

Fungal diversity and its potential in environmental clean up

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Abstract:

Biodegradation is the general term used for all biologically mediated break down of chemical compounds and complete biodegradation leads to mineralization. On the other hand, bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. The fungi are unique among microorganisms in that they secrete a variety of extracellular enzymes. The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them in bioremediation. Fungi are good in the accumulation of heavy metals and the role of fungi in the degradation of complex carbon compounds such as starch, cellulose, pectin, lignin, lignocellulose, inulin, xylan, araban etc., is well known. The white rot fungi are unique among eukaryotes for having evolved nonspecific methods for the degradation of lignin. They do not use lignin as a carbon source and is, therefore, essentially a secondary metabolic process, not required for the main growth process. A wide variety of fungi has been shown to degrade a number of toxic xenobiotics such as aromatic hydrocarbons, chlorinated organic, nitrogen aromatics and several miscellaneous compounds such as sulfonatedazo dyes etc. Several enzymes that are released such as laccases, polyphenol oxidases, lignin peroxidases etc. play a role in the degradative process. In addition, a variety of intracellular enzymes such as reductases, methyl transferases and cytochrome oxygenases are known to play a role in xenobiotic degradation.

Keywords:

Biodegradation; bioremediation; fungi; extracellular enzyme; heavy metals; xenobiotics; intracellular enzyme etc

Introduction

In nature, fungi do much of the dirty work. Fungi have an astonishing potential to clean up contaminated environment. Fungi are the decomposers in the global cycle of life and death. They are particularly efficient at degrading the major plant polymers, cellulose and lignin, but they also decompose a huge array of other organic molecules including waxes, rubbers, feathers, insect cuticle and animal flesh. Paradoxically despite their notoriety, the use of fungi in bioremediation and biodegradation has been limited compared to that of bacteria.

Bioremediation and biodegradation are not terms always used with appropriate sensitivity to their subtle differences in meaning. Bioremediation is the use of any organism's metabolism to remove pollutants. Microorganisms used to perform the function of bioremediation are known as bioremediators. Mycoremediation is the form of bioremediation in which fungi are used to decontaminate the area. The term mycoremediation refers specifically to the use of fungal mycelia in bioremediation. Stimulating microbial and enzymatic activities mycelium reduces toxins in-situ. Some fungi are hyperaccumulators, capable of absorbing and concentrating heavy metals in the mushroom fruit bodies.

Biodegradation is the biologically mediated breakdown of chemical compounds. When biodegradation is complete the process is called mineralization i.e. total breakdown of organic molecules into water, CO₂ and/or other inorganic end products.

One of the primary roles of fungi in ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant. Virtually all natural organic compounds can be degraded by one or more fungal species for the production of a variety of enzymes such as amylases, lipases and proteases.

There are many factors that affect the rate and ability of fungi to break down pollutants. Temperature controls the rate at which contaminants are broken down. Low temperature slows the process and warm temperature speed it up. Fungi prefer slightly acidic p H for biodegradation and bioremediation. Oxygen is also essential for fungal metabolism. The initial step of hydrocarbon degradation involves adding oxygen to the hydrocarbon. So, lack of oxygen in the environment slows the process. Fungal colonization is also affected by soil texture, p H and presence of inhibitory compounds. In one conducted experiment, a plot of soil contaminated with diesel oil was inoculated with mycelia of oyster mushroom. After 4 weeks, more than 95% of many of the PAHs (polycyclic aromatic hydrocarbons) had been reduced to non-toxic components in the mycelia-inoculated plots. It appears that natural microbial community participates with the fungi to break down contaminants, eventually into carbon dioxide and water. Wood-degrading fungi are particularly

effective in breaking down aromatic pollutants (toxic components of petroleum), as well as chlorinated compounds. Two species of Eucadorian fungus *Pestalotiopsis* are capable of consuming polyurethane in aerobic and anaerobic conditions, such as found at bottom of landfills. Mycofiltration is a similar process, using fungal mycelia to filter toxic wastes and microorganisms from water in soil.

