditions, biostimulation of the mixture with inorganic fertilizers (N, P, K) at low concentrations (0.1% and 0.5%) resulted in a significant increase in the degradation of chlorpyrifos in the early days of incubation. However, increasing N, P and K concentrations (0.5% and 1.0%) resulted in the accumulation of TCP (the main metabolite of the pesticide), which caused significant changes in the bacterial communities and an increased the risk of leaching (Tortella *et al.* 2010).

Bioaugmentation is a process that increases the soil microbiota by inoculating external microorganisms for the remediation of soil contaminated by a xenobiotic. To improve the efficiency of biodegradation in the BB, Diez *et al.* (2012) used bioaugmentation with pellets of *Anthracophyllum discolor,* a fungus with highly efficient ligninolytic activity on atrazine degradation and obtained an increase of 18 % in the degradation of the pesticide. Recent studies demonstrated that the addition of terpenes at relatively low concentrations (50 mg/kg) significantly enhances the degradation of atrazine (Tortella *et al.* 2013).

Despite its benefits, there are certain limitations. Due to exhaustion, in general, the maturity of the BM affects the performance of the BB, but this possibility requires further study. A study of the three stages of biomix maturity (0, 15 and 30 days) with regard to the degradation of different concentrations of chlorpyrifos (200, 320 and 480 mg/L) showed that the maturity did not interfere with the degradation (Tortella *et al.* 2012). In the field, the mixture should be replaced after 6-10 years and composted to remove pesticide residues (Castillo *et al.* 2008). Although the efficiency of biodegradation is depen-

dent on the dissipation of pesticides in the BB, little is known about the microbiota and its interaction with pesticides (Marinozzi *et al.* 2012). Specific studies are needed on this subject to discern these metabolic processes and enhance the efficiency of the degradation.

CONCLUSIONS

Chemical pesticides are widely used around the world and have historically increased the crop yields for food production. However, they have also been introduced into the food chain, with effects on human health and ecosystems. Therefore, it is important that efforts are made for the disposal of waste and for the remediation of contaminated sites. Biodegradation of pesticides with specific microorganisms is economic and environmental and socially acceptable. By understanding the mechanisms for degradation, it is possible to develop technologies to increase the efficiency of degradation, such as the immobilization of cells in different support systems and the construction and use of biobeds for waste degradation *in situ*.

REFERENCES

Abdel-Razek S.A.R.M., Folch-Mallol J.L., Perezgasga-Ciscomani L., Sánchez-Salinas E., Castrejón-Godínez M.L. and Ortiz-Hernández M.L. (2013). Optimization of methyl parathion biodegradation and detoxification by cells in suspension or immobilized on tezontle expressing the opd gene. J. Environ. Sci. Heal. B. 48, 449-461.

Abo-Amer A.E. (2012). Characterization of a strain of *Pseudomonas putida* isolated from agricultural soil that degrades cadusafos (an organophosphorus pesticide). World J. Microbiol. Biotechnol. 28, 805-814.

Abraham W.R., Nogales B., Golyshin P.N., Pieper D.H. and Timmis K.N. (2002). Polychlorinated biphenyl-degrading microbial communities and sediments. Curr. Opin. Microbiol. 5, 246-253.

Agrawal A., Pandey R.S. and Sharma B. (2010). Water pollution with special reference to pesticide contamination in India. J. Water Resource and Protection. 2, 432-448.

Aislabie J. and Lloyd-Jones G. (1995). A review of bacterial-degradation of pesticides. Aust. J. Soil. Res**.** 33, 925-942.

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

Alves S.A., Albergaria J.T., Fernandes D.V., Alvim-Ferraz M.C. and Delerue-Matos C. (2010). Remediation of soils combining soil vapor extraction and bioremediation: Benzene. Chemosphere 80, 823-828.

Amin A. and Latif Z. (2013). Detoxification of mercury pollutant by immobilized yeast strain *Candida xylopsoci.* Pak. J. Bot. 45, 1437-1442.

Araya M. and Lakhi A. (2004). Response to consecutive nematicide applications using the same product in *Mussa AAAcv*. Grande naine originated from in vitro propagative material and cultivated in virgin soil. Nematologia Brasileira 28, 55-61.

