

# Bacteria and fungi in pesticide degradation: a review.

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**Abstract.** After the Second World War, the use of pesticides, substances used to combat pests that attack agricultural crops, became increasingly used because they helped increase productivity over the years. However, their intense use turned out to be dangerous and offensive, both to the environment and to mankind. In an attempt to put an end to areas that have been used pesticides in larger quantities than indicated, studies tend to the use of decomposing agents, such as bacteria and fungi, which have the enzymatic capacity of remediation, helping to remove or detoxify some pesticides. Among bacteria, the major bacterial genera include: *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Moraxalla*, *Acinetobacter*, *Arthrobacter*, *Paracoccus*, *Aerobacter*, *Alkaligens*, *Burkholderia* and *Sphingomonas*. Fungi include the genera *Fusarium*, *Aspergillus niger*, *Penicillium*, *Lentinulaedodes*, *Lecanicillium* and *Oxysporum*. Along with this, we can use the bioremediation in flooded crops that contributes effectively so that this production system does not contaminate soil and water resources. The studies on the use of microorganisms such as bacteria, fungi and acidimicrotots have been developed since the last century and currently has already been established the bioremediation of intoxicated areas with efficient pesticides and efficient molecules, such as: pesticides 2,4-D, parathion, carbofuran, phenol, benzene, toluene, xylene and others.

**Keywords.** Pesticides, bacterial, fungi, biodegradation, bioremediation.

## 1. Introduction

The vertiginous increase in world population and the blind quest for development have led man to seek more space and take more and more resources from nature. In recent decades, this population increase has generated a growing demand for food and caused impacts on the environment that are difficult to quantify. As one of the observable consequences, in agricultural production, the use of pesticides has become more and more frequent in the world.

Pesticides are organic chemical purposely presumably intended for increasing agricultural yield, soil productivity, products quality, minimizing losses of agricultural products caused by crop pest and to control the insect vectors for prevention of the outbreak of human and animal epidemics. Increased use of the pesticide and herbicides in agriculture has also been implemented for food storage. Recently, over 500 compounds are registered and used worldwide as pesticides or metabolites of pesticides. As per definition of ideal pesticide, a pesticide must be lethal to the targeted pests, but not to non-target species, including man but unfortunately, this is not so, hence the controversy of use and abuse of

pesticides has come into the light. However, due to their unplanned and indiscriminate use, only 10% of applied pesticides reach the target organism and the remaining high percentage of it is deposited on non target areas such as soil, water, sediments and causes serious environmental pollution. Similarly they also causes impacts onto non target organism such as wild life, besides affecting public health. There are now overwhelming evidence of some of these chemicals that cause unwanted side effects to the environment and do pose potential risk humans and other life forms. <sup>(1-3)</sup>.

The fate of pesticides in the environment is determined by both biotic and abiotic factors. Pesticides are degraded in the environment principally by the action of microorganisms, a process termed biodegradation, where iodegradation is defined as the breakdown of a substance to smaller products caused by microorganisms or their enzymes. <sup>4</sup>

To predict the fate of pesticides in soils it is important to have an understanding of those microbes able to degrade pesticides, their activities and the factors that limit their activity in situ. Microbial metabolism of pesticides has been reviewed extensively <sup>5</sup>.

This review aims to gather data on the efficiency of bacteria and fungi in the decomposition of pesticides based on results of recent research related to this topic for comparison of results.

## 2. Metodology

I conducted a literature search using online platforms to survey studies conducted with . I used Google Scholar, ISI Web of Knowledge, and Scopus databases using the keywords : "Pesticides", "bacteria", "fungi", "biodegradation", "bioremediation". in the title, abstract, keywords, and/or text. Since many studies in Brazil were published in Portuguese, we selected articles in both English and Portuguese.