Many environments are characterized by low or elevated temperatures, acidic or alkaline pH, high salt concentrations, or high pressure. Extremophilic microorganisms are adapted to grow and thrive under these adverse conditions. Hydrocarbon degrading extremophiles are thus ideal candidates for the biological treatment of polluted extreme habitats. In this review we summarize the recent developments, obtained both in laboratory and field studies, in fungal biodegradation and bioremediation of contaminants that are of environmental concern in extreme habitats.

Mechanism of fungal degradation:

Fungi share a unique nutritional strategy, i.e their cells secrete extracellular enzymes which break down potential food sources, which are then absorbed back into the fungal colony. Any discussion on fungal biodegradation must cover an extraordinary amount of catalytic activity. The decomposition of ligninocellulose is probably the single most important degradative event in earth's carbon cycle. The utilization and transformation of dead remains of other organism is essential to the earth's economy. An enormous ecological literature exists on the role of fungi as primary and secondary decomposers in these classic cycles of nature.

From human perspective the power of fungal enzymes is Janus-faced. Molds destroy more foods than any other microorganisms. They destroy standing

timbers, finished wood products, fibers and a wide range of noncellulosic products such as plastics, fuels, paints, glues, drugs and other human artifacts. On the hand, many of the old biotechnological practices are also based on fungal catalytic power: baking, brewing, wine fermentation, production of certain cheeses and the koji process are ancient examples of the way humans have employed fungi for their own benefit. In the 20th century, numerous fungal hydrolytic enzymes involved in the degradation of relatively simple biopolymers such as starch and proteins which were utilized within the industrial settings. These include fungal amylases, glucoamylases, lipases, pectinases and proteases. Fungal cellulases provide a good example of the contrasting faces of a single enzymatic capability. During World

War II, research by the US army on the microbial destruction of military clothing and tents led to the characterization of the cellulolytic mold *Trichoderma reesei*. Continuing research on *T. reesei* identified a complete set of cellulase enzymes required for breakdown of cellulose to glucose. These enzymes now promise the potential of converting waste cellulose into foods for our burgeoning population and have been the subject of intense molecular biology research. Although cellulose produced glucose is not yet economically competitive, another traditional fungal process is mushroom cultivation. These and some other examples of economically advantageous uses of fungal degradation are displayed in the following table:

Process	Substrate	Species
Composting	Straw, manure, agricultural waste, bark	Consortia of fungi, usually uncharacterized
Mushroom cultivation	Lignocellulose, animal manure, sawdust, wood logs	<i>Agaricus bisporus</i> , <i>Pleurotus ostreatus</i> , <i>Leptinus odoides</i>
Single cell protein production	Alkanes, brewery wastes, molasses, sulphate waste liquid	Yeasts e.g. <i>Candida tropicalis</i> , <i>C. utilis</i> , <i>Saccharomyces carlsbergensis</i> , <i>Paecilomyces varioti</i>
Solid waste treatment	Sludge/sweage pulp and paper mill effluents	<i>Coriolus versicolor</i> , <i>Phanerochaete chrysosporium</i>
Waste water treatment	Distillery waste, Kraft bleaching effluent, Tannery effluent	Yeasts especially <i>Candida utilis</i> , <i>Aspergillus</i> , <i>Penicillium</i> .

Fungi are also good at bioaccumulation of heavy metals. Many species can absorb cadmium, lead, copper, mercury and zinc into their mycelium and spores. Sometimes the walls of dead fungi bind better than living ones. Systems using *Rhizopus arrhizus* have been developed for treating uranium and thorium. Spent fungal biomass from industrial fermentations is an available resource for the concentration of heavy metal contamination.

Fungi play several ecological roles in rhizosphere such as symbiotic, pathogenic and saprotrophic ones. In particular, the last one is strongly related to the bioremediation potentiality of rhizosphere fungi. Among pioneer saprotrophic fungi, there are several species of *Mucor*, *Rhizopus* and high sporulating mitosporic fungi (deuteromycetes) and species of zygomycetes are characterized by their ability to decrease the concentration of organic pollutants. They accumulate toxic compounds in intracellular lipid vesicles and

these vesicles have roles in biodegradation too. Species belonging to the genera *Trichoderma*, *Fusarium*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Mortierella*, *Beauveria*, and *Engyodontium* are some examples of the fungi that have recently been described as tolerant to a variety of pollutants and indicated as potential bioremediation agents in soil.