Arroyo M. (1998). Inmovilización de enzimas. Fundamentos, métodos y aplicaciones. Ars. Pharmaceutica. 39, 23-39.

Badii M. and Landeros J. (2007). Plaguicidas que afectan la salud humana y la sustentabilidad. CULCYT/ Toxicología de Plaguicidas 4-19.

Bansal O.P. (2012). Degradation of pesticides. In: Pesticides Evaluation of environmental pollution (S. Hamir, Rathore and L. M. L. Nollet, Ed.). CRC Press, New York, United States of America, pp. 47-77.

Barragán H.B., Costa C., Peralta J., Barrera J., Esparza F. and Rodríguez R. (2007). Biodegradation of organochlorine pesticides by bacteria grown in microniches of the porous structure of green vean coffee. Int. Biodeter. Biodegr. 59, 239-244.

Bass C. and Field L.M. (2011). Gene amplification and insecticide resistance. Pest. Manag. Sci. 67, 886-890.

Becker J.G. and Seagren E.A. (2010). Biorremediation of hazardous organics. In: Environmental microbiology (R. Mitchell y G. Ji-Dong, Ed.). Wiley-Blackwell, New Jersey, United States of America, pp. 177-212.

Blackburn J.W. and Hafker W.R. (1993). The impact of biochemistry, bioavilability, and bioactivity on the selection of bioremediation technologies. Trends. Biotechnol. 11, 328-333.

Briceño G., Palma G. and Duran N. (2007). Influence of organic amendment on the biodegradation and movement of pesticides. Crit. Rev. Env. Sci. Tec. 37, 233-271.

Castillo M.D.P. and Torstensson L. (2007). Effect of biobed composition, moisture, and temperature on the degradation of pesticides. J. Agric. Food Chem. 55, 5725-5733.

Castillo M.D.P., Torstensson L. and Stenström J. (2008). Biobeds for environmental protection from pesticide uses: A review. J. Agric. Food Chem. 56, 6206-6219.

Chen H., He X., Rong X., Chen W., Cai P., Liang W., Li S. and Huang Q. (2009). Adsorption and biodegradation

of carbaryl on montmorillonite, kaolinite and goethite. Appl. Clay Sci. 46, 102-108.

Chen S., Liu C., Peng C., Peng C., Liu H., Hu M. and Zhong G. (2012). Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by a new fungal strain *Cladosporium cladosporioides* Hu-01. PLoS ONE 7, 1-12.

Chen W. and Georgiou G. (2002). Cell-surface display of heterologous proteins: from high throughput screening to environmental applications. Biotechnol. Bioeng. 5, 496-503.

Chevillard A., Coussy H. A., Guillard V., Gontard N. and Gastaldi E. (2012). Investigating the biodegradation pattern of an ecofriendly pesticide delivery system based on wheat gluten and organically modified montmorillonites. Polym. Degrad. Stabil. 97, 2060-2068.

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

Chivita L. and Dussán J. (2003). Evaluación de matrices para la inmovilización de *Pseudomonas spp.* en bioremediación de fenol. Revista Colombiana de Biotecnología 5, 5-10.

Coppola L., Castillo M.D.P., Monaci E. and Vischetti C. (2007). Adaptation of the biobed composition for chlorpyrifos degradation to southern Europe conditions. J. Agric. Food Chem. 55, 396-401.

Damalas C.A. (2009). Understanding benefits and risks of pesticide use. Sci. Res. Essays. 4, 945-949.

Damalas C.A. and Eleftherohorinos I.G. (2011). Pesticide exposure, safety issues, and risk assessment indicators. Int. J. Environ. Res. Public Health 8, 1402-1419.

Dasgupta S., Meisner C. and Wheeler D. (2010). Stockpiles of obsolete pesticides and cleanup priorities: A methodology and application for Tunisia. J. Environ. Manage. 91, 824-830.

Davey M.E. and O'Toole G.A. (2000). Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. R. 64, 847-867.