## 3. Main Strategy For Pesticide Remediation

### 3.1 Bioremediation

Bioremediation is an innovative technology that is frequently being used for the clean-up of polluted sites. This technology is cost effective and becoming an increasingly attractive clean-up technology. The solid sludge, soil, and sediment as well as groundwater pollution can be treated by bioremediation. The rate of the natural microbial degradation of contaminants is enhanced by bioremediation. This enhancement is carried out by supplementing these microorganisms with nutrients, carbon sources or electron donors. The process can be carried out by using indigenous microorganisms or by adding an enriched culture of microorganisms. Microbes utilize their inherent specific characteristics to degrade desired contaminant at a quicker rate. The result of bioremediation is the complete mineralization of contaminants to H<sub>2</sub>O and CO<sub>2</sub> without the build-up of intermediates. For effective bioremediation, microorganisms must enzymatically attack the pollutants and convert them to less toxic products. An effective bioremediation can be achieved only where environmental conditions permit microbial growth and activity; its application often involves the manipulation of environmental conditions to allow microbial growth and degradation to proceed at a faster rate.<sup>(6,7)</sup> Bioremediation processes can be broadly classified into two categories, ex situ and in situ. The ex situ bioremediation technologies involves the use of bioreactors, biofilters, land farming and some composting methods whereas in situ includes biostimulation, bioventing, biosparging liquid delivery systems and some composting methods. The low cost and its effectiveness are the most positive parts of this technology.

## 4. Microorganisms in the degradation of pesticides.

Regarding the diversity of microorganisms in the

soil, about 160,000 species are known and described in the literature. Each year, an average of 1,700 and 120 new species of fungi and bacteria, respectively, are described in the literature. Estimates, considered by some as conservative, point to a total of around 1.8 million species.<sup>8</sup> However, perhaps less than 0.1 to 10% of microbial species, depending on the habitat studied, have been discovered and named so far.<sup>8</sup>

There are various sources of microorganisms having the ability to degrade pesticides. Generally, microorganisms that have been identified as pesticide degraders have been isolated from a wide variety of pesticide contaminated sites. The soil is the medium that mostly gets these chemicals, when they are applied to agricultural crops; additionally, the pesticide industry's effluent, sewage sludge, activated sludge, wastewater, natural waters, sediments, areas surrounding the manufacture of pesticides are also rich source of pesticide degrader. In different laboratories around the world, presently there are collections of microorganisms identified and characterized for their pesticides degradation ability. The isolation and characterization of pesticides degrading microorganisms that is able to give the possibility to count with new tools to restore polluted environments or to treat wastes before the final disposition. Upon complete biodegradation of the pesticide, the carbon dioxide and water are formed by the oxidation of the parent compound and this process provides the energy to the microbes for their metabolism. The intracellular or extracellular enzymes of the microbes play major role in the degradation of chemical compounds.

In several countries, the main genera of bacteria isolated from rice-growing areas are: *Arthrobacter*, *Bacillus*, *Clostridium*, *Flavobacterium*, *Micrococcus*, *Mycobacterium* and *Pseudomonas*.<sup>9</sup> In a typical eutrophic hydromorphic PLANOSSOLO cultivated with flood irrigated rice in the Lowlands Experimental Station (ETB) of Embrapa Clima Temperado, Mattos and Thomas (1996) identified a degrading bacterium of the herbicide clomazone: *Pseudomonas fluorescens* (Figure 1). Also at ETB, fungi isolated from samples of flooded rice straw collected from plots desiccated with glyphosate herbicide were identified as glyphosate degrading bacteria: *Nigrospora sphaerica*, *Cochliobolus heterostrophus*, *Fusarium anthophilum* and *Micelia sterilia* (Figure 2).<sup>10</sup>



Photo: Maria Laura Turino Mattos

**Figure 1.** *Pseudomonas fluorescens*, a degrading

bacterium of the herbicide clomazone.

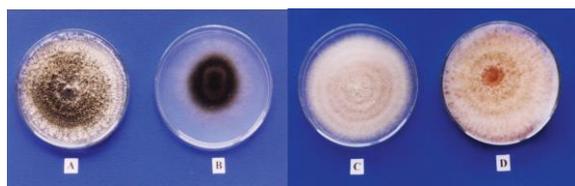


Photo: Maria Laura Turino Mattos

**Figure 2.** Plating of glyphosate herbicide degrading fungi: *Nigrospora sphaerica* (A), *Cochliobolus heterostrophus* (B), *Fusarium anthophilum* (C) and *Micelia sterilia* (D).