To date, the most sophisticated fungal approaches to environmental clean-up have grown out of prior research on degradation of petroleum hydrocarbons and on the adaptation of research on fungal treatment of lignocellulosic wastes in the pulp and paper industry. The ability to grow on petroleum hydrocarbons is widely spread among the fungi. Jet crashes caused by blocked fuel lines due to growth of *Cladosporium resinae*, first reported during World War II, are among the most dramatic negative consequences of the ability of fungi to thrive in extreme habitats. Considerable information is available about the mycological flora associated with marine petroleum spills. On the industrial side, the years of research on single cell protein, instituted with the goal of turning petroleum hydrocarbons into feed, have paid off in the study of the enzymatic mechanisms used by yeasts and other microorganisms in the biodegradation of petroleum wastes for environmental remediation. Cytochrome P-450s are mixed function oxidases (monooxygenases), derived from a superfamily of genes, which are involved in many steps of petroleum degradation and biotransformation of a variety of environmental pollutants. Both detoxification and activation are associated with the action of cytochrome P-450. Fungal monooxygenases are more similar to mammalian than to bacterial counterparts. Sarialani has presented a particular review of these enzymes, and Kellner et al. have discussed their use in bioremediation. Extensive biochemical and genetic data are

available for several yeasts. There is also a large literature surrounding the aseptate filamentous species *Cunninghamella elegans*. Similarly and even to a greater extent, research on pulp waste treatment, such as decolorization of effluent from kraft pulp mills, and the subsequent mushrooming of research on white rot fungi have shown the power of wood-decaying species against a surprisingly large battery of environmental contaminants. Recent advances in the use of fungi in environmental remediation and biotechnology have been summarized by Paszczyński and Crawford.

Among other fungi used in bioremediation, the yeasts, e.g., *Candida tropicalis*, *Saccharomyces cerevisiae*, *S. carlbergensis* and *Candida utilis* are important in clearing industrial effluents of unwanted chemicals. *Agaricus bisporus* and *Lentinus olivoides* are important in lignocellulose decomposition. *Coriaria versicolor* is important in cleaning up pulp and paper mill wastes. Consortia of fungi are used in composting, the most useful waste disposal practice. Phenolic azo dyes have been shown to be oxidized by the enzyme laccase produced by *Pyricularia oryzae*.

***Phanerochaete chrysosporium* - an emerging model system for bioremediation:**

Among filamentous fungi, *Phanerochaete chrysosporium* has emerged as the model system for studying xenobiotic degradation. A great deal remains to be learned about the fundamentals of how this white rot fungus mineralizes pollutants. Not surprisingly, even less is known about the degradative mechanisms used by fungi in general. Oxidative enzymes play a major role, but organic acids and chelators excreted by the fungus also contribute to the process. Many of the toxic chemicals mineralized by fungi are already highly oxidized. The ability of fungi to lower the

pH of their environment appears to be involved in the reduction of some of these compounds.

Phanerochaete chrysosporium is a higher basidiomycete belonging to the white rot group of fungi. It is the best studied ligninolytic fungi, a group whose natural habitat is forest litter and rotting wood. White rot fungi are unique among eukaryotes in having evolved nonspecific mechanisms for degrading lignin. Lignin is unlike many natural polymers in that it consists of irregular phenylpropanoid units united by nonhydrolysable carbon-carbon and ether bonds. Lignin contains chiral carbons in the both L and D configuration, and this stereo irregularity renders it still more resistant to attack by most microorganisms. Nevertheless, many extracellular ligninolytic enzymes produced by white rot fungi can catalyze the breakdown of lignin.

Phanerochaete chrysosporium has been shown to degrade a number of toxic xenobiotics such as aromatic hydrocarbons (Benzo alpha pyrene, Phenanthrene, Pyrene) chlorinated organic (Alkyl halide insecticides, Chloroanilines, DDT, Pentachlorophenols, Trichlorophenol, Polychlorinated biphenyls, Trichlorophenoxyacetic acid), nitrogen aromatics (2,4-Dinitrotoluene, 2,4,6-Trinitrotoluene-TNT) and several miscellaneous compounds such as sulfonated dyes. Several enzymes that are released such as laccases, polyphenol oxidases, lignin peroxidases etc. play a role in the degradative process. In addition, a variety of intracellular enzymes such as reductases, methyl transferases and cytochrome oxygenases are known to play a role in xenobiotic degradation.