De Roffignac L., Cattan P., Mailloux J., Herzog D. and Le Bellec F. (2008). Efficiency of a bagasse substrate in a biological bed system for the degradation of glyphosate, malathion and lambda-cyhalothrin under tropical climate conditions. Pest. Manag. Sci. 64, 1303-1313.

de-Bashan L.E. and Bashan Y. (2010). Immobilized microalgae for removing pollutants: Review of practical aspects. Bioresource Technol. 101, 1611-1627.

Diez M.C. (2010). Biological aspects involved in the degradation of organic pollutants J. Soil Sci. Plant. Nutr. 10, 244-267.

Diez M.C., Elgueta S., Fernandez S. and Gallardo F. (2012). Bioaugmentation of biobed biomixture with fungal pellets of *Anthracophyllum discolor* to improve pesticides degradation. New Biotechnol. 29, S195.

Dua M., Singh A., Sethunathan N. and Johri A.K. (2002). Biotechnology and bioremediation: successes and limitations. Appl. Microbiol. Biotechnol. 59, 143-152.

Duda-Chodak A., Wajda L. and Tarko T. (2013). The immobilization of *Arthrospira platensis* biomassin different matrices-A practical application for lead biosorption. J. Environ. Sci. Heal. A. 48, 509-517.

EPA (2012). What is a Pesticide? [on line]. http://www.epa.gov/opp00001/about/. 16/07/2012.

Ettayebi K., Errachidi F., Jamai L., Tahri-Jouti M.A., Sendide K. and Ettayebi M. (2003). Biodegradation of polyphenols with immobilized *Candida tropicalis* under metabolic induction. FEMS Microbiol. Lett. 223, 215-219.

Farrera P.R. (2004). Acerca de los plaguicidas y su uso en la agricultura. Ceniap Hoy. 6. [on line]. www.ceniap.gov.ve/ceniaphoy/articulos/n6/arti/farrera_r/arti/farrera_ r.htm. 20/03/2012.

Flemming H.C. and Wingender J. (2010). The biofilm matrix. Nat. Rev. Microbiol. 8, 623-33.

Fogg P., Boxall A., Walker A. and Jukes A. (2004). Degradation and leaching potential of pesticides in biobed systems. Pest. Manag. Sci. 60, 645-654.

Fuentes M.S., Briceño G.E., Saez J.M., Benimeli C.S., Diez M.C. and Amoroso M.J. (2013). Enhanced removal of a pesticides mixture by single cultures and consortia of free and immobilized *Streptomyces* Strains. Biomed Res. Int. 1-9.

Gao Y., Chen S., Hu M., Hu Q., Luo J. and Li Y. (2012). Purification and Characterization of a Novel Chlorpyrifos Hydrolase from *Cladosporium cladosporioides* Hu-01. PLoS ONE 7, 1-8.

Garzón J.R.C. (2009). Cinética de degradación de colorantes textiles de diferentes clases químicas por hongos y bacterias inmovilizados sobre fibra de *Agave tequilana* Webber var. Azul. Tesis de Licenciatura. Facultad de Ciencias, Pontificia Universidad Javeriana. Bogotá, Colombia. 124 pp.

Ghanem E. and Raushel F.M. (2005). Detoxification of organophosphate nerve agents by bacterial phosphotriesterase. Toxicol. Appl. Pharmacol. 207, 459-470.

Grube A., Donaldson D., Kiely T. and Wu L. (2011). Pesticides industry sales and usage: 2006 and 2007 Market Estimates. U.S. Environmental Protection Agency; Washington, D.C., United States of America. 1-33.

Ha J., Engler C.R. and Lee S.J. (2008). Determination of diffusion coefficients and diffusion characteristics for chlorferon and diethylthiophosphate in Ca-alginate gel beads. Biotechnol. Bioeng. 100, 698-706.

Ha J., Engler C.R. and Wild J. (2009). Biodegradation of coumaphos, chlorferon, and diethylthiophosphate using bacteria immobilized in Ca-alginate gel beads. Bioresource Technol. 100, 1138-1142.

Harms H., Schlosser D. and Wick L.Y. (2011) Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals. Nature Rev. Microbiol. 9, 177-192.

Heitkamp M., Camel V., Reuter T. and Adams W. (1990). Biodegradation of p-nitrophenol in an aqueous waste stream by immobilized bacteria. Appl. Environ. Microbiol. 56, 2967-73.

