Fungi and Bioremediation

Bioremediation

- **Bioremediation is an environmentally friendly process using many different microbes to weaken and detoxify harmful pollutants in a parallel or sequential manner**
- **Microorganisms (e.g., fungi and bacteria), green plants, or combinations of them used together can convert toxic pollutants into carbon dioxide (CO2) and water (H2O), inorganic salts, microbial biomass, and other products that are less toxic and accelerating natural metabolic processes**
- **In recent years, interest in exploring microbial biodegradation of toxins has been amplied by human attempts to achieve a sustainable approach to purication and restoration of polluted habitats**
- **In bioremedial technologies, microbes are introduced to improve decomposition or elimination of organic and inorganic pollutants and harmful contaminants**

Why fungi?

- **Fungi play crucial roles as degraders and symbionts in the environment due to their morphology and versatile metabolic ability**
- **Fungi can survive in a variety of habitats with complex soil matrix to freshwater as well as marine habitats with stable colonization**
- **Fungi can also flourish in the soils of different climatic conditions including the extreme ones**
- **They propagate through the dispersal of spores in the air and also help in maintaining the balance of ecosystem**
- **They have also been reported to survive in effluent treatment plants (ETPs) treating various waste waters**
- **The diversity of habitats and ability for secreting multitude of enzymes makes fungi potential candidates for bioremediation at various sites**

Fungal Mycelium

Fungi are organized as mycelia which provide flexibility and resilience not found in any other organism group

- **Mycelia are multicellular**
- **Mycelia are able to grow around barriers**
- **Mycelia avoid areas with adverse condition**
- **Mycelia can quickly reallocate resources (i.e. more mycelium) to areas with preferential conditions (e.g. high amount of nutrients)**
- **Mycelia are able to recycle dead hyphae**
- **Hyphae allow fungi to expand their surface area, making it easier to contact the pollutant**
- **Extracellular enzymes can then go to work**