Under the condition of nitrogen, sulphur and carbon deprivation *P.*

chrysosporium produces families of ligninolytic enzymes including lignin peroxidase and manganese dependent peroxidase. The peroxidases use hydrogen peroxides generated by glyoxal oxidase, glucose oxidase and cellobiose oxidase to promote the oxidation of lignin to free radicals that then undergo spontaneous reactions with water or oxygen which leads to depolymerization. The depolymerization of lignin by nonspecific extracellular peroxidases is sometimes called enzymatic combustion. Both LiP and MnP are encoded by families of structurally related genes that have been cloned and sequenced. These genes are differentially regulated in response to a variety of environmental signals, especially starvation.

During 1980s, it became apparent that *P. chrysosporium*, in addition to degrading lignin, is capable of degrading a wide variety of xenobiotics. Polyaromatic hydrocarbons, chlorinated phenols, nitroaromatics, dyes and many other environmental toxins have been biotransformed or mineralized by *P. chrysosporium*, sometimes in complex mixtures of xenobiotics. The ability to degrade such broad spectrum of highly toxic and generally recalcitrant substrate is unusual for a single species. It is often assumed that this broad spectrum xenobiotic degradation is effected by the same extracellular enzymes used in lignin biodegradation. In addition, a variety of other factors are thought to contribute, such as intracellular enzymes (reductases, methyltransferases and cytochrome P-450 oxygenases), plasma membrane potential and bioabsorption onto mycelia. Postulated mechanisms used by the white rot fungi to degrade pollutants have been summarized by Barr and Aust.

Examples of xenobiotics degraded or transformed by *P. chrysosporium*:

Type of compound	Examples
Aromatic hydrocarbons	Benzopyrene, phenanthrene, pyrene
Chlorinated organics	Alkyl halide insecticide, atrazine, DDT, 2,4,5-T, aroclor, pentachlorophenol
Nitrogen aromatics	2,4-dinitrotoluene, TNT, hexahydro-1,3,5-trinitro-1,3,5-triazine
Miscellaneous	Dyes

Fungal diversity in biodegradation:

The attributes that distinguish filamentous fungi from other life forms determine why they are good biodegraders. First, the mycelial growth habit gives a competitive advantage over single cells such as bacteria and yeast, especially with respect to the colonization of insoluble substrates. In 2001, Anersson and collaborators showed that a brown rot fungi *A. vaillantii* was able to efficiently colonize contaminated soil. Fungi can rapidly ramify through substrates, literally digesting their way along by secreting a battery of extracellular degradative enzymes. Hyphal penetration provides a mechanical adjunct to the chemical break down affected by the secreted enzymes. The high surface to cell ratio characteristics of filaments maximizes

both mechanical and enzymatic contact with the environment. Secondly, the extracellular nature of degradative enzymes enables fungi to tolerate higher concentration of toxic chemicals than would be possible if these compounds had to be brought into the cell. In addition, insoluble compounds that cannot cross the cell membrane are susceptible to attack. Fungi even solubilize low rank coal, a particularly persistent, irregular and complex polymeric substrate, although they do so slowly. Finally, since the relevant enzymes are usually induced by nutritional signals, independent of the target compounds during secondary metabolism, they can act independently of the concentration of the substrate, and their frequently non-specific nature means that they can act on chemically diverse substrate.

Representative xenobiotic-degrading filamentous fungi:

Group	Species	Substrate
White rot fungi	<i>Agrocybeaegarita</i> , <i>A. praecox</i> , <i>Coriolopsisgallica</i> , <i>Dichomitussqualens</i> , <i>Ganodermaapplanatum</i> , <i>Hypholomafasciculare</i> , <i>Lentinusedodes</i> , <i>Lenzitesbetulina</i> , <i>Nematolomafrowardii</i> , <i>Pleurotussajor-caju</i> , <i>P. pulmonarius</i> , <i>Pycnoporuscinnabarinus</i> , <i>Stropharia sp.</i> , <i>Trameteshirsuta</i> .	Benzanthracene, pyrene, phenanthene, fluoranthene, lignite, dinitrotoluene, trinitrotoluene, lignite-coal, pentachlorophenol, dibenzofuran, textile dyes etc.
Mycorrhizal fungi	<i>Morchellaconica</i> , <i>Tylosporafibrilosa</i> .	Anthracene, fluoranthene, phenanthene, flurobiphenyl.
Others	<i>Agaricusbisporus</i> , <i>Coprinuscomatus</i> , <i>Gleophyllum striatum</i> , <i>Marasmiellusrotula</i> , <i>Crinipellisstipitana</i> .	Anthracene, fluoranthene, phenanthene, pyrene, dichlorophenol, pentachlorophenol etc.

