Biodegradation of Halogenated Organic Pollutants by White Rot Fungi

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White rot fungi have been shown to be capable of mineralizing a wide variety of halogenated environmental pollutants (1-4). The list of halogenated chemicals which can be mineralized by the fungi include both aliphatic (i.e., carbon tetrachloride, Lindane and toxaphene) and aromatic chemicals (i.e., PCB, DDT, pentachlorophenol, 2,4,5-trichlorophenoxyacetic acid, dioxin, etc.).

The white rot fungi use a variety of mechanisms to accomplish the mineralization of such a wide variety of chemicals. Some chemicals are already so highly oxidized by the electron withdrawing halides that they require reduction before they can be oxidized. Other highly oxidized chemicals, such as the nitro explosives, are also mineralized by the fungi (5). These chemicals also require reduction before they can be oxidized.

The white rot fungi primarily use extracellular processes to degrade chemicals. These processes evolved to degrade lignin, an insoluble, high molecular weight polymer that gives physical strength to woody plants (6). Lignin varies between plants and there are many different bonds of even different stereochemistry configurations. A number of non-specific extracellular mechanisms evolved for these fungi to degrade lignin. They obtain little or no energy from the degradation of lignin. Their energy comes from cellulose and some cellulose must be used to generate the energy required to degrade lignin to gain access to more cellulose. Since lignin is also a highly oxidized polymer, it is obvious that the fungi would need reductive mechanisms for the complete degradation of lignin.

The extracellular oxidative power comes from hydrogen peroxide and a series of peroxidases (7,8). The peroxidases are oxidized by the hydrogen peroxide and the oxidized enzymes are reduced back to what is termed resting state by the chemicals that are being oxidized. The classical example is with veratryl alcohol, the chemical that is frequently used to assay the oxidative activity of the major peroxidases secreted by the fungi. The highly oxidized enzymes are reduced, one electron at a time, by veratryl alcohol, producing the veratryl alcohol cation radical and then veratryl aldehyde.

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Interestingly, the white rot fungi synthesize and secrete veratryl alcohol (9). The question arises as to why the fungi would synthesize a chemical which is a substrate for enzymes which are presumably secreted to oxidize other chemicals, such as lignin. Another interesting observation is that the fungi seem to require veratryl alcohol for the oxidation of lignin (10). We have also shown that veratryl alcohol is required for the metabolism of aminotriazole (11). Another very interesting observation was that the fungi also secrete a chemical which appears at first analysis to be an inhibitor of veratryl alcohol oxidation (12). Thus the fungus appears to produce a substrate for the lignin degrading enzymes and also an inhibitor of the veratryl alcohol oxidation. At first glance this seems very confusing. The inhibitor turned out to be oxalic acid and it inhibits veratryl alcohol oxidation by reducing the veratryl alcohol cation radical (formed by the enzyme catalyzed, one-electron oxidation of veratryl alcohol) back to the alcohol (13). This produces the oxalate radical. Thus a cationic radical (veratryl alcohol cation radical) has been converted to an anionic radical (the oxalate anion radical). This is an example of cooxidation. The mediator is veratryl alcohol. Iodide can also serve as a mediator (14). For the manganese-dependent peroxidases secreted by the fungi, manganese is the mediator (15). Oxidized manganese can oxidize a number of chemicals. The reduced manganese is oxidized again by the peroxidase to complete the cycle.

In the case of the lignin peroxidases we are now left with the anionic oxalate radical. This is an excellent reductant. It can, for example, cause the reductive dechlorination of carbon tetrachloride giving the trichloromethyl radical (16). The oxalate radical can also reduce molecular oxygen to superoxide (12), which is known to dechlorinate a variety of environmental pollutants such as PCB in aprotic situations (17). Alternatively, in the presence of iron, superoxide is known for its ability to generate the hydroxyl radical by the Haber-Weiss series of reactions. The hydroxyl radical is a powerful oxidant, capable of oxidizing almost any organic molecule. Previous investigators had provided evidence for its involvement in the oxidation of lignin (18). It is thought to play a major role in the oxidation of environmental pollutants in the atmosphere.

The manganese-dependent peroxidases appear to use quinones as a source of reducing power to also catalyze reductions (19). Quinones and quinone reductases are produced extracellularly by the fungi (20). The oxidation of hydroquinones to semiquinones results in the production of an excellent reductant. Thus both forms of peroxidases secreted by the fungi have mechanisms for oxidation, cooxidation, or reduction. All of the enzymes, mediators and electron donors are secreted by the fungi.

A plasma membrane redox potential can also be used by the fungi to reduce a number of chemicals. This system has been studied mostly for the metabolism (21) and detoxification of TNT (22) and other nitro explosives. This would allow metabolism of chemicals that would normally be very toxic.

Another mechanism to accomplish reductions is by methylation of phenols. This mechanism is used to reduce pentachlorophenol (to pentachloroanisole) (23). Pentachloroanisole is then much easier to oxidize by the peroxidases.

These results would suggest that several factors are important to consider when applying this technology for the biodegradation of environmental pollutants. First, the fungi should be able to metabolize a wide variety of chemicals, starting with either oxidation or reduction. The reaction could be used in repeated sequences to accomplish complete oxidation (mineralization) of chemicals. Secondly, mineralization will require the fungi to be in their "normal" state of metabolism for the degradation of lignin. It will be almost impossible to get degradation of chemical with pure enzyme or enzyme systems. Many factors other than just the enzymes will be important. Conditions or fungi that give greater enzyme activities may not be superior in terms of mineralization of a chemical. Also, disappearance of chemicals may occur under conditions where the peroxidases are not present. However, complete oxidation may not occur under these conditions. This would require the complete, ligninolytic system.

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