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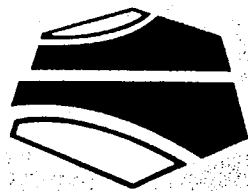
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**Decontamination of polluted soils with white-rot fungi : aromatic hydrocarbons  
and chloride compounds**

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Organic molecules originating from human activity are generally considered as biodegradable if their structures are close to the natural ones. The need for more efficient products has conducted chemical industry to produce molecules with structures unknown in nature called « xenobiotics ». Among xenobiotics released in the natural environment, aromatic compounds are almost the most persistent.

Some fungal species called « white-rot » use non-specific extracellular enzymes to degrade aromatics. These fungi grow on wood and can degrade lignin, an aromatic polymer that gives rigidity to wood fibres. Lignin is a tridimensional polymer constituted from phenylpropan units bounded by ether or carbon-carbon bonds. Despite the natural origin of lignin, its reticulated structure, its heterogeneity and its hydrophobic properties give it a great resistance to biodegradation.

The non-specific degrading mechanism of lignin by white-rot fungi depends on the synthesis of lignolytic enzymes, produced by the fungus in response to low levels of carbon, nitrogen, or other nutrients. These enzymes can also degrade monoaromatic compounds and therefore white-rot fungi are of a great interest for xenobiotic biodegradation.

Lignolytic enzymes produced by white-rot are mainly peroxidases and oxygenases. Two peroxidases are generally produced, a lignin peroxidase and a manganese dependent peroxidase. Both are hemoproteins containing a protoporphyrin IX associated to an iron atom. Oxidases produced are mainly laccases, blue-copper enzymes. They are involved in lignin depolymerisation by the oxidation of phenolic compounds. However, oxidation of non-phenolic aromatic compounds can occur in the presence of a chemical mediator such as ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate)) or HBT (1-hydroxybenzotriazole) as demonstrated by Bourbonnais *et al.* (1).

*Phanerochaete chrysosporium*, the most widely studied basidiomycete, produces only peroxidases, while *Trametes versicolor* et *Phlebia radiata* also produce laccases. *Pycnoporus cinnabarinus*, another white-rot strain, produces only laccases which resemble closely to *Trametes versicolor* laccases.

MnP production in bioreactors has been studied for several years at INRA-Marseille. The use of the mutant strain *Phanerochaete chrysosporium* I-1512 and the design of new types of immobilised bioreactors should allow large scale production of these enzymes (2).

Laccase production in classical mechanically stirred bioreactors using a monokaryotic strain of *Pycnoporus cinnabarinus*, by producing large quantities of enzymes (3), allows the *in-vitro* study of the biodegradation mechanisms. These studies have shown that lignolytic enzymes are active *in-vitro* on a number of aromatic compounds such as polycyclic aromatic hydrocarbons (PAH), chlorophenols, PCBs and dioxins.

For example, laccases of *Pycnoporus cinnabarinus* degraded benzo(a)pyrene in the presence of ABTS at 30°C after some hours of incubation in liquid medium in an enzymatic aerated and agitated bioreactor (4). Metabolites, identified by mass spectrometry and U.V. spectrophotometry, corresponded to benzo(a)pyrene 1,6-, 3,6- and 6,12-quinones.

In all cases, complete mineralisation cannot occur because the presence of the living fungus is essential to carry out the large number of biochemical reactions needed for the full depollution.

Mineralisation experiments with pentachlorophenol (PCP) performed at laboratory scale with *Phanerochaete chrysosporium* I-1512 showed that the mode of culture was an essential parameter. Results clearly established the advantage of an immobilised culture for the mineralisation of PCP: 23% of total carbon was mineralised by the fungus immobilised on stainless steel mesh rings as compared with 11% for static cultures. Pentachloroanisole

(PCA), a metabolite of PCP, was formed only in static cultures and underwent a limited mineralisation.

The use of white-rot fungi for soil depollution appears advantageous for several reasons :

- the lignolytic system is extracellular, the fungus can degrade poorly soluble products (poor biodisponibility of the pollutant),
- the induction of lignolytic enzyme depends only on the presence of a limiting factor, the fungus activity is independent of the pollutant concentration or composition,
- many xenobiotics are toxic to the micro-organisms; the excretion of lignolytic enzymes that modifies the pollutant outside the cell allows the fungus to tolerate high level of toxic products. For example, PCP is very toxic to the spores of *P. chrysosporium* while three days old cultures tolerate and degrade high concentration of this product (5).
- when growing in soil, white-rot fungi develop only on lignocellulosic support and can colonise the soil by this way without competition with the endogenous microflora. This ability can be used to produce inocula for polluted soils. This technique, called « bioaugmentation », is based on the selection of the better degrading strains and their massive inoculation in soils. In a first step, the fungus is produce in liquid cultures in bioreactors. Then, the crushed mycelium is added to granulated wheat-bran. This innoculum, after three days of incubation, is mixed to the soil to be treated to form a biopile. The colonisation of a soil by several fungus was followed by ergosterol titration . In all cases, the presence of such a carrier provided better results (6).

Some problems regarding the use of fungi in polluted soil are not yet resolved and restrain the development of such techniques. Fungi found in small quantities in polluted soils are not the most useful strains for the *in-situ* biodegradation, and biostimulation techniques are frequently of poor efficacy. It is then necessary to screen, for each type of pollutant, the best adapted strains able to degrade it in a representative sample of polluted soil.

Enzymatic activity and biomass measurement show that fungal strain cultivated in solid medium often use metabolic pathways different from that of the same strains growing in liquid medium. As a consequence, it is very important to reproduce in the laboratory, soil conditions while alleviating the analytical difficulties due to the complexity of the matrix.

A screening system was developed by replacing soil by inert sand moistened by an adapted nutritive medium (7). The screening of fungal strains, based on their ability to degrade model molecules, phenanthrene and benzo(a)pyrene, allowed to select two strains, *Trametes versicolor* and *Cunninghamella elegans*, showing a great potential activity versus PAH. The activity of these two strain was followed by using <sup>14</sup>C radiolabelled molecules. The first one produces high quantities of hydrosoluble metabolites when the other produces volatile metabolites.

Polluted soils are very heterogeneous and the bioavailability of the pollutant varies markedly with each situation. The feasibility of bioremediation by fungus or other micro-organisms needs always a complete study, taking into account the local conditions. In many cases, biodegradation is not achieved by a single organism but is due to the combined activities of fungal and bacterial stains. However, the use of white-rot fungus seems very promising owing to the ability of lignolytic enzyme to carry out the first steps of the degradation of aromatic compounds.

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