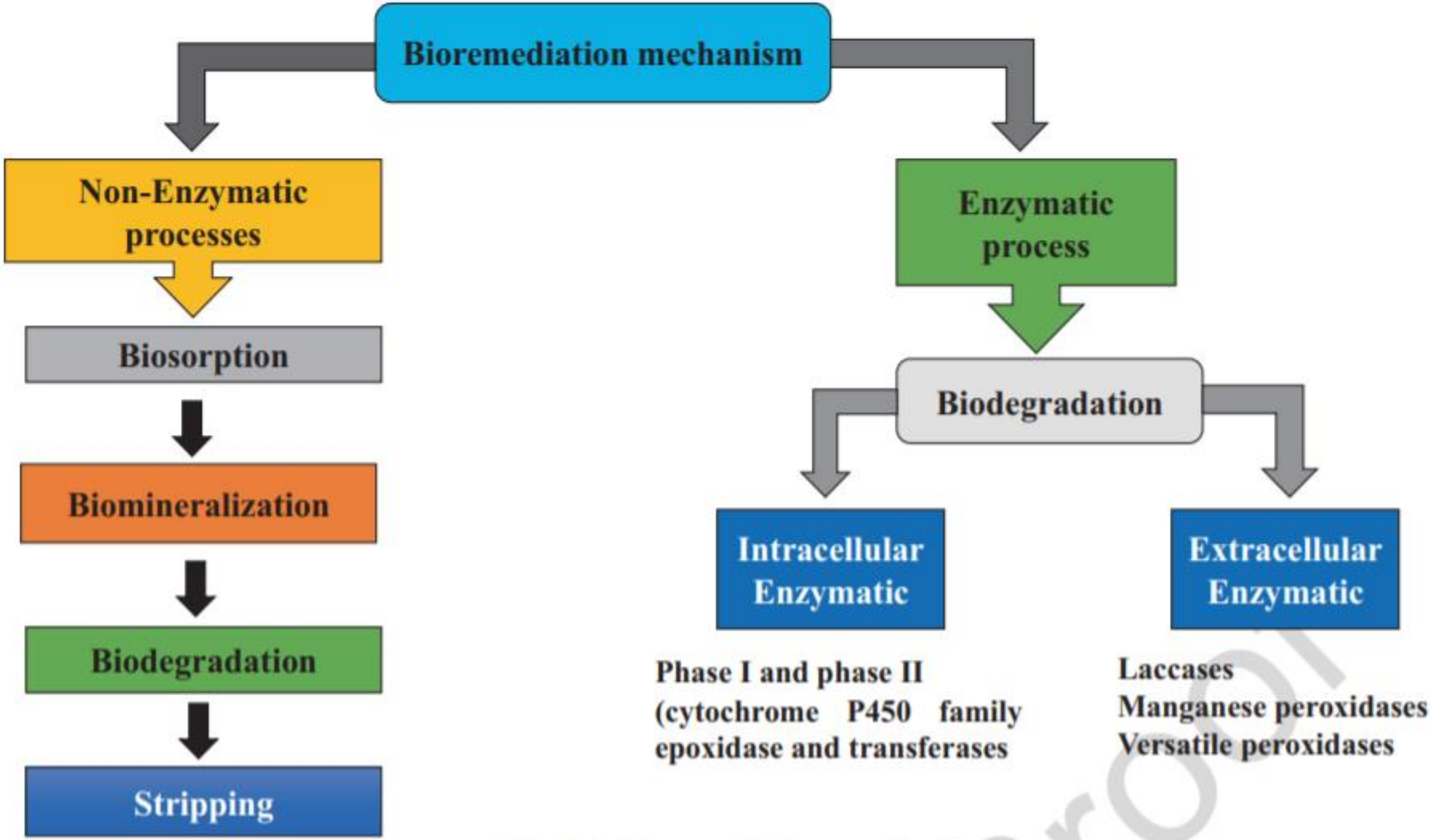
Mycoremediation

- **Mycoremediation is bioremediation with fungi**
- **It is a method of bioremediation using fungi to decontaminate contaminated areas**
- **Fungal nutrition depends on the release of enzymes by the mycelium into the substrate**
- **Those fungal enzymes are able to degrade many pollutants through various mechanisms**

The Mechanism

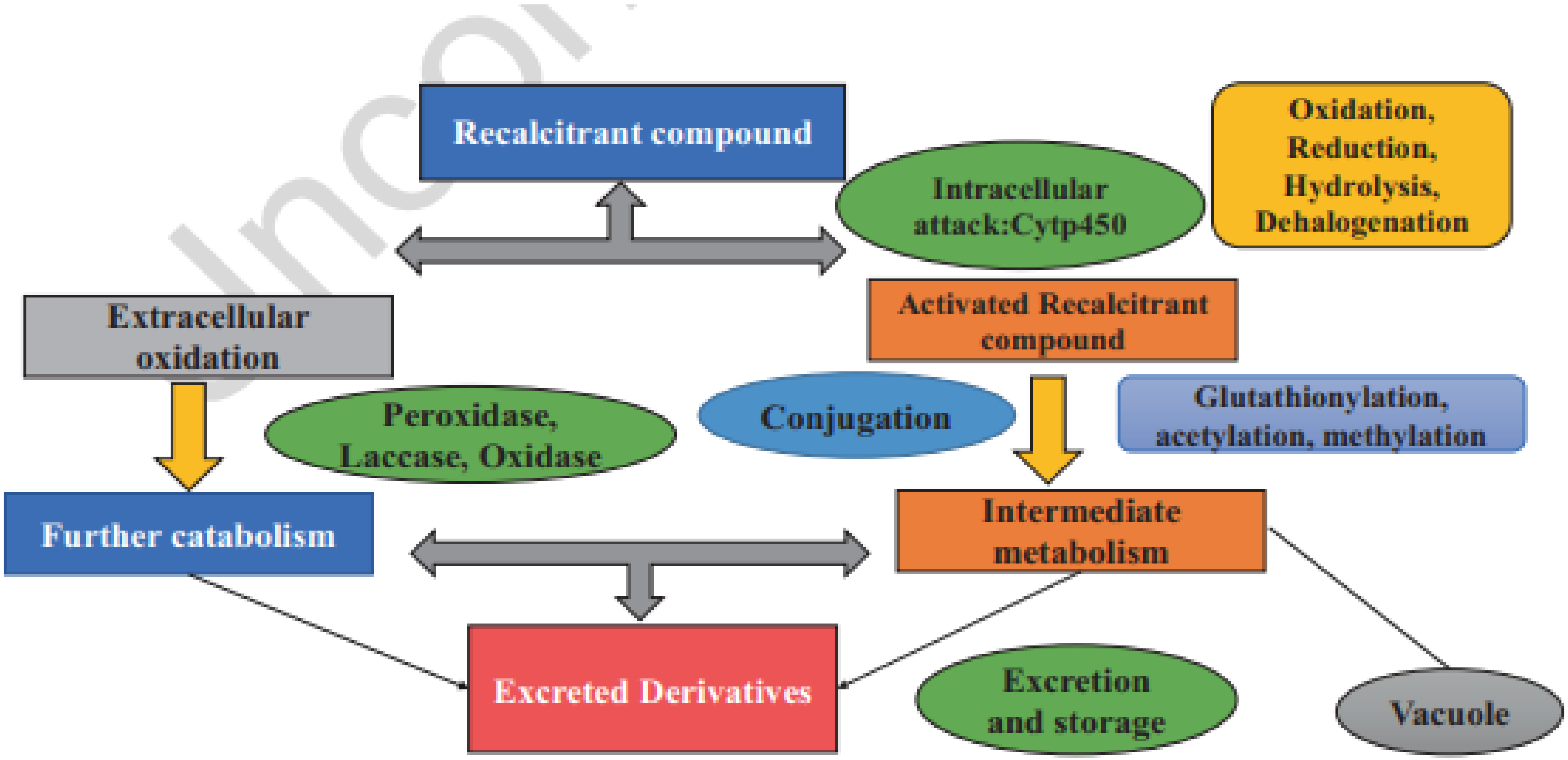
Various mechanisms of detoxication exist: Hydrolysis, dehalogenation, ether cleavage, methylation, hydroxylation, deamination and others

