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Mycoremediation of Environmental Pollutants

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Abstract : A wide number of fungal species have shown incredible abilities to degrade a growing list of persistent and toxic industrial waste products and chemical contaminants to less toxic form or non-toxic form. Mycelium reduces toxins by different enzymatic mechanism to restore the natural flora and fauna. White rot fungi has successfully been utilized in degradation of environmental pollutant like polyaromatic compounds, pesticides etc. The present review gives a insights on degradation aspects of heavy metals, PAH especially using different fungal species. White rot fungi has potential to degrade contaminants using wide range of enzymes. Mycoremediation is promising alternative to replace or supplement present treatment processes.
Keywords: Mycoremediation, Heavy metal, PAH, White rot fungi, Contaminant.

Introduction

Recent rapid and progressive development of technology and industry has led to increasing the proportion of various environmental pollutants, such as pesticides, toxic xenobiotic, metals, metalloids and halogenated and polycyclic aromatic hydrocarbons[1]. Microbial remediation of metal is a complex process that depends on the chemistry of metal ions, cell wall composition of microorganism, cell physiology, and physic-chemical factors like pH, temperature, time, ionic strength and metal concentration[2]. The retention time of metals in soil is thousands of years because, unlike organic pollutant, metals are not degraded biologically. They rather are transformed from one oxidation state or organic complex into another and therefore persist in soil[3].

Microorganisms have the ability to bind metals from aqueous solution. This phenomenon is known as bio sorption. Yeast and fungi are unique in metal bio sorption, and this process is known as mycosorption. Mycosorption is a topic of great interest for researchers all over the world[4]. Fungi possess the biochemical and ecological capacity to degrade environmental organic chemicals and to decrease the risk associated with metals, metalloids and radionuclides, either by chemical modification or by influencing chemical bioavailability. Furthermore, the ability of these fungi to form extended mycelial networks, the low specificity of their catabolic enzymes and their independence from using pollutants as a growth substrate make these fungi well suited for bioremediation processes[5].The ability of most fungi to produce extracellular enzymes for the assimilation of complex carbohydrates without prior hydrolysis makes possible the degradation of a wide range of pollutants[6]. Mushrooms are vegetal organisms with the ability to accumulate heavy metals. This ability is explained by the presence of a rich network of hyphae which occurs in a considerable volume in the upper layer of soil. This allows mushrooms to collect required water and minerals from the soil for production of fruiting body[7]. Every species of mushroom has a specific capacity, genetically controlled for absorption of one or another heavy metal from the soil[8]. Mushroom can be successfully utilized in mycoremediation technologies, where their feature concerning the uptake of heavy metal is beneficial[9]. A number of mushrooms have been proven to mycoremediate heavy metals which include *Pleurotus platypus, Agaricus bisporus, Calocybe indica, Calvatiaexci puliformis, Hygrophorus virgineus, Boletus edulis, Lepiota rhacodes, Lepis tanuda, Pleurotus sajor-caju, Pleurotus ostreatus, Psalliota campetris, Russula delica*[10,11].

Bioremediation of heavy metals using Fungi:

Heavy metals are another group of toxins of environmental concern with a possible solution arising from fungal treatments. It is reported that 389 of the 703 National Priority List sites in the USA contain toxic metal contamination and at least 100000 sites are estimated in Europe[12]. Heavy metal contamination which has increased tremendously resulting from rapid industrialization which has led to environment and human health problems due to their non-degradable and persistent nature. Nowadays the scientific attention is mainly focused on four sources of heavy metals, as a consequence of their environmental impact[13].

- acid mine drainage (AMD), associated with mining operations;
- electroplating industry waste solutions;
- coal-based power generation (high coal quantities);
- nuclear power generation (uranium mining and waste generation).