Hsu F.Y., Wang Z.Y. and Chang B.V. (2013). Use of microcapsules with electrostatically immobilized bacterial cells or enzyme extract to remove nonylphenol in wastewater sludge. Chemosphere 91, 745-750.

Hussain S., Siddique T., Saleem M., Arshad M. and Khalid A. (2009). Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. Adv. Agron. 102, 159-200.

Iqbal M. and Edyvean R.G.J. (2004). Biosorption of lead, copper and zinc ions on loofa immobilized biomass of *Phanerochaete chrysosporium*. Minerals Engineering 17, 217-223.

Iranzo M., Sain-Pardo I., Boluda R., Sanchez J. and Mormeneo S. (2001). The use of microorganisms in environmental remediation. Ann. Microbiol. 51, 135-143.

Jo M.S., Lee J.B., Kim J.E., Sohn H.Y., Jeon C.P., Choi C.S. and Know G.S. (2010). Biodegradation of endosulfan by *Klebsiella oxytoca* KE-8 immobilized on Activated Carbon. *Korean J. Environ. Agric.* 29, 176-183.

Juwarkar A.A., Singh S.K. and Mudhoo A. (2010). A comprehensive overview of elements in bioremediation. Rev. Environ. Sci. Biotechnol. 9, 215-288.

Karamanev G., Chavarie C. and Samson R. (1998). Soil immobilization: new concept for biotreatment of soil contaminants. Biotechnol. Bioeng. 4, 471-476.

Karanasios E., Tsiropoulos N.G. and Karpouzas D.G. (2012a). On-farm biopurification systems for the depuration of pesticide wastewaters: recent biotechnological advances and future perspectives. Biodegradation 23, 787-802.

Karanasios E., Papadi-Psyllou A., Karpouzas D.G. and Tsiropoulos N.G. (2012b). Optimization of biomixture composition and water management for maximum pesticide dissipation performance in peat-free biobeds. J. Environ. Qual. 23, 787-802.

Karanasios E., Tsiropoulos N.G., Karpouzas D.G. and Ehaliotis C. (2010). Degradation and adsorption of pesticides in compost-based biomixtures as potential substrates for biobeds in Southern Europe. J. Agric. Food Chem. 58, 9147-9156.

Karstensen K.H., Nguyen K.K., Le B.T., Pham H.V., Nguyen D.T., Doan T.T., Nguyen H.H., Tao M.Q., Luong D.H. and Doan H.T. (2006). Environmentally sound destruction of obsolete pesticides in developing countries using cement kilns. Environ. Sci. Policy. 9, 577-586.

Kennedy J.F. and Cabral J.M.S. (1983). Immobilized enzymes. In: Solid Phase Biochemistry. (W. H. Schouten, Ed.). Wiley, New York, United States of America. pp. 253-391.

Klerks P.L., Xie L. and Levinton J.S. (2011). Quantitative genetics approaches to study evolutionary processes in ecotoxicology; a perspective from research on the evolution of resistance. Ecotoxicology 20, 513-523.

Laocharoen S., Plangklang P. and Reungsang A. (2013). Selection of support materials for immobilization of *Burkholderia cepacia* PCL3 in treatment of carbofuran contaminated water. Environ. Technol. DOI: 10.1080/09593330.2013.781226 (on line first).

Latifi A.M., Khodi S., Mirzaei M., Miresmaeili M. and Babavalian H. (2012). Isolation and characterization of five chlorpyrifos degrading bacteria. Afr. J. Biotechnol. 11, 3140-3146.

Lee S.H., Lee S.H., Ryu S.J., Kang C.S., Suma Y. and Kim H.S. (2013a). Effective biochemical decomposition of chlorinated aromatic hydrocarbons with a biocatalyst immobilized on a natural enzyme support. Bioresource Technol. 141, 89-96.

Lee Y., Hu H.F. and Chiu C.Y. (2013b). Using agar–alginate immobilized cyanobacteria (*Dermocarpella* sp.) arranged in tubular chains to treat swine farm waste water. J. Appl. Phycol. 1-6.