- **Many basidiomycetes use enzyme systems such as phenoloxidases**
- **The free radical ions are formed in the surrounding of mycelial network which attack various chemical bonds**
- **Fungi have ligninase and cellulase enzymes that break down woody materials, which allows fungi to get the needed carbon and energy they require for growth**
- **Some enzymes are non-specific, they can act on substrates like environmental pollutants**



Bioremediation mechanism
Non-Enzymatic processes
Biosorption
Biomineralization
Biodegradation
Stripping
Enzymatic process
Biodegradation
Intracellular Enzymatic
Extracellular Enzymatic
Phase I and phase II (cytochrome P450 family epoxidase and transferases
Laccases
Manganese peroxidases
Versatile peroxidases

Mechanism adopted by fungi for bioremediation of toxic, recalcitrant compounds



White-Rot Fungi

- **White-rot fungi are chief agents of biodegradation of ligninninous material in nature which contribute in the global carbon recycling**
- **Endocrine disrupting chemicals (EDCs) and TrOCs such as pharmaceuticals and personal care products (PPCPs) which can result in effects such as bioaccumulation, acute and chronic toxicity to aquatic organisms, and possible adverse effects on human health have generated a lot of interest with reference to their degradation by white-rot fungi**
- **Majority of the studies have demonstrated the bioremediation potential of white-rot fungi; *Phanerochaete chysosporium*, *Trametes versicolor*, *Bjerkandera adjusta* and *Pleurotus* sp., by virtue of producing different ligninolytic enzymes such as laccases and peroxidases**
- **The ligninolytic enzymes from white-rot fungi have been applied for transformation of variety of organic pollutants such as pesticides from contaminated wastewaters by promoting microbial activity using a biopurification system (BPS)**

- **Extracellular ligninolytic enzymes also have capacity for adsorption of dyes which has made white-rot fungi, a dominating force in the area of dye degradation or decolourization as demonstrated in case of decolorization of Direct Blue 14 by various species of *Pleurotus*, and Remazol Brilliant Blue-R by *Agaricomycete***
- **Diverse fungal groups such as *Coriolus versicolor*, *Hirschioporus larincinus*, *Inonotus hispidus*, *Phanerochaete chrysosporium*, *Phlebia tremellosa* have been reported for decolourization of dye**
- **The strains *T. versicolor* and *Lentinus tigrinus* are known involve by biostimulation with lignocellulosic substrate along with bioaugmentation on the cresolate-polluted soil, contaminated with residual recalcitrant petroleum hydrocarbons and high molecular weight PAH fraction remaining after a biopiling treatment**
- **Attempts have been made for increasing the laccase production in white-rot fungi, *T. versicolor* and *P. ostreatus* by solid state fermentation on orange peels followed by further testing of its capacity for bioremediation of PAHs such as phenanthrene and pyrene**
- **Laccase production from *T. versicolor* cultures was 3000 U/L and though, *P. ostreatus* produced 2700 U/L laccase, it showed better removal of phenanthrene and pyrene**