Biosorption involves a number of external factors (e.g., type of metal, ionic form in solution, and the functional site) and tends to be exothermic. Other factors such as pH, temperature, biomass concentration, type of biomass preparation, initial metal ion concentration and metal characteristics, and concentration of other interfering ions, are also important in evaluating the extent of biosorption. Biosorption and recovery can be intensified in the presence of stirring induced by magnetic field[14]. Fungi have been investigated as a bio sorbent because of its capability to sequester metal ions from aqueous solutions. Fungal sorption performs well in comparison to sorption on commercial ion-exchange resins, activated carbon, and metal oxides. *Penicillium janthinellum* F-13 on different media reduces Al toxicity, but tolerance of the high external concentration of Al appears to be due to a different mechanism[15]. *Aspergillus fumigatus* was found to be suitable bio sorbent for Pb ions, especially when metal content in the aqueous solution was in concentration of 100mg/l[16]. Mushrooms interact with heavy metals physiological and morphologically. Some heavy metals have important biological roles in the fungal metabolism and some are considered toxic at certain concentration[17]. *R. atropurpurea* was confirmed as an effective Zn-absorbing species[18]. Mycoremediation properties of oyster mushroom in cultivation on soil what is contaminated by solutions with radioactive isotopes of 235Pu and 241Am was determined distributive coefficients between the ground and the fruiting body of oyster mushrooms. The average value of which was obtained for the transfer factor for plutonium was 0.72 and for americium 3.97[19]. *Cortinarius* genus have shown to accumulate heavy metal like Sn, Cu, Bi in fruiting body of mushroom, cap and stipe[20]. The marine fungi *Corollospora lacera* and *Monodictyspelagica* have been found to accumulate lead and cadmium extracellularly in mycelia[21]. *Alternaria alternate* causes volatilization of substantial amounts of selenium to the dimethyl selenide form[22]. Recently, the high potential of *P. simplicissimum* to remove Cd(II), Zn(II) and Pb(II) from aqueous solutions was reported[23]. Fluorine degradation by *P. italicum* in the presence of several cyclodextrins was reported[24]. *Fusarium oxysporum* reduces silver ions in solution thus forming stable silver hydrosol[25]. Silver nanoparticles of 5-15 nm are stabilized by proteins of the fungus. It seems that the reduction of ions occurs due to an enzymatic process.

Bioremediation of polyaromatic hydrocarbon:

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds that have accumulated in the natural environment mainly as a result of anthropogenic activities such as the combustion of fossil fuels. Interest has surrounded the occurrence and distribution of PAHs for many decades due to their potentially harmful effects to human health[26]. Low molecular weight PAHs, such as naphthalene, acenapthene, acenaphthylene, fluorine, anthracene, and phenanthrene are transformed rapidly by many bacteria and fungi. High molecular weight PAHs, however are more recalcitrant in the environment and resist both chemical and microbial degradation[27]. Extracellular peroxidases and laccases have been shown to oxidize recalcitrant compounds in vitro. Degradation of anthracene and pyrene in spiked soil by straw-grown explorative mycelium of *Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus* showed the importance of MnP and LAC levels secreted into the soil[28]. Deuteromycete fungus, *Cladosporium sphaerospermum* was able to degrade PAHs in non-sterile soils (average 23%), including high molecular weight PAHs, after 4 weeks of incubation[29]. In a study degradation of PAH was determined against concentration of PAH in non-treated contaminated soils after 14 weeks of incubation results showed removal of PAH in the two industrial soils by*I. lacteus* were: fluorene (41 and 67%), phenanthrene (20 and 56%), anthracene (29 and 49%), fluoranthene (29 and 57%), pyrene (24 and 42%), chrysene (16 and 32%) and benzo[*a*]anthracene (13 and 20%). In the same two industrial soils *P.ostreatus* degraded the PAH with respective removal figures of fluorene (26 and 35%), phenanthrene (0 and 20%), anthracene (19 and 53%), fluoranthene (29 and 31%), pyrene (22 and 42%), chrysene (0 and 42%) and benzo[*a*]anthracene (0 and 13%)[30]. After 192 h of incubation, *Cyclothyrium* sp. was able to degrade simultaneously 70, 74, 59 and 38% of phenanthrene, pyrene, anthracene and benzo[a]pyrene[31].