There are more than 1500 different species of white rot fungi. In addition, there are thousands of other fungal species loosely categorized as brown rots, dry rots, litter rots, soft rots, mycorrhiza, terricolous and so forth. In recent years, several groups have done comparative studies of white rot fungi with the expectation of finding better lignin-degrading systems. Many new species with bioremediation potential have been identified. In a number of cases, these fungi show more practical promise than *P. chrysosporium* since their growth strategies offer better sustainability in natural habits; for example, they do not require constant addition of wood or other substrates. In addition, their enzymatic repertoires offer fresh approaches to xenobiotic degradation. In the survey by Gramsset et al., 58 species from different physioecological groups, viz. wood degrading, wood and straw degrading, terricolous, ectomycorrhizal and mitosporic, were grown in liquid cultures and tested against a battery of polyaromatic hydrocarbons. On average, wood degrading species were best at metabolizing PAH, but competent fungi were found in all five groups. Polyaromatic hydrocarbon conversion was correlated with the production of MnP, peroxidase and laccase. Other fungi that can be used in bioremediation are obviously the members of Zygomycetes e.g., the mucoraceous fungi and the arbuscular mycorrhizal fungi. Aquatic fungi and anaerobic fungi are the other candidates for bioremediation.

Whiterotfungi: White rot basidiomycetes that degrade all cell wall polymers are generally considered to be the most effective lignocelluloses degraders. The ligninolytic activity of white rot fungi makes them the most interesting taxa of fungi for use in bioremediation. When used for bioremediation, it is the fungal mycelia and not their fruit bodies that transform lignocellulosic wastes and transform

aromatic pollutants. Two particular white rot species that have received considerable attention are *Pleurotus ostreatus* and *Trametes versicolor*. They are both efficient at mineralizing PAH and at degrading polychlorinated biphenyls. *P. ostreatus* are better able to colonize soil than *P. chrysosporium*, and although it is a successful lignin-degrading species, it does not exhibit LiP activity. *P. ostreatus*, however, produce several laccase isozymes encoded by a family of laccase genes. Laccase (p-diphenoldioxygenoxidoreductase) is a member of "blue copper oxidase" family. Many phenols and chlorophenols are transformed by these enzymes to radicals that subsequently undergo spontaneous polymerization. Laccase mediator combinations also show activity against acenaphthene, acenaphthylene and anthracene. A purified laccase isolated from *T. versicolor* could oxidise a variety of polyaromatic hydrocarbons in vitro, including anthracene, benzopyrene, fluoranthene and chrysene.

In addition to laccase, an enzyme with properties of both LiP and MnP has been isolated from species of *Pleurotus* and *Bjerkandera*. This third type of lignin peroxidase has a high affinity for substituted hydroquinones. A cell free system of MnP of the white rot fungus *Nematoloma frowardii* is capable of mineralizing pentachlorophenol, catechol and pyrene. Fungal heme containing peroxidases e.g. LiP, MnP and versatile peroxidase (VP) use hydrogen peroxide to promote the one-electron oxidation of chemicals to free radicals; they are involved in the biodegradation of lignocelluloses and participate to the bioconversion of diverse recalcitrant compounds. Soft rot fungi, on the other hand, are a specialized group of organisms that grow in a localized niche within the secondary wood cell wall and degrade the cell wall polymers slowly.