Marine Fungi

- The potential of marine fungi for production of secondary metabolites, biosurfactants, novel enzymes, polysaccharides and polyunsaturated fatty acids in addition to their application in bioremediation of hydrocarbons and heavy metals has been documented
- Their ability to adapt to high saline conditions and pH extremes provides a biological advantage to these fungi over terrestrial fungi
- The role of marine fungi from mangrove areas has been reviewed
- Marine fungi have even been found to tolerate high concentrations of heavy metals such as lead and copper
- These fungi possess the ability to synthesize nanoparticles both extra and intracellularly which are being used for diverse applications in areas ranging from textile industries, food preservations, to medicines and clinical microbiology etc.

- **The attribute of marine fungi for producing laccase tolerant to high salinity and phenolics by *Trichoderma viride* isolated from an estuary polluted with phenolics**
- **Some enzyme mediated bioremediation was demonstrated for decolorizing Remazol Brilliant Blue-R dye using three basidiomycetes isolated from marine sponges, and anthraquinone dye Reactive Blue 4 by *C. unicolor*, a marine white-rot basidiomycete**
- **The biotransformation of persistent organic pollutants (POPs) by *Penicillium* and *Trichoderma harzianum* has been reported**
- **Other marine derived fungi including *Mucor*, *Aspergillus*, *Penicillium* and slime mold demonstrated bioremediation potential for water soluble crude oil fractions between 0.01 and 0.25 mg/mL**

Extremophilic Fungi

- **Fungi from extreme environments are very important from industrial point of view owing to their extremophilic enzymes which posses several special characteristics such as thermotolerance, pH tolerance, and tolerance to other harsh conditions**
- **These species are extremely useful in bio-conversions of raw materials such as in food industries, leather processing, textiles manufacture, animal feed preparation, and bio-remediation**
- **A psychrophilic fungus, *Cryptococcus* sp. isolated from deep-sea sediments showed tolerance and growth in presence of high levels of heavy metals (upto 100 mg/L) $ZnSO_4$, $CuSO_4$, $Pb(CH_3COO)_2$ and $CdCl_2$**
- **Extreme acting laccases were observed to be responsible for bioremediation activity in *Pestalotiopsis palmarum* when wheat bran was present and lignin peroxidases were produced when extra heavy crude oil was the only carbon and energy source**
- **Other enzymes such as chitinases produced by a psychrophilic fungus, *Lecanicillium muscarium*, could be applied for enhancing the activity of insecticides owing to their ability for acting on insect chitin exoskeleton**

Symbiotic Fungi with Plants and Bacteria

- **Fungi are known to forge close association with plants and bacteria in order to overcome the barrier of restricted growth under different environmental conditions**
- **Arbuscular mycorrhizal fungi (AMF) represent the most common symbiotic relationship between fungi and plants wherein, fungal partner promotes pollutant removal by providing higher surface area for absorption of pollutants through its hyphae and spores by mobilizing the pollutants and binding to the root**
- **AMF colonization was observed in root samples from plants used for phytoremediation of groundwater contaminated with various pollutants in a constructed wetland**
- **Certain plant-associated fungi (*A. nidulans*, *Bjerkandera adusta*, *Trametes hirsuta*, *T. viride*, *Funalia trogii*, *Irpex lacteus*, *P. ostreatus*) could survive in presence of and decolorize textile industry effluents**
- **Colonization of AM fungus *Rhizophagus custos* under root-organ cultures was responsible for high levels of tolerance to PAHs especially anthracene with lower formation of toxic by-product anthraquinone**
- **Enhanced ^{137}Cs uptake by quinoa plants on loamy soil after inoculation with a commercial AM product was also shown to be associated with mycorrhizal effect due to root colonization**
- **Recently, ectomycorrhizal fungi, *Suillus bovinus* and *Rhizopogon roseolus* in association with *Pinus* have been shown to be helpful for cadmium removal which was also subject to the effect of other environmental factors like the type of nutrients and pH**