Liang W.Q, Wang Z.Y., Li H., Wu P.C., Hu J.M., Luo N., Cao L.X. and Liu Y.H. (2005). Purification and characterization of a novel pyrethroid hydrolase from *Aspergillus niger* ZD11. J. Agric. Food. Chem. 53, 7415-7420.

Lu P., Li Q., Liu H., Feng Z., Yan X., Hong Q. and Li S. (2013). Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by *Cupriavidus* sp. DT-1. Bioresource Technol. 127, 337-342.

Lusta K.A., Starostina N.G. and Fikhte B.A. (1990). Immobilization of microorganisms: cytophysiological aspects. In Proceedings of an International Symposium: Physiology of Immobilized Cells. (J. A. M. de Bont, J. Visser, B. Mattiasson and J. Tramper, Ed.). Wageningen, The Netherlands, 10-13 Dec., 1989. 557-562.

Mansee A.H., Chen W. and Mulchandani A. (2005). Detoxification of the organophosphate nerve agent coumaphos using organophosphorus hydrolase im-

mobilized on cellulose materials. J. Ind. Microbiol. Biotechnol. 32, 554-560.

Marinozzi M., Coppola L., Monaci E., Karpouzas D.G., Papadopoulou E., Menkissoglu-Spiroudi U. and Vischetti C. (2012). The dissipation of three fungicides in a biobed organic substrate and their impact on the structure and activity of the microbial community. Environ. Sci. Pollut. Res. Int. 20, 2546-2555.

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

Martinez J. (2004). 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. 65 pp.

Mazmanci M. and Unyayara. (2005). Decolourisation of reactive black 5 by *Funalia trogii* immobilized on *Loofa cylindrical* sponge. Process Biochem. 40, 337-342.

Megharaj M., Ramakrishnan B., Venkateswarlu K., Sethunathan N. and Naidu R. (2011). Bioremediation approaches for organic pollutants: A critical perspective. Environ. Int. 37, 1362-1375.

Meleiro-Porto A.L., Zelayarán M. G., Consiglio-Kasemodel M. and Nitschke M. (2011). Biodegradation of Pesticides. In: Pesticides in the Modern World-Pesticides Use and Management. (M. Stoytcheva, Ed.). InTech. Croatia, pp. 408-438.

Moreno M.D.A. (2011). Evaluación de la degradación de paratión metílico y coumafos por *Metarhizium anisopliae* y un consorcio bacteriano inmovilizados en *Luffa aegyptiaca* Mill. Tesis de Maestría. Centro de Investigación en Biotecnología. Universidad Autónoma del Estado de Morelos. Cuernavaca, Morelos, México.

Moslemy P., Guiot S.R. and Neufeld R.J. (2002). Production of size-controlled gellan gum microbeads encapsulating gasoline-degrading bacteria. Enzyme Microb. Technol. 30, 10-18.

Myresiotis C.K., Vryzas Z. and Papadopoulou-Mourkidou E. (2012). Biodegradation of soil-applied pesticides by selected strains of plant growth-promoting rhizobacteria (PGPR) and their effects on bacterial growth. Biodegradation 23, 297-310.

Nicolella C.M., Van Loosdrecht and Heijnen J.J. (2000). Wastewater treatment with particulate biofilm reactors. J. Biotechnol. 80, 1-33.

Nyakundi W.O., Magoma G., Ochora J. and Nyende A.B. (2011). Biodegradation of diazinon and methomyl pesticides by white rot fungi from selected horticultural farms in rift valley and central Kenya. J. Appl. Tech. Environ. Sanit. 1, 107-124.

Ortiz-Hernández M.L., Quintero-Ramírez R., Nava-Ocampo A. A. and Bello-Ramírez A.M. (2003). Study of the mechanism of *Flavobacterium* sp. for hydrolyzing organophosphate pesticides. Fundam. Clin. Pharmacol. 17, 717-723.

Ortiz-Hernández M.L. and Sánchez-Salinas E. (2010). Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México. Rev. Int. Contam. Ambie. 26, 27-38.