Properties of fungal heme peroxidase

Name	Mol. Wt. (kDa)	Pi	Substrates	Redox potential(V)
Lip	35-48	3.1-4.7	Non-phenolics(veratryl compounds) and phenolic aromatic compounds, organo-pollutants (PAHs, Chlorophenol,nitroaromatics,dyes and explosives)	1.4-1.5
MnP	38-50	2.9-7.1	Manganese, phenols and non-phenolic lignin moieties via lipid radicals.	1.0-1.2
VP	43-45	3.4-3.9	Non-phenolic, phenolic, dye substrate and manganese.	1.4-1.5

Brown rot fungi: Brown rot fungi degrade the cellulose and hemicellulose components of wood, leaving a residue of modified lignin that is dark brown. Although, they are enormously destructive of wood products, the mechanism of wood decay by brown rot fungi has received far less attention than that of wood decay by white rot fungi. It is believed that early steps in brown rot decay are nonenzymatic. Interestingly, only 6% of all the known wood decay fungi are now known to cause a brown rot and are almost exclusively associated with conifers. The ability of brown rots to degrade xenobiotics is relatively a new avenue of research. Current studies have implicated two species of *Gloeophyllum* in the degradation of chlorophenols and *G. trabeum* is active against polyethylene glycol and ciprofloxacin, a fluroquinolone antibiotic. More recently, 12 species of brown rot fungi have been investigated for their ability to degrade DDT. *G. trabecium*, *Fomitopsis pinicola* and *Daedalea dickinsi* showed high ability to degrade DDT via a chemical Fenton reaction. No doubt, many other interesting brown rot fungi will be found to demonstrate activity against a spectrum of xenobiotics.

Litter decomposing fungi: Litter decomposing fungi are another

ecophysiological group of basidiomycetes which play a pivotal role in the ecology of forests since they are deeply involved in wood and litter decomposition, humification, mineralization of soil organic matter. Typical litter basidiomycetes such as species of the genera *Mycena*, *Clitocybe* and *Collybia* are prominent during the stage of decomposition. Litter decomposing basidiomycete fungi have substantial ability to degrade PAH. *Stropharia rugosoannulata* was found to be the most efficient strain of basidiomycete for the removal of a variety of PAHs, doing away with over 85% of them after six weeks in experimental culture. This shows that manganese peroxidase, one of the extracellular lignolytic enzymes, is an important component of the degradation that the species performs. In further testing of eight promising basidiomycetous fungi by Steffen et. al, (2007), 60% of all PAHs present were decomposed by *S. rugosoannulata*, again the top-performing species.

Very recently, the involvement of laccases from *Marasmius quercophilus* and peroxygenases from *Coprinellus radians* in the degradation of PAHs, methyl naphthalenes and dibenzofurans were also reported. The ability of litter decomposing fungi to colonize soil, to

survive there for long periods and to compete with other microorganisms should be considered as ecological features that can make them even more suitable for bioremediation applications compared with the wood decomposing fungi which usually prefer to colonize compact wood and have poor capability to grow in different niches such as soil.

Mycorrhizal fungi: Mycorrhizal fungi (both VAM & EC) may benefit plants that grow in contaminated soils providing greater access to water and nutrients, possibly protecting them from direct contact with the toxic contaminants. The capability to degrade pollutants by mycorrhizal fungi is attributed to the production of a variety of polyphenol oxidases (laccase, catechol oxidase and tyrosinase) and peroxidase.

Fungi attack plastic polymers as well; these come in a wide range of structures as lignins and are acted upon by different fungal species for different polymers. This decomposing ability is perhaps even more impressive than PAH decomposition. It was found that the white rot basidiomycetes known for lignin degradation especially *P. ostreatus* could effectively break down polyacrylimide. *Cladosporium resinae* was found in another study to also degrade polyurethane. Jecuet. al, (2010) examined polyvinyl alcohol films under a scanning electron microscope and found substantial degradation by fungi, most notably *Aspergillus niger*. Copolymerization with natural polymers such as starch and collagen increase biodegradation. These provide an additional carbon source for the fungi and may also provide access points for the fungi to invade the synthetic polymers.

Sometimes the same fungi that degrade PAHs have been found to remediate toxic metals as well, which are commonly found in the same polluted sites and can reduce the effectiveness of some degradative microorganisms. Hong et. al, (2009)

surveyed gas station soil and found strains of *Fusarium* and *Hypocreathat* could degrade one carcinogenic high weight PAH, pyrene, as well as uptake copper and zinc. These strains were able to use the pyrene as their sole carbon source.