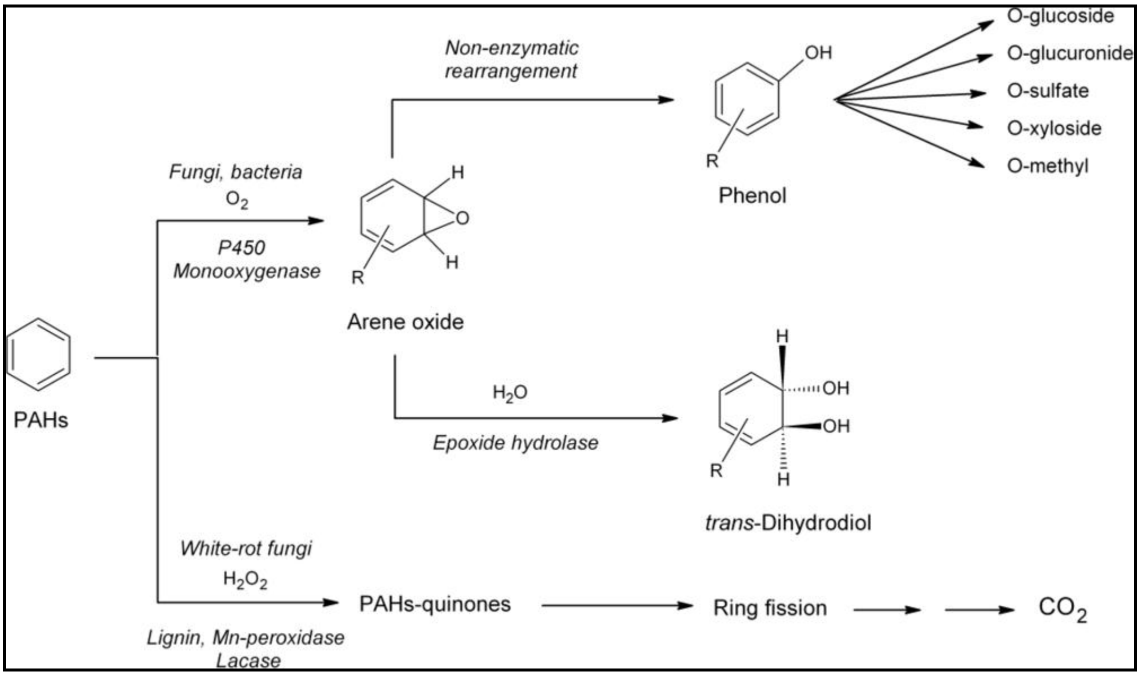

Figure 1. Initial steps in the degradation pathways of polycyclic aromatic hydrocarbons (PAHs) by fungi[32].

White rot fungi in bioremediation: enzyme system of wrf

White-rot fungi are basidiomycetes that are capable of degrading a lignocellulose substrate. Extracellular enzymes involved in the degradation of lignin and xenobiotics by white-rot fungi include several kinds of laccases, peroxidases, and oxidases producing H_2O_2[33]. Lignin peroxidases are capable of mineralizing a variety of recalcitrant aromatic compounds[34]. The ability of fungi to degrade lignocellulosic materials is due to their highly efficient enzymatic system. Fungi have two types of extracellular enzymatic systems; the hydrolytic system, which produces hydrolases that are responsible for polysaccharide degradation and a unique oxidative and extracellular ligninolytic system, which degrades lignin and opens phenyl rings[35]. A white-rot basidiomycete, isolated from decayed acacia wood (from Northwest of Tunisia) and identified as *Trametestrogii*, was selected in a broad plate screening because of its ability to degrade commercial dyes[36]. *Phanerochaetechrysosporium*, *Pleurotus ostreatus*,*Trametes versi-color* and *Bjerkandera* sp. BOL13 were tested for their ability to degrade the endocrine-disrupting compound nonylphenol at an initial concentration of 100 mg l^{-1}. The highest removals were achieved with *T. versicolor* and *Bjerkandera* sp. BOL13, which were able to degrade 97 mg l^{-1} and 99 mg l^{-1} of nonylphenol in 25 days of incubation, respectively[37]. In a study *Coriolusversicolor* decolorised reactive dye Remazol Brilliant Violet to almost 90%. The fungal mycelia removed color as well as COD up to 95% and 75%, respectively[38]. White rot fungi are excellent mycoremediators of toxins held together by hydrogen-carbon bonds. Enzymes secreted by white rotters include lignin peroxidases, and laccases. Extra-cellular lignin modifying enzymes have very low substrate specificity so they are able to mineralize a wide range of highly recalcitrant organapollutants that are similar in structure to lignin.

Laccase:

Laccase (benzenediol, oxygen oxidoreductases, EC 1.10.3.2) is one of the few lignin-degrading enzymes that have been extensively studied since 18th century. Until recently laccases were reported from eukaryotes e.g. fungi, plants and insects[39]. Laccase due to their broad substrate specificity and to the fact that they use molecular oxygen as the final electron acceptor instead of hydrogen peroxide as used by peroxidases. This makes laccases highly interesting for a wide variety of processes, such as textile dye decolouration, pulp bleaching, effluent detoxification, biosensors and bioremediation[40]. Laccase is involved in the pigmentation process of fungal spores, the regeneration of tobacco protoplasts, as fungal virulence factors, and in lignification of cell walls and delignification during white rot of wood[41].