Bioremediation of Toxic Recalcitrant Compounds

- **Bioremediation of many toxic, organic compounds from industrial effluents is an essential pre-requisite for release of such effluents into the environment**
- **PAHs are complex organic compounds with fused, highly stable, polycondensed aromatic rings, which have efficiently bioremediated through *Aspergillus*, *Curvularia*, *Drechslera*, *Fusarium*, *Lasiodiplodia*, *Mucor*, *Penicillium*, *Rhizopus*, *Trichoderma* through high lipase production**
- **Degradation ability to several fungi for explosives such as TNT in presence of co-substrates including cellulose and lignin**
- **Conventionally, many toxic chemicals are used in agro-industrial operations such as in bleaching of agro-residual pulp in paper mills resulting in toxic effluent, which can be remediated through a green technology using fungal laccase-mediator system**

- **White-rot fungi are extensively studied for their variable degradative capacities of chemicals from dyeing industries**
- **The toxicity of chemicals used in dyeing industries is manifested in the form of decreased carbohydrate, protein and chlorophyll and increased proline content in exposed plants in addition to reduction in the rate of seed germination and growth of crop plants**
- **Basic and acid dyes are the most toxic for aquatic organisms including algae and fishes and have the tendency to pass through food chain and ultimately reach human body resulting in various physiological disorders**

- **Removal of Reactive Remazol Blue at pH 6 and pH by co-culture of *Aspergillus versicolor* and *Rhizopus arrhizus***
- ***Schizophyllum commune* IBL-06, a white-rot fungus, was able to completely decolorize direct dye Solar Brilliant Red 80, while, *C. versicolor* was shown to degrade an azo dye, Acid Orange 7**
- **Among the other toxic compounds, pesticide chlorpyrifos and its major metabolites were completely degraded by *Aspergillus terreus***
- **A *Mucor racemosus* strain DDF was found to degrade dieldrin, heptachlor (94 %), heptachlor epoxide (67.5 %), endosulfan (80 %) and endosulfan sulfate (95 %)**

Bioremediation of Heavy Metals

- **Due to high degree of toxicity leading to health effects such as multiple organ failure and carcinogenic effects, heavy metals; arsenic, cadmium, chromium, lead, and mercury are considered as priority metals which need to be removed from environment in order to reduce their impact on public health and environment**
- **Different microbes show the ability to tolerate the presence of heavy metals and possess different mechanisms for their removal from environment**
- **High tolerance and remediation capacity of filamentous fungi towards heavy metals like Cd, Cu and Ni (up to 1500 mg/L) assumes significance for bioremediation of these metals from contaminated soil and waste water**
- **Members of genus *Aspergillus* are known for their versatility to degrade a diversity of toxic compounds ranging from heavy metals, textile dyes, aromatic compounds, pesticides etc.**
- ***A. flavus* and *A. niger* have been reported for their capacity to reduce heavy metals such as Cr^{6+} to Cr^{3+}**

- ***A. foetidus*** **isolated from a wastewater treatment plant was found to be tolerant to high concentrations of lead (Pb) through biosorption**
- **Bioremediation of aqueous substrates containing mercury (II) by *A. flavus* has also been reported**
- **The potential of fungi like *Aspergillus*, *Cryptococcus*, *Penicillium* and *Curvularia* for bioremediation of uranium contaminated soils through uranium binding ability has been demonstrated**
- **Symbiotic association of AM fungi with the roots of plants promoted immobilization of heavy metals and hence provided ability to plants to grow in metal-contaminated soils as observed in case of enhanced Cd tolerance of plants**
- **Exposure of *Aspergillus* sp. to gamma rays (20–100 Gy) in Cd supplemented media resulted in an increase in growth and higher Cd removal in comparison to un-irradiated controls**

Bioremediation of Municipal Solid Waste (MSW)

- **Although, incineration and land-filling are commonly used methods for disposal of MSW, incineration is an expensive process, while, land filling sites are a main source of secondary environmental pollution including fouling of air, bad odour, and increased pathogen content in soil**
- **Biocomposting and bio-methanation by anaerobic digestion are the desirable solution for managing MSW due to two benefits, management of MSW and production of value added products such as volatile fatty acids (VFAs), biogas, and organic residue/compost for application as a soil conditioner or fertilizer**
- **Fungi and their hydrolytic enzymes such as cellulases, proteases, amylases, and lipases could be applied for the conversion of complex polymeric substances to simple compounds**