In upland conditions, bacteria and fungi are considered to be primarily responsible for the transformations of pesticides in soils. In flooded soils, fungi are involved but are probably the less important than microalgae which have a significant task.<sup>9</sup>

Microbial diversity, present in soils of irrigated rice fields, contributes effectively to this production system does not contaminate soil and water resources. In the rhizosphere of irrigated rice plants, there are microorganisms (bacteria, fungi and actinomycetes) that, through their exudates, form biofilms that act as a filter where pesticide residues are degraded. It has been found that, in the rhizosphere, microbiological degradative processes operate more rapidly than chemical ones. Thus, degradation is modelled in the root zone primarily as a biological process. In the subsoil zone, or on the soil surface, chemical degradation pathways are more important.<sup>11</sup>

When a comparison is made between the stability of pesticides in flooded and non-flooded soils, a greater persistence is observed in non-flooded soils than in flooded ones.<sup>9</sup> This behaviour is due to the relationship between soil redox potential (Eh) and pesticide degradation.

The review by KAUFMAN; KEARNEY (1970) lists 42 species of bacteria and fungi degrading simazine (6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine), atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] and ten other herbicides. Species from 16 genera of bacteria, two of actinomycetes and eight of fungi can be tabulated for their ability to degrade 20 of the most commonly used herbicides.<sup>12</sup> (Tab 1).

**Tab. 1 -** Pesticide-degrading microorganisms isolated from rice fields.

microorganisms	Pesticide
<i>Achromobacter</i> spp.	MCPA - 2,4,D - 2,4,5,T -
<i>Agrobacterium</i> sp.	Dalapon - TCA
<i>Alcaligenes</i> sp.	Dalapon
<i>Arthrobacter globiformis</i>	2,4,D
<i>Arthrobacter</i> spp.	MCPA-2,4D-Dalapon-

<i>Azotobacter</i> sp.	TCA- Endothall
<i>Brevibacterium</i> sp.	Allyl Alcohol
<i>Crynebacterium</i> sp.	2,4,5,T
<i>Flavobacterium</i> spp.	MCPA - 2,4,D - PCP
<i>Micrococcus</i> sp.	MCPA - 2,4,D - Dalapon
<i>Mycoplana</i> sp.	Dalapon
<i>Pseudomonas putida</i>	MCPA - 2,4,D -2,4,5,T
<i>P. dehalogenans</i>	Allyl Alcohol
<i>P. cruciviae</i>	Dalapon - TCA -
<i>Pseudomonas</i> spp	2,4,D - 2,4,5,T - PCP
<i>Sporocytophaga congregata</i>	MCPA-2,4,D-Dalapon-TCA- PCP
	2,4,D
<i>Nocardia</i> spp.	2,4D-MCPB-Dapalon-
<i>Streptomyces</i> spp.	TCA- Allyl Alcohol 2,4,D - Dalapon
<i>Aspergillusniger</i>	MCPA - 2,4,D - MCPB
<i>Aspergillus</i> sp.	Dalapon PCP
<i>Cephaloasca fragrans</i>	Dichlobenil Dichlobenil
<i>Fusarium</i> sp.	Dalapon - PCP-
<i>Geotrichum</i> sp.	Dichlobenil Dalapon -
<i>Penicillium</i> spp.	TCA
<i>Trichoderma viride</i>	PCP - Dichlobenil - Allyl
<i>Trichoderma</i> spp.	Alcohol

Fonte: Brown (1978)

The pesticides 2,4-D, paraethion and carbofuran have been extensively studied with respect to degradation by microorganisms. Degrading bacterial accessions of these pesticides have been isolated, and the responsible genes have been cloned and sequenced. The most studied degrading accessions for 2,4-D are *Alcaligenes eutrophus* JMP134; for paration, they are *Flavobacterium* sp. ATCC 27551 and *Pseudomonas diminuta* MG, and, for carbofuran, *Pseudomonas* spp., *Bacillus* sp., *Arthrobacter* spp., *Micrococcus* sp., *Azospirillum lipoferum* and *Streptomyces* spp.<sup>13</sup> The author highlights that *Flavobacterium* sp. access MS2d, isolated from soil, also exhibited ability to degrade the insecticide carbofuran.