Environmental pollutants degraded by white rot fungi:

Polychlorinated biphenyls

Doratomyces nanus, *Doratomyces purpureofuscus*, *Doratomyces verrucisporus*, *Myceliophthora thermophila*, *Phomaeupyrena*, and *Thermoascus crustaceus* showed remarkable degradation ability (>70 %) regardless of the number of chlorine substituents on the biphenyl nucleus and a high tolerance towards PCBs[42].

Dyes:

Synthetic dyes are widely used in the textile industries environmental legislation are imposed for the control the release. Dyes are mostly recalcitrant in nature. These dyes have got serious impact on human health. Dyes used in the textile industry are designed to resist fading upon exposure to sweat, light, water, oxidizing agent and microbial attack. During processing 15% of the total world textile dye production (about 800000 tons/year) are released into the process water[43].

Degradation experiments were carried out in N-rich (C:N ratio, 11.6:1) and N-limited, 116:1) conditions at a dye concentration of 100 mg/liter. *B. adusta* degraded 85% of the dyes in 7 days and *P. tremellosa* 79% in 9 days in N-rich media. 86% of the effluent was degraded in 9 days by *B. adusta* and 74% by *P. tremellosa* in 11 days in N-limited conditions[44]. In a study white rot fungus *Thelephora* sp. Showed decolourization of azo dyes such as orange G (50 μM), congo red (50 μM), and amido black 10B (25 μM). Decolourization using the fungus was 33.3%, 97.1% and 98.8% for orange G, congo red and amido black 10B, respectively[45].

Pesticides :

Many xenobiotic compounds have medium to long-term stability in soil, and their persistence results in significant impact on the soil ecosystem[46]. For fungal systems, bioremediation requires the soil to be aerobic with the provision of enough oxygen to enable effective colonization to occur. Very often, urban application of pesticides is carried out at an excessively high concentration, resulting in pesticide waste characterized by prolonged persistence[47]. Filamentous fungi are also more tolerant of environmental stress and can produce copious amounts of extracellular enzymes during hyphal colonization of soil, resulting in enhanced rates of bioremediation[48]. *P. chrysosporium* in liquid culture have reported biotransformation of the insecticide lindane independently of the production of ligninolytic enzymes[49].

Polycyclic aromatic compound:

Polycyclic aromatic hydrocarbons (PAHs) are widespread in various ecosystems and are pollutants of great concern due to their potential toxicity, mutagenicity and carcinogenicity. Because of their hydrophobic nature, most PAHs bind to particulates in soil and sediments, rendering them less available for biological uptake[50].The high hydrophobicity of polycyclic aromatic hydrocarbons (PAHs) greatly hamper their degradation in liquid media. The use of an organic solvent can assist the degradative action of ligninolytic enzymes from white rot fungi. Anthracene was degraded to phthalic acid. A ring cleavage product of the oxidation of dibenzothiophene, 4-methoxybenzoic acid[51]. Degradation of four representatives of PAHs (phenanthrene, anthracene, fluoranthene, and pyrene) was tested and the enzyme showed the ability to degrade them in vitro. The role of MnP in PAH degradation by I.lacteus, including cleavage of the aromatic ring (Baborova *et al.*, 2006). Agrocybe sp. CU-43, a white-rot fungus isolated from Thailand, showed a high potential for degrading both low- and high-molecular weight polycyclic aromatic hydrocarbons. At 100 ppm fluorene was degraded by 99% within six days while at the same concentration 99 and 92% degradation of phenanthrene and anthracene, respectively[53]. *A. cylindrospora* and maltosyl-cyclodextrin could be used successfully in fluorene bioremediation systems[54].

Conclusion:

Fungi are considered as natural decomposers which can significantly reduce and degrade persistent and highly toxic pollutant. Mycoremediation can be augmented by adding carbon sources at polluted sites and providing optimum condition to increase degradation process. Naturally present community of microbes acts in concert with the fungi to decompose the contaminants. White rot fungi are extremely effective in decomposing toxic aromatic pollutants, Heavy metals, Dyes, chemical pollutants etc.

Further studies may be helpful in understanding the mechanism and optimizing the process of degradation. Benefit is offered that land that is contaminated and unfit for agriculture could be both restored and made to yield a nutritious food crop.

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