INTRODUCTION
The word lignin is derived from the Latin word "Lignum", meaning wood and was used to describe the non-cellulosic encrusting substances present in wood. Lignin does not represent a definite uniform compound, is a collective form for substances that have very similar chemical properties but very different molecular weights. The molecular weight of lignin may reach the range of 100,000 daltons or even greater. A considerable part of the photosynthetic activity in plants is devoted to the conversion of atmospheric carbon dioxide to lignin. Lignin constitutes about 40% of the solar energy stored in plants. Hence, it plays a highly significant role in the carbon cycle.

Lignin is the second most abundant renewable biopolymer on earth next to cellulose. It is concentrated mainly in the spaces between the cell walls of adjacent cells (middle lamella) and in to the $S_2$ layer of the cell wall where it is deposited during the lignification process of the plant (wood) tissues. Lignin in the cell wall not only encrusts the cellulose microfibrils in a sheath like manner, but is also bonded physically and chemically to hemicelluloses. Physically lignin forms a barrier against the penetration of cellulases and hemicellulases (Fenegel and Wegener, 1983). Lignocellulosic materials comprise about 95% of the earth's land-based biomass, about 25% of which is lignin.
Biosynthetically, lignin is an amorphous, three-dimensional aromatic polymer composed of oxyphenylpropane units. It is formed by polymerization of cinnamyl alcohols or monolignols, viz., \( p \)-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Fig.1.1) through a variety of non-hydroxyl, stable C-C and C-O-C bonds. \( p \)-coumaryl alcohol is a minor component of grass and forage type lignins. Coniferyl alcohol is the predominant lignin monomer found in softwoods. Both coniferyl and sinapyl alcohols are the building blocks of hard wood lignin.

Phenylalanine is a biosynthetic precursor for all the three cinnamyl alcohols. Once the cinnamyl alcohols are formed, enzymes (peroxidases and laccases) initiate the process of polymerization by breaking the covalent bond between the phenolic oxygen and its hydrogen. This yields free radical species in mesomeric forms. These radicals then couple in a non-enzymatic and random fashion to form dilignols, oligomeric intermediates and finally lignin macromolecules.

There are three main types of lignin, depending on its structural monomeric units:

1. Guaiacyl lignin, which occurs in conifers, is mainly a dehydrogenation polymer of coniferyl alcohol.
Fig. 1.1

Phenyl propanoid monomers of lignin

\[ \text{p-coumaryl alcohol} \]

\[ \text{coniferyl alcohol} \]

\[ \text{sinapyl alcohol} \]
2. Guaiacyl-syringyl lignin, which occurs in angiosperms, is composed of a mixed dehydrogenation polymer of coniferyl and sinapyl alcohols.

3. Guaiacyl-syringyl-p-hydroxyphenyl lignin, which found in grasses, is composed of a mixed dehydrogenation polymer of coniferyl, sinapyl and p-coumaryl alcohols (Higuchi, 1980).

Lignin performs a number of functions essential to the life of plants. It serves as energy storage system, permanent bonding agent between cells, impediment to penetration of destructive enzymes, inhibitor to enzymic degradation of other plant components, antioxidant, UV light stabilizer, flame retardant and water proofing agent.

**Microbiology of lignin degradation:**

There are some compounds, which are naturally occurring and are also recalcitrant. They are resistant to biodegradation in the sense that they are not rapidly degraded in nature. Lignin is a complex polymeric compound released during decomposition of plant materials falling in this group. Its biological degradation is important phase of the biospheric carbon-oxygen cycle. The efficiency of lignin decomposition in nature is evident from the lack of accumulation of lignin on a year-to-year basis. Efforts to
find and isolate microorganisms with lignin decomposing ability have already born fruits and the range of microorganisms known to attack lignin has been expanded.

Microorganisms respond to the presence of organic materials by growing rapidly and using the easily available parts of the organic material. Different populations of microorganisms then use more resistant parts until a recalcitrant or resistant fraction remains. The order of use is often, carbohydrates→proteins→cellulose and hemicellulose→lignin and associated compounds. There are distinct populations of microorganisms able to degrade each of these categories of organic materials.

**Bacteria:**

Most of the literature on bacterial degradation of lignin deals with the decomposition of monomeric and dimeric compounds such as methoxylated aromatic acids. Only a few strains have been shown to be capable of attacking lignin and lignin derivatives. The most active strains are those from actinomycetes, such as *Streptomyces* and *Nocardia* sp. Noncellulolytic gram negative aerobic bacteria viz. *Pseudomonas*, *Xanthomonas* and *Acinetobacter*, have been reported to be capable of degrading dioxane and milled wood poplar lignins at rates ranging
between 4% to 20%. Two strains of *Bacillus polymyxa* caused a loss of 42% in lignin content of Scots pine sapwood (Benner et al., 1984). Antai and Crawford (1981) showed that *Streptomyces* could degrade grass and corn lignin more extensively than hard wood (maple) and soft wood (spruce) lignins.

Bacteria have also been investigated for their use in the degradation of industrial lignin. Chlorinated lignin from bleachery discharges, which is known to be resistant to biodegradation, was used, but no bacterial strain was found to depolymerize this lignin. However, isolation of several mesophilic and thermophilic bacteria capable of degrading kraft lignin at significant rates (16-98% in 5 Days) has been reported.

Some bacteria may degrade lignin only in association with other strains. Mixed cultures of *Bacillus* and *Cellulomonas* strains delignified untreated bark chips, where as pure cultures of these bacteria did not show any delignifying ability. Lignin degradation is primarily an aerobic process, and in an anaerobic environment lignin can persist for very long periods (Van Soest, 1994). Benner et al. (1984) reported that only 4.5% degradation of wood occurred after 246 days under strict anaerobic conditions. Bacteria are unable to compete with fungi if conditions are
favourable for fungal attack. Thus, extensive bacterial attack will only occur where fungal attack is limited (Blanchette et al., 1990).