- **The potential of kitchen waste residues to serve as substrate for solid-state fermentation of cellulolytic, hemicellulolytic, pectinolytic, amylolytic enzymes by a locally isolated strain of *Aspergillus niger* was demonstrated**
- **Application of a fungal consortium composed of two fungi *Armilleria gemina* and *Pholiota adipose* for remediation of MSW has been approved**
- **The tolerance of wood-rotting fungi *Antrodia xanthan* and *Fomitopsis palustris* to copper was exploited in bioremediation of copper deposited wood**

Fungal Enzymes in Bioremediation

- Fungal enzymes of industrial importance include cellulases, xylanases, amylases, proteases, lipases, laccases, peroxidases, catalases etc. which can find potential applications in managing organic waste
- These enzymes can be used for hydrolyzing the polymeric substances such as cellulose, xylan, starch, protein, and lipid present in wastes including food, kitchen, vegetable market, leaf litter etc. which could be further subjected to composting, or used for production of value added products such as VFAs and biogas
- Ligninolytic enzymes secreted by white rot fungi for oxidation of lignin in the extracellular environment have been categorized into two groups: peroxidases-manganese and lignin peroxidases (MnP and LiP) and laccases
- Much interest is currently focused on developing tailor-made enzymes through protein engineering techniques and recombinant expression of genes from white-rot fungi which are effective tools for eco-friendly treatment of toxic wastes

Laccase

- **Laccases are copper containing extracellular enzymes belonging to group of blue oxidases which use copper as co-factor and molecular oxygen as co-substrate**
- **Laccases are capable of oxidizing most of the phenolic and non-phenolic compounds, and their activity has been observed to be more than 20 times greater in fungi such as *T. versicolor* than other organisms**
- **The non-specific nature of their activity on a variety of substrates makes them ideal catalyst for different industrial applications of which these enzymes have been extensively explored for their efficient bioremediation potential**
- **One such application was its demonstration in recycled paper industry for deinking of offset printed paper wherein laccases from three basdiomycetes (*Trametes villosa*, *Coriolopsis rigida*, *Pycnoporus coccineus*) and one ascomycete (*Myceliopthora thermophila*) were assayed for decolourization of flexographic inks in presence of synthetic and artificial mediators**
- **In spite of their tremendous potential in bioremediation, the utility of laccases is restricted by their low shelf life. This drawback can be overcome by immobilization of the enzyme on nanoparticles thus providing high residual activities over a broad range of pH and temperature**

Catalase

- The reactive oxygen species (ROS) accumulation results in damage to cellular macromolecules, which is deleterious for cellular integrity
- Primary defense mechanism to ROS generation in fungi consists of monofunctional catalases and bifunctional peroxidase/catalase enzymes
- Inhibition of catalase in presence of pesticide lindane has been reported to manifest in the form of increased ROS generation and hence in ROS-mediated damage resulting in inhibition of growth of *Saccharomyces cerevisiae*
- Heavy metals such as lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn) have been reported to be among the major reasons for ROS induction in microbial cells
- The tolerance of *Aspergillus foetidus* in presence of 200 mg/L Pb(II) increase in levels of catalase for detoxifying malondialdehyde and H_2O_2
- Similar observations on enhancement of *Aspergillus* spp. tolerance to oxidative stress induced by heavy metals-100 mg/L Cu(II) and 750 mg/L Zn(II)
- The fungal consortia consisting of *A. niger*, *Penicillium* sp. and *Rhizopus* sp. at about 50 mg/L could be used as monitoring tool for monitoring bioremediation efficiency through catalase activity
- The catalase activity decreased with increasing oil concentration during bioremediation of oil contaminated soil

Peroxidase

- **Peroxidases are classified into lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP) depending on their source and activity**
- **These are mostly reported for degradation of toxic compounds by white-rot and basidiomycetes fungi**
- **One such peroxidase produced by a polyporous fungi *B. adusta* was shown to disrupt the phthalocyaninic ring in phthalocyanine dyes by cleavage of azo bond thus leading to decolorization of azo and phthalocyanine dye**

