BIODEGRADATION OF CHEMICAL PESTICIDES AS A STRATEGY TO RESIDUE TREATMENT: EXPERIENCES IN MEXICO

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For the last 150 years, man has fabricated different chemical compounds in order to satisfy the growing needs of the technological development and to improve his life quality. Since the beginning of the industrial revolution, there is an estimation of 120,000 chemical substances of new synthesis. The sub products derived from these, produced by human activity, census that increases day by day and that doesn't seem to have and end, if we consider that about 2000 new compounds are incorporated each year to the list (Olea y Fernández 2001).

Among these compounds we can find pesticides, which are defined as any substance or mixture of substances with the purpose of prevent, destroy or control any plague, including vectors of animal and human diseases, plant species or undesirable animals. They potentially cause damage or interfere in any way in any other way in the production, elaboration, storage, transport, or commercialization of food, agricultural products and wood products or animal food, or that may be given to animals to prevent insects, arachnids or other plagues in or over their bodies (FAO 2002).

The application of pesticides may cause adverse effects among the different forms of life and among the ecosystems; this will depend on the sensibility grade of the organisms and the pesticide (CICOPLAFEST 2004). The inadequate practice of application is one of the most important way of pollution, which has a profound impact not only on the soils of the areas in which they are applied, but they arrive through the rivers to the coastal zones, affecting marine species. The pesticide application generates social conflicts because of the elevated number of workers from the country who are intoxicated by these products, with a high mortality rate, as well as for the suspicion of adverse effects on the health of surrounding communities, flora and fauna (Lichtinger *et al*. 2001).

From the millions of tons of pesticides applied annually in a worldwide level, there's a generation of liquid and solid wastes, besides containers, which are placed directly and without control on the soil and water mainly, polluting and affecting the trofic chains (Pasillos *et al.* 2001). Among the main consequences derived from the soil pollution, we find the loss of fertility and others, which directly or indirectly allow the survival of the flora and fauna, given the tight interrelationships among the different elements, which constitute the ecosystems.

On the other hand, 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. An obsolete pesticide may be recognized as one that is undesirable or impossible to use and has to be eliminated, these include (Martinez 2004):

1. Technical pesticides and formulations passing the expiration date (generally two years after their manufacture)
2. Pesticides whose use has been forbidden or strictly restricted
3. Damaged products:
   a) Those who suffered physical or chemical changes which make them phytotoxic for the crops, or with non acceptable dangerousness for human and environment health
   b) Those who suffered a loss of biological efficiency
   c) Those who present changes in their physical properties which make them incompatible with the habitual equipments of application
4. Pesticides which are undesired by their owners, even if they are in good conditions for their use
5. Products without identification
6. Products which are polluted with other substances

It is also included:

a) Pesticide wastes generated on fire and other accidents

b) Materials which are strongly polluted with pesticides
c) Wastes which are generated by the fabrication or formulation of pesticides

Because of their characteristics, obsolete pesticides are a dangerous waste, that's why they should be managed as such. 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 (Farrera 2004). In Mexico there are obsolete pesticides, some of which have been inventoried and others, which remain unknown. In 2002 there was knowledge of the existence of obsolete pesticides in different states of Mexico, achieving a total of 1040 tons, besides 84,000 tons of highly polluted soil (Cortinas 2002).

The damages caused to the environment and health, such as the existence of obsolete pesticides, make necessary the development of technologies that guaranteed their elimination in a safe, efficient and economic way. Among the existent technologies there are those which apply physic treatments, such as adsorption and percolator filters; chemical treatments such as the advanced of oxidation or inverse osmosis, and the incineration. However, one which promises to be efficient, economic and safe is the biological treatment, because several reactions catalyzed by enzymes of specific microorganisms take place.

To give a treatment to wastes and polluted sites with organophosphate pesticides, biological processes have been used (Moens *et al.* 2004, Araya y Lakhi 2004). Among them, the microbial metabolism is the primary force of transformation or degradation. In many cases it has been reported that the microorganisms are more important in the degradation of xenobiotic compounds (xenobiotic comes from the greek "xeno" which means "odd" and "bio" which means "life"). It is applied to the compounds whose chemical structure in the nature is infrequently or inexistent because they are compounds synthesized by man at the laboratory (Ortiz-Hernández 2002). The importance of the microorganisms lays on their great diversity and metabolic plasticity, which allows them to use diverse ecologic niches. The microorganisms may live in any medium because of their remarkable capacity of mutation and adaptation; besides, they seem to have a great potential to acquire capacities of degradation when they are exposed to xenobiotics. Additionally, the isolated microorganisms with the capacity to degrade xenobiotic compounds have the potential to be used at the bioremediation of other compounds that doesn't have any microbial system known for their degradation (Singh y Walker 2006).

The soil microorganisms are capable of degrade a wide variety of chemical compounds, from polysaccharides, amino acids, proteins, lipids to more complex materials such as pesticides (Ortíz-Hernández 2002). Diverse bacterial genuses are adapted to develop in polluted soils with pesticides. These microorganisms present enzymes involved in the hydrolysis of P-O, P-F, P-S and P-C bonds, which are found in a wide variety of organophosphate pesticides (Singh and Walker 2006). Some soils isolated bacteria are capable of degrading pesticides such as methyl parathion and ethylic parathion

Diverse microorganisms have the organophosphate degrading enzymes, which are codified by different genes such as *opd, mpd, opdA* found in different genres as *Flavobacterium, Pseudomonas* and *Agrobacterium*, respectively, amongst others. The studied and reported enzymes so far, are related to the phosphotriesterase, which is capable of hydrolyzing organophosphate pesticides in the central atom of pesticides' phosphorus. Hydrolysis is fundamental for the complete degradation of the molecule (Chino-Flores 2007)

Development of strategies for the treatment of organophosphate pesticides

For the development of strategies that allow either to give treatment and final disposition to organophosphate pesticides wastes or to restore polluted soils with these products, this problem has been treated form a biotechnological and complete point of view in order to be able to have a methodology that is safer and more economic than the conventional treatments, as well as avoiding additional damages to the environment.