Ortiz-Hernández M.L., Sánchez-Salinas E., Olvera-Velona A. and Folch-Mallol J.L. (2011). Pesticides in the Environment: Impacts and its Biodegradation as a Strategy for Residues Treatment. En: Pesticides-Formulations, Effects, Fate. (M. Stoytcheva, Ed.). InTech. Croatia, pp. 551-574.

Paul D., Pandey G., Pandey J. and Jain R.K. (2005). Accessing microbial diversity for bioremediation and environmental restoration. Trends. Biotechnol. 23, 135-142.

Pedersen S. and Christensen M.W. (2000). Immobilized biocatalysts. In: Applied Biocatalysis. (A. Straathof and P. Adlercreutz, Ed.). Harwood Academic Publishers. Amsterdam, Netherlands, pp. 213-228.

Porzio E., Merone L., Mandricha L., Rossia M. and Manco G. (2007). A new phosphotriesterase from *Sulfolobus acidocaldarius* and its comparison with the homologue from *Sulfolobus solfataricus.* Biochemie 89, 625-636.

Prigent-Combaret C., Zghidi-Abouzid O., Effantin G., Lejeune P., Reverchon S. and Nasser W. (2012). The nucleoid-associated protein Fis directly modulates the synthesis of cellulose, an essential component of pellicle–biofilms in the phytopathogenic bacterium *Dickeya dadantii.* Mol. Microbiol. 86, 172-186.

Qiu X., Zhong Q., Li M., Bai W. and Li B. (2007). Biodegradation of p-nitrophenol by methyl parathion-degrading *Ochrobactrum* sp. B2. Int. Biodeter. Biodegr. 59, 297-301.

Ramakrishnan B., Megharaj M., Venkateswarlu K., Sethunathan N. and Naidu R. (2011). Mixtures of environmental pollutants: effects on microorganisms and their activities in soils. Reviews of Environmental Contamination and Toxicology 211, 63-120.

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

Rivelli V., Franzetti A., Gandolfi I., Cordoni S. and Bestetti G. (2013). Persistence and degrading activity of free and immobilized allochthonous bacteria during bioremediation of hydrocarbon-contaminated soils. Biodegradation 24, 1–11.

Riya P. and Jagatpati T. (2012). Biodegradation and bioremediation of pesticides in soil: its objectives, classification of pesticides, factors and recent developments. World J. Sci.Technol. 2, 36-41.

Santacruz G., Bandala E. and Torres L. G. (2005). Chlorinated pesticides (2,4 –D and DDT) biodegradation at high concentrations using immobilized *Pseudomonas fluorescens*. J. Environ. Sc. Heal. 40, 571-583.

Scott C., Pandey G., Hartley C.J., Jackson C.J., Cheesman M. J., Taylor M.C., Pandey R., Khurana J.L., Teese M., Coppin C.W., Weir K.M., Jain R. K., Lal R., Russell R.J. and Oakeshott J.G. (2008). The enzymatic basis for pesticide bioremediation. Indian J. Microbiol. 48, 65-79.

Shah B.P. and Devkota B. (2009). Obsolete Pesticides: Their Environmental and Human Health Hazards. J. Agr. Envir. 10, 51-56.

Sharari M., Latibari A.J., Guillet A., Aurousseau M. and Sharari A. (2013). Treatment of bagasse preparation effluent by *Phanerochaete chrysosporium* immobilized on polyurethane foam: Enzyme production versus pollution removal. Ind. Crop. Prod. 46, 226-233.

Shen Y.J., Lu P., Mei H., Yu H.J., Hong Q. and Li S.P. (2010). Isolation of a methyl-parathion degrading strain *Stenotrophomonas* sp. SMSP-1 and cloning of the *ophc2* gene. Biodegradation 21, 785-792.

Shukla K.P., Singh N.K. and Sharma S. (2010). Bioremediation: developments, current practices and perspectives. Genet. Eng. Biotechnol. J. 3, 1-20.

Singh B.K. and Walker A. (2006). Microbial degradation of organophosphorus compounds. FEMS Microbiol. Rev. 30, 428-471.

Singleton I. (2004). Microbial metabolism of xenobiotics: fundamental and applied research. J. Chem. Technol. Biot. 59, 9-23.