Fungal Cytochromes in Bioremediation

- **Certain fungi possess intracellular networks which constitute the xenome, consisting of cytochrome (CYP) P450 monooxygenases and the glutathione transferases for dealing with diverse range of pollutants**
- **The members of the detoxification pathways which generally belong to multigenic families such as cytochrome P450 monooxygenases and glutathione transferases together constitute the xenoma**
- **The fungal cytochrome P450 system can serve as versatile catalyst for region- and stereospecific oxidation of non-activated hydrocarbons, and can be ideal substitutes for chemical catalysts**
- **Separate cytosolic and mitochondrial iso-forms of P450 found in *Fusarium oxysporum* and other fungi are employed by fungi in degradation of dioxins**
- **CYP63A2 P450 monooxygenase from white-rot fungus *P. chrysosporium* oxidized crude oil aliphatic hydrocarbon *n*-alkanes, endocrine-disrupting long-chain alkylphenols (APs), mutagenic/carcinogenic fused-ring high molecular weight PAHs (HMW-PAHs)**
- ***F. oxysporum* CYP monooxygenases were promising catalysts in significant production of ω-hydroxy fatty acids**
- **Pre-induction of the P450 monooxygenase before application in degradation studies could result in enhanced PAH removal**

	Compound	Fungi
1	**POPs**	
	Polychlorinated biphenyls	Doratomyces nanus, D. purpureofuscus, D. verrucisporus, Myceliophthora thermophila, Phoma eupyrena, and Thermoascus crustaceus, Aspergillus niger
	Polychlorinated dibenzofurans	White rot fungi: Phanerochaete sordida
	Phenylurea herbicide diuron	Mortierella
2	**Textile dye decolourization**	Aspergillus niger, A. foetidus, A. sojae, Geotrichum candidium, Penicillium sp., Pycnoporus cinnabarinus, Trichoderma sp., T. viride, White rot fungi: Bjerkandera adusta, Ceriporia metamorphosa, Ganoderma sp.
3	**Petroleum products**	
	Crude oil	A. niger, Rhizopus sp., Candida sp., Penicillium sp., Mucor sp., Fusarium sp.
	Gasoline	Exophiala xenobiotica
4	**Bleached kraft pulp mill effluent**	Rhizopus oryzae or Pleurotus sajor caju
5	**Effluent from leather tanning**	Aspergillus flavus, Aspergillus sp. and A. niger, Aspergillus jegita

6	**PAHs**	
	Diphenyl ether	White rot fungi: Pleurotus ostreatus, Trametes versicolor
	Anthracene	Armillaria sp.
	Naphthalene	White rot fungi: Pleurotus eryngii
7	**PPCPs**	
	Caffiene	Chrysosporium keratinophilum, Gliocladium roseum, Fusarium solani, A. restrictus, Penicillium and Stemphylium
	Citalopram, fluoxetine, sulfamethoxazole	Bjerkandera sp. R1, Bjerkandera adusta, and Phanerochaete chrysosporium
8	**Fungicide**	
	Metalaxyl and Folpet	Gongronella sp. and R. stolonifer
9	**Pesticide**	
	Chlorinated hydrocarbons: Heptaclor	P. ostreatus
	Chloropyriphos	Aspergillus terreus

10	**Heavy Metals**	
	Heavy metals, lead, mercury and nickel	Aspergillus, Curvularia, Acrimonium, Pythyme, Aspergillus flavus, Cunninghamella elegans, Saccharomyces cerevisiae
	Cadmium	A. versicolor, A. fumigatus, Paecilomyces sp., Paecilomyces sp., Terichoderma sp., Microsporum sp., Cladosporium sp.
11	**Other Hydrocarbons**	A. niger, A. fumigatus, F. solani and P. funiculosum, Tyromyces palustris, Gloeophyllum trabeum, Trametes versicolor
12	**Phenanthrene, benzopyrene**	Candida viswanathii
13	**Striatum Pyrene, anthracene, 9-metil anthracene, Dibenzothiophene Lignin peroxidasse**	Gleophyllum striatum

Abbreviations:

PAHs: Polycyclic Aromatic Hydrocarbon

POPs: Persistent organic Pollutants

TOC: Total Organic Carbon

PPCPs: Pharmaceuticals and Personal Care Products

PCBs: Polychlorinated Biphenyls

DDT: Dichlorodiphenyltrichloroethane