Obtaining isolated strains from agricultural soil in the state of Morelos, Mexico.

Several projects have been created for the search of microorganisms capable of degrading pesticides. Several samples of agricultural soil from different communities in the state of Morelos have been collected. The frequent usage of pesticides whose active agents are organophosphates is reported by these projects. Microbial population in one gram of soil was cultivated during 2 weeks at ambient temperature and in constant agitation in flasks of 125 mL with 80 mL of mineral salts medium with organophosphates as the only source of carbon.

This procedure was repeated several times, increasing the concentration of pesticides to ensure the adaptation of bacteria to the conditions of the culture in the laboratory as well as the growth of those that used the pesticide as their only source of carbon. After the adaptation time, several bacterial consortiums have been obtained. They do not need an additional source of carbon, only the pesticide they were adapted.

At present, we have a collection of bacteria characterized by their identification, growth and degradation of organophosphate pesticides. The isolation and characterization of microorganisms that are able to degrade organophosphate pesticides give the possibility to count with new tools to restore polluted environments or to treat wastes before the final disposition.

Biodegradation of pesticides with isolated bacteria and analysis of the responsible genes.

Once we have the biological material, pesticides degradation tests are run to identify the strains with the best degrading characteristics. The responsible genes for the phosphotriesterases (PTEs) enzymes that degrade the organophosphate pesticides, for example the methyl parathion, do it through a hydrolysis reaction. They have been reported in the plasmid (*opd* gene*)* and in the chromosome (*mpd*, *opdA*, *hocA* genes) of different bacteria such as *Pseudomonas, Pleisemonas, Bacillus* and *Flavobacterium.* From a previously isolated consortium of agricultural soil in the state of Morelos, a bacterial strain was chosen based on the results of specific activity on the PM pesticide. This strain belongs to the genus *Enterobacter sp.*, it doesn't have plasmids and it has the size of a 4.87 Mb genome. The gene *mpc* (*methyl parathion catabolism*) was isolated. It is located in the *Enterobacter* chromosome, from the strain that codifies for a metal dependent hydrolase that can be responsible for the PM pesticide hydrolysis. It has been proved that this gene is responsible for the biodegradation, for the mutated strain does not have any activity on the PM, so we can confirm that the *mpc* gene is involved in the degradation or PM pesticide.

There are reports on other enzymes, such as esterases (carboxilesterases B) that are in the organisms as acarus and that have resistance to organophosphate pesticides. This research group is searching for these enzymes, to be able to express them in a heterologist way and to achieve better tools for the biodegradation of pesticides.

Development of proposals for massive pesticides degradation

We are also making efforts to build bioreactors with bacteria and/or immobilized yeast in order to have a strategy for the treatment of pesticide wastes. There are different materials for the immobilization of cells, but we have looked for some materials that might be economic and with different advantages to design a reactor that can be used on big scale.

Tezontle is a volcanic rock that is highly porous. It provides a great contact surface and it can be sterilized and reused. The presence of micropores allows the establishment of bacterial micro colonies. The immobilization method with this material is based on the colonization of the tezontle micropores through the formation of a biofilm

For the formation of the biofilm, tezontle rocks are crushed to obtain particles of about 3 mm. Tezontle is autoclaved in an intermittent way, at 121°C for 20 minutes, leaving it rest for 24 hours. Then, the microorganisms are planted to allow the formation of the 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.

Ecotoxicology studies

The ecotoxicology is a toxicology extension which was proposed by Truhaut in 1969 and it studies the adverse effects of toxic substances on ecosystems. This is by the analysis of the exposure routes, the entry to the organism and the harmful effects on individuals, populations and communities, their way of action, such as the prevention or combat of their harmful effects. A toxic substance is that one that after its penetration in the organism causes immediately or later on the suppression of any function of the organism or death.

Soil pollution by pesticides represents a worldwide scale problem, that's why it's necessary to deal with it with an Ecotoxicological point of view, this discipline represents an useful tool to study the destiny and effects of pesticides in the environmental compartments (soil, water, air) and it has the objective to explain the cause and anticipate possible risks of pollution of environmental compartments, and the toxic effects (mortality, immobility, growing and enzymatic inhibition, among others) that pesticides may cause. Different markers have been used (lethality, enzymatic activity ACE, loss of weight and behaviour) to prove the pesticides toxicity in laboratory conditions. For their study all the systems of

biological organization can be used, from molecules, tissues, organisms and communities, with the purpose of evaluate the pollutant effects. The annelids (oligochaeta) which include the earthworms are key species in the earth ecosystems, that's why they have been widely used as biomarkers in enzymatic studies (acetylcholinesterase) and in studies about the behaviour depending of the specific composition of the communities, the competition among species and the digging of galleries (Lavelle *et al.* 1997, Capowiez 2000).

In this working group, the earthworms have been used to measure the effects of pesticides when they are in the soil, on the earthworms, such as the effects of the products that are obtained after the pesticide degradation.

Pesticide degradation studies in agricultural soils

The study of pesticides behaviour in the soil is really interesting, because it is a heterogeneous, complex and dynamic system, in which different reactions (chemical and biochemical) take place and also work as receptors of polluting substances. Including ecotoxicologic studies and using tools with experimental microcosmos we have ventured to elucidate the pesticide fractions (percentage of the concentration of the pesticide applied to the soil) in different compartments (solid and liquid fraction) of the soil.

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