Bacteria of the genus *Pseudomonas* have the ability to utilise a large number of complex and rare organic compounds as a source of carbon and energy.

Moreover, *Pseudomonas* are capable of developing new metabolic activities in response to changes in environmental conditions.<sup>14</sup> As an example, carbofuran degrading *Pseudomonas* species accumulate polyhydroxybutyrate (PHB) from the use of sucrose as a substrate.<sup>15</sup>

The degradation of clomazone herbicide by a species of *Pseudomonas* may initially indicate that other components of the microbial population may also degrade this herbicide. This approach highlights the importance of maintaining microbial diversity in soils, as a way to preserve this natural resource, allowing the exploration of an economically and environmentally sustainable agriculture.

Some isolated bacteria capable of degrading atrazine have been classified as facultative anaerobes<sup>16</sup> that can reduce nitrate. The degradation rate of atrazine is slower under low oxygen conditions than under aerobic conditions in estuarine and wetland sediments.<sup>17</sup>

Regarding the spectrum of microorganisms, it is not an exaggeration to state that most xenobiotics can be metabolized - although, in different degrees - under appropriate conditions as long as, naturally, the compound is not lethally toxic. However, normally toxic compounds such as carbon monoxide, cyanide, toluene and fluoroacetate can be metabolised by bacteria.<sup>18</sup>

Fungi stand out in the biological treatment of oil refinery effluents due to their enzymatic capacity to degrade long-chain polycyclic aromatic compounds into compounds that can be assimilated by their metabolism. The phenolic compounds present in these waters are potentially toxic to some aquatic species and may also alter, even at low concentrations, the taste and odour in the chlorination treatment process of water supplies.<sup>19</sup> The great plasticity of this group allows them to survive and grow in conditions practically impossible for other beings seem capable of utilising recalcitrant compounds using them as an energy source.<sup>(20-22)</sup> In these conditions,<sup>(20-22)</sup> suggested that for an intensification of the fungal activation, glucose should be added as primary substrate. However, Santella et al., (2009), pointed out that it should only be added for the start-up of the reactors, facilitating the initial fungal growth.<sup>29</sup> ,added that to further optimise removal efficiency is the use of immobilised cells in the course of the process.

The filamentous fungi were presented by<sup>30</sup> as more effective in the production of extracellular oxidative enzymes (ligninases, proteases, cellulases, among others). The *A. niger*, working in an optimum range of pH between 3.0 and 4.0<sup>23</sup> stood out among the filamentous fungi for the proven capacity of degradation of recalcitrant compounds of diverse industries (breweries, cashew nuts, pharmaceutical, petroleum refineries, degradation of paration in water.<sup>(24-27)</sup>

The *A. niger* species has a great performance in the removal of COD, as well as in the degradation of recalcitrant composts such as atrazine, methyl paration

as well as other organophosphorus pesticides.<sup>(24-26,27)</sup> Studying four batch reactors with the objective of evaluating the potential of degradation of atrazine in a mixed culture of anaerobic microorganisms,<sup>31</sup> observed that the best result occurred in the reactor where atrazine was employed as the only source of carbon and nitrogen, with a percentage of mineralization of 43 to 45% of the pesticide with a DTH of 5 days.

## 5. Conclusion

Pesticides are compounds widely used in agriculture, and they are increasingly developed and specific in order to combat weeds, pests and plant diseases present in major crops. However, their continuous and increasing use may cause impact on the environment, both in soil and water regions. These natural resources are being compromised by the intense disposal of these pesticides and their misuse. Aiming to recover areas that have been contaminated, several research are being developed in an attempt to use decomposing agents, such as fungi and bacteria, in remediation. Bioremediation has proved to be a valid process, as it has the potential to reduce certain compounds that are contaminants.