A few papers discuss the potential applications for fungal bioremediation which were reviewed by Fernandez-Luqueno et. al, (2010). Bioaugmentation involves bringing selections of effective fungi to contaminated sites, either from the lab or from older contaminated sites in which fungi have had ample chance to evolve degradative abilities. Biostimulation is adding nutrients such as N,P,Cu, K or S to stimulate activity of the fungi that are native to the location. Steffen and Hatakka (2002) utilized this method when they added manganese to a mixture of litter decomposing fungi and contaminated soil. A host of organic materials could be added that would contain some of these nutrients as well, such as compost, food waste and sewage sludge. Additionally plants (especially grasses) can be added to increase fungal activity.

Many workers divide bioremediation strategies into three general categories: 1. The target compound is used as a carbon source, 2. The target compound is enzymatically attacked but is not used as a carbon source (co-metabolism), and 3. The target compound is not metabolized at all but is taken up and concentrated within the organism (bioaccumulation). Although, fungi participate in all three strategies, they are often more proficient at co-metabolism and bioaccumulation than at using xenobiotics as sole carbon sources.

Conclusion:

What about the future? Various brown rots, litter rots, aquatic fungi, anaerobic fungi and mycorrhizal fungi, in conjunction with the pollution tolerant plants, all provide

opportunities for new research. Genetic engineering is another frontier. Fungal genes for degradative enzymes can be added to bacteria; alternatively, competent fungi can be modified to grow in an extended range of environments. For example, several groups have investigated the recombinant expression of fungal peroxidases in order to facilitate large scale commercial production of these enzymes. LiP and MnP have been produced in *Aspergillusniger* host and in the insect baculovirus expression system, although LiP was not active. Another development is that of large scale DNA sequencing. The US department of energy is using a whole shotgun approach to sequence the genome of *P. chrysosporium*. The availability of DNA sequence data for the model white rot fungus, coupled with the capacity to build DNA microarrays for transcriptional profiling and gene discovery, will provide powerful tools for identifying genes for hitherto undiscovered degradative enzymes from other filamentous fungi.

As we get better at recognizing what can and cannot be done with bioremediation, we will create a menu of choices using a broad range of organisms. In some situations, bioconcentration of toxic wastes is the best we can do. In others, the nonspecificity of the white rot fungi is ideally suited to treating low concentrations of mixed wastes in a nutrient deficient habitat. Yet, in others, anaerobic bacteria are clearly the best candidates. Complete pathways of degradation are more likely to occur through the combined effects of many organisms. For example, cocultures of the bacterium *Stenotrophomonasmaltophilia* and the fungus *Penicilliumjanthinellum* degrade high molecular weight polycyclic aromatic hydrocarbons more efficiently than does either microorganism alone.

Judicious combinations of chemical and physical processes with biological schemes also offer promise. Filamentous fungi, yeasts and nonphotosynthetic bacteria are

the workhouses of biological degradation. Therefore, it is ironic that the popular press has chosen the word “green” to describe environmentally friendly technologies such as bioremediation. Decidedly not green in color and most certainly underappreciated, the fungi possess the most varied and most efficient battery of depolymerizing enzymes of all decomposers. When joined with their bacterial brethren in cooperative catabolism, fungal-bacterial consortia will foster the ecological recovery of contaminated habitats world-wide. Filamentous fungi will always be the major players in the “greening” of toxic waste sites and other polluted habitats. It is also clear that different ecophysiological groups of fungi are strictly interconnected creating communities that underpin survival and productivity of the different ecosystems. In addition, it appears that the redundancy of biodegradation capacity of different groups of fungi is essential to compensate for the depletion of microbial communities due to soil contamination and hence, it is a key aspect for the ecosystem recovery. Further, efforts are needed to better understand how the soil ecosystem works as a whole. A better knowledge of the complexity of this heterogeneous environment and of the interactions between different organisms present in the soil will make it possible to formulate more effective bioremediation strategies.

Fungi have been shown to even solubilize partially coal, a highly polymeric substance more complex than lignin. There is no doubt, therefore, regarding fungi being harnessed more and more in environmental bioremediation work in future.

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