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

Taha T.H., Taha Alamri S.A., Mahdy H.M. and Hafez E.E. (2013). The effects of various immobilization matrices on biosurfactant production using hydrocarbon (HC)-degrading marine bacteria via the entrapment technique. J. Biol. Sci. 13, 48-57.

Tampion J. and Tampion M.D. (1987). Immobilized cells: principles and applications. Cambridge University Press. Cambridge, Great Britain. 249 pp.

Tao X.Q., Lu G.N., Liu J.P., Li T. and Yang L.N. (2009). Rapid degradation of phenanthrene by using *Sphingomonas* sp. GY2B immobilized in calcium alginate gel beads. Int. J. Environ. Res. Public Health. 6, 2470-2480.

Theriot C.M. and Grunden A.M. (2010). Hydrolysis of organophosphorus compounds by microbial enzymes. Appl. Microbiol. Biot. 89, 35-43.

Torres R.D. (2003). El papel de los microorganismos en la biodegradación de compuestos tóxicos. Ecosistemas 2, 1-5.

Torstensson L. (2000). Experiences of biobeds in practical use in Sweden. Pestic. Outlook. 11, 206-212.

Torstensson L. and Castillo M.D.P. (1997). Use of biobeds in Sweden to minimize environmental spillages from agricultural spraying equipment. Pestic. Outlook. 8, 24-27.

Tortella G.R., Rubilar O., Cea M., Wulff C., Martínez O. and Diez M.C. (2010). Biostimulation of agricultural biobeds with npk fertilizer on chlorpyrifos degradation to avoid soil and water contamination. J. Soil Sci. Plant. Nutr. 10, 464-475.

Tortella G.R., Rubilar O., Castillo M.D.P., Cea M., Mella-Herrera R. and Diez M.C. (2012). Chlorpyrifos degradation in a biomixture of biobed at different maturity stages. Chemosphere 88, 224-228.

Tortella G.R., Rubilar O., Stenström J., Cea M., Briceño G., Quiroz A., Diez M.C. and Parra L. (2013). Using volatile organic compounds to enhance atrazine biodegradation in a biobed system. Biodegradation 24, 711-720.

Trigo A., Valencia A. and Cases I. (2009). Systemic approaches to biodegradation. FEMS Microbiol. Rev. 33, 98-108.

Van Eerd L.L., Hoagland R.E., Zablotowicz R.M. and Hall J.C. (2003). Pesticide metabolism in plants and microorganisms. Weed Sci. 51, 472-495.

Vidali M. (2001). Bioremediation. An overview. Pure Appl. Chem. 73, 1163-1172.

Vijgen J. and Egenhofer C. (2009). Obsolete pesticides a ticking time bomb and why we have to act now. Centre for European Policy Studies and the International HCH & Pesticides Association. Brussels, Belgium. 28 pp.

Vischetti C., Casucci C. and Perucci P. (2002). Relationship between changes of soil microbial biomass content and imazamox and benfluralin degradation. Biol. Fertil. Soil. 35, 13-17.

Vischetti C., Monaci E., Cardinali A., Casucci C. and Perucci P. (2008). The effect of initial concentration, co-application and repeated applications on pesticide degradation in a biobed mixture. Chemosphere 72, 1739-1743.

Vischetti C., Capri E., Trevisan M., Casucci C. and Perucci P. (2004). Biomassbed: a biological system to reduce pesticide point contamination at farm level. Chemosphere 55, 823-828.

Wang P., Sergeeva M., Lim L. and Dordick J. (1997). Biocatalytic plastics as active and stable materials for biotransformations. Nat. Biotechnol. 15, 789-793.

Yañez-Ocampo G., Penninckx M., Jiménez-Tobon G. A, Sánchez-Salinas E. and Ortiz-Hernández M.L. (2009). Removal of two organophosphate pesticides employing a bacteria consortium immobilized in either alginate or tezontle. J. Hazard. Mater. 168, 1554-1561.

Yañez-Ocampo G., Sánchez-Salinas E. and Ortiz-Hernández M.L. (2011). Removal of methyl parathion and tetrachlorvinphos by a bacterial consortium immobilized on tezontle-packed up-flow reactor. Biodegradation 22, 1203-1213.