The bacteria have a greater number of species for the decomposition of simple pesticides and the fungi, even if in smaller quantities, are extremely important because they have the ability to degrade contaminating compounds of large chains and broad spectrum of compounds.

Thus, it is valid the indication of bioremediation using bacteria and fungi for the detoxification of areas with higher number of deposited pesticides.

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## 7. References

- [1] Jeyaratnam J (1985). Health Problems of Pesticide Usage in The Third World. Br. J. Ind. Med. 42:505-506.
- [2] Igbedioh SO (1991). Effects Of Agricultural Pesticides on Humans, Animals, And Higher Plants in Developing Countries. Archiv. Environ. Health 46:218-224.
- [3] Forget G (1993). Balancing The Need for Pesticides with The Risk to Human Health. In: Impact of Pesticide Use on Health in Developing Countries by Eds. G. Forget G, Goodman T, De Villiers A. Pp. 2-16.
- [4] Atlas, R.M. (1988). 'Microbiology. Fundamentals And Applications.' 2nd Edn. (Macmillan: New

York.)

- [5] Hill, I. R., And Wright, S. J. L. (1978). 'Pesticide Microbiology.' (Academic Press: London.) Holben, W. E., Schroeter, B. M., Calabrece, V. G. M., Olsen, R. H., Kukor, J. J., Biederbeck, V. O., Smith, A. E., And Teidje, J. M. (1992). Gene Probe Analysis of Soil Microbial Populations Selected by Amendment With 2,4-Dichlorophenoxyacetic Acid. *Applied And Environmental Microbiology* 58, 3941-8.
- [6] Lacey LA, Goettel MS (1995). Current Developments in Microbial Control of Insect Pests and Prospects for The Early 21st Century. *Entomophaga*. 40:3-27.
- [7] Vidali M (2001). Bioremediation. An Overview. *Pure Appl. Chem.* 73(7):1163-1172.
- [8] Coutinho, H. L. C.; Oliveira, V. M.; Manfio, G. P. Diversidade Microbiana Em Amostras Ambientais. In: Garay, I. E. G.; Dias, B. F. S. Conservação Da Biodiversidade Em Ecossistemas Tropicais: Avanços Concentuais E Revisão De Novas Metodologias De Avaliação E Monitoramento. Petrópolis: Editora Vozes, 2001. 430 P.
- [9] Pingali, P. L.; Roger, P. A. Ed. Impact of Pesticides on Farmer Health and The Rice Environmental, Philippines: Kluwer Academic Publishers, 1995. 664 P.
- [10] Mattos, M. L. T.; Machado, M. I.; Santos, F. O.; Martins, F. S.; Santos, S. C. A. Microrganismos Do Solo Envolvidos Na Degradação Dos Herbicidas Clomazone E Glifosate, Em Lavouras De Arroz Irrigado, No Rio Grande Do Sul. In: Workshop Sobre Biodegradação, 2., 2001, Campinas. Resumos... Campinas: Embrapa-Cnpma, 2001. P. 361-364.
- [11] Torstensson, L. Role of Microorganisms in Decomposition. In: Hance, R.J., Ed. Interactions Between Herbicides and The Soil. London: Academic Press, 1980. 349 P.
- [12] Brown, A. W. A. Ecology Of Pesticides, New York : J. Wiley, 1978. 525 P.
- [13] Head, I. M.; Cain, R. B.; Suett, D. L. Molecular Aspects of Enhanced Microbial Degradation of Pesticides. In: Brighton Crop Protection Conference-Pests and Diseases, 3., 1990, Proceedings. 1990. P. 907-916.
- [14] Barbieri, S. M. Regulation and Expression of Degradative Plasmids in Pseudomonas. *Ciência E Cultura, São Paulo*, V. 42, N. 5/6, P. 317-324, 1990.
- [15] Crochemore, A. G.; Mattos, M. L. T.; Vendruscolo, C. T.; Castro, L. A. S. De; Moreira, A. S. Identification of Pesticide-Degrading Pseudomonas Strains as Poly- B-Hydroxybutyrate Producers. *African Journal of Biotechnology*, V. 11, N. 85, P. 15144-15149. 2012.
- [16] Jessee, J. A.; Benoit, R. E.; Hendricks, A. C.; Allen, G. C.; Neal, J. L. Anaerobic Degradation of Cyanuric Acid, Cysteine, And Atrazine by A Facultative Anaerobic Bacterium. *Applied Environmental Microbiology*, Washington, V. 45, P. 97-102, 1983.
- [17] Papiernik, S. K.; Spalding, R. F. Atrazine, Deethylatrazine, And Deisopropylatrazine Persistence Measured in Groundwater in Situ Under Low-Oxygen Conditions. *Journal Of Agricultural and Food Chemistry*, Easton, V. 46, P. 749-754, 1998.
- [18] Neilson, A. H. Organic Chemicals in The Aquatic Environment: Distribution, Persistence, And Toxicity. Boca Raton: Lewis Publishers, 1994. 438 P.
- [19] Araña, J. Et Al. Highly Concentrated Phenolic Wastewater Treatment by The Photo-Fenton Reaction, Mechanism Study By FTIR-ATR. *Chemosphere*. V. 44, P.1017-1023. 2001.
- [20] Eggen, T.; Majcherczyk, A. Removal of Polycyclic Aromatic Hydrocarbons (PAH) In Contaminated Soil by White Rot Fungus *Pleurotus Ostreatus*. *International Biodeterioration and Biodegradation*. V. 41, N. 2, P. 111-117. 1998.
- [21] Esposito, E.; Azevedo, J. L. De. Fungos: Uma Introdução À Biologia, Bioquímica E Biotecnologia. 2èed. Revisada. Caxias Do Sul: Educs, 2010.
- [22] Santos, V.L; Linardi, V.R. Biodegradation of Phenol by A Filamentous Fungi Isolated from Industrial Effluents - Identification and Degradation Potencial. *Process Biochemistry*, V. 39, P. 1001-1006. 2004.
- [23] GRIFFIN, D. Fungal Physiology. New York: Wiley Liss. 1994.
- [24] Garcia, I. G. Et Al. Removal of Phenol Compounds from Olive Mill Wastewater Using *Phanerochaete Chrysosporium*, *Aspergillus Niger*, *Aspergillus Terreus* and *Geotrichum Candidum*. *Process Biochemistry*, V. 35, P. 751-758. 2000.
- [25] Hernández, M.S. Et Al. Amylase Production by *Aspergillus Niger* in Submerged Cultivation on Two Wastes from Food Industries. *Journal Of Food Engineering*, V. 73, N. 1, P. 93-100. 2006.
- [26] Miranda, M.P. Et Al. Color Elimination from Molasses Wastewater by *Aspergillus Niger*. *Ioresource Technology*, V. 57, N. 3, P. 229-235. 1996.
- [27] Vassilev, N. Et Al. Olive Mill Waste Water Treatment By Immobilized Cells Of *Aspergillus Niger* And Its Enrichment With Soluble Phosphate. *Process Biochemistry*, V. 32, N. 7, P. 617-620. 1997.
- [28] Santos, E.M.A Et Al. Influência Do Tempo De Detenção Hidráulica Em Um Sistema UASB Seguido De Um Reator Biológico Com Fungos Para Tratar Efluentes De Indústria De Castanha De Caju. *Revista Engenharia Sanitária E Ambiental*, V. 11, N. 1, P. 39-45, Jan./Mar. 2006.

- [29] Godjevargova, T. et al. Biodegradation of toxic organic components from industrial phenol production waste water by free and immobilized *Trichosporon cutaneum* R57. *Process Biochemistry*, v. 38, n. 6, p. 915-920. 2003.
- [30] Eggen, T.; Majcherczyk, A. Removal of polycyclic aromatic hydrocarbons (PAH) in contaminated soil by white rot fungus *Pleurotus ostreatus*. *International Biodeterioration and Biodegradation*. v. 41, n. 2, p. 111-117. 1998.
- [31] Ghosh, P. K.; Philip, L. Atrazine degradation in anaerobic environment by a mixed microbial consortium. *Water Research*, V. 38, pp. 2277-2284. 2004.