**Fungi:**

Although it is supposed that lignin degradation is the result of the cooperative action between different fungi, bacteria and other microflora in the soil, fungi still remain the most studied organisms of all the other inhabitants of soil. Wood-rotting fungi are the only organisms that have clearly demonstrated extensive degradation of lignin to carbon dioxide.

Based solely on the components utilized and type of decay they cause, fungi are classified as white rot (basidiomycetes and a few ascomycetes), brown rot (basidiomycetes) and soft rot (ascomycetes and fungi imperfecti) fungi (Bakshi, 1976).

**Soft rot fungi:** This group contains species that are capable of degrading cellulose and hemicellulose and may partially digest lignin. The soft rots are particularly prevalent at the early stages of wood decay and in conditions of high moisture and increased nitrogen content. Wood affected by soft rot may appear wet, spongy or pitted. There are over 300
species of known soft rots. These include many filamentous micro fungi (the molds) such as *Cephalosporium*, *Acremonium*, and *Chaetomium*.

**Brown rot fungi**: The brown rot fungi are also capable of degrading cellulose and hemicellulose but are unable to digest the lignin component of wood. They invade the lumens of wood cells where they secrete enzymes. In this case, the inner layers of the wall are heavily degraded and lignin remains intact (Bravery, 1971). As a result, infected portion appears as a brown, crumbly matrix. Unlike the soft rots, the brown rot fungi are relatively few in numbers, comprising less than 6% of all wood-decay fungi. All of these species are members of the Basidiomycota (the mushrooms).

**White rot fungi**: This group of fungi contains mostly macro fungi that produce visible reproductive structures. They are important especially in the forest ecosystem since they are the only fungi capable of degrading all cell wall components (lignin, cellulose, hemicelluloses) of wood. Some white rot fungi were found to remove all cell wall components simultaneously (simultaneous rot) while others were found to remove lignin and hemicelluloses first, leaving the cellulose (corrosive rot or preferential rot) (Otjen and Blanchette, 1986; Eaton and Hale, 1993).
Coriolus versicolor is one of the most efficient lignin degrading white rot basidiomycetes, causing simultaneous attack on lignin, cellulose and hemicellulose (Addleman and Archibald, 1993). Trametes versicolor, Heterobasidion annosum, Phanerochaete chrysosporium, Phlebiopsis gigantea, Pleurotus ostreatus, Pycnoporus sanguineus, Schizophyllum commune, Nia vibrissa, Donkioporia expansa, Daldinia concentrica, Asterostroma ochroleucum, Phellinus contiguus and Ganoderma (Fomes) applanatum are other examples of white rot fungi (Eaton and Hale, 1993).

Visual symptoms of white rot decay:

At the early colonisation phase of decay, damage is limited. As decay develops, slight changes in color, wood texture, and fibre brashness may appear. A bleached or mottled appearance is a common feature of the early stages of some white rots (e.g. T. versicolor, G. applanatum). In the advanced stage, gradual thinning of the cell walls from the lumina toward the middle lamella occur and the late stage is when the wood structure is totally disrupted and the residual wood has become a brownish amorphous, whitish punky or fibrous material (Zabel and Morrell, 1992; Eaton and Hale, 1993).
Chemical changes in wood caused by decay fungi:

Carbohydrate components of wood are depolymerized primarily by a series of hydrolytic enzymes, where as lignin is degraded primarily by oxidative enzymes.

Lignin is efficiently degraded in nature primarily by white rot fungi, including many litter decomposing basidiomycetes. Lignin breakdown is accomplished by a limited group of specialised fungi (white roters). Lignin decomposition proceeds by oxidative reactions that separate carbon to carbon bonds or ether linkages and separate various functional groups, side chains, and aromatic rings randomly from the huge, amorphous lignin macromolecule.

Looking to the history of white rot fungus and bioremediation, in the 1970s, the main focus was to define laboratory conditions under which white rot fungi, especially *P. chrysosporium*, maximally mineralize lignin. In the 1980s, biochemistry of lignin modifying enzymes was predominantly studied and the enzyme, "ligninase" was thought to be directly involved in lignin biodegradation (Hatakka, 1994). In the 1990s, in addition to detailed studies on catalytic and enzymatic properties of lignin modifying peroxidases and their molecular biology, major lines of
research have involved applications of enzymes in biopulping and pulp bleaching, remediation of xenobiotics and a search for the enzymes responsible for lignin degradation in more selective lignin degraders i.e. fungi which degrade larger amounts of lignin relative to carbohydrates.

Much of the research on the lignin degrading enzyme systems has been centred on the white rot fungi, then also, there is little information available on the lignolytic enzymes of brown rot fungi, soft rot fungi and actinomycetes (Orth and Tien, 1995; Ruttimann et al., 1992). Crawford et. al. (1993) reported that a recombinant actinomycete, *Streptomyces lividans* TK 231, expressing a pIJ702-encoded extracellular lignin peroxidase showed mineralization of lignin. Sinegani et. al. (2000) showed production of lignolytic enzymes by the imperfect fungi, *Aspergillus terreus* and *Trichoderma reesei* and the yeast.

**Biochemical features of white rot:**

The major features of lignin include phenolic and methoxy substitutions on the aromatic rings and a wide variety of bonds linking the monomeric phenylpropanoid units that are polymerised to a high molecular weight lignin. Such a diverse substrate can not be degraded by a single enzyme, but requires multiple enzymes to effect depolymerisation, mainly through
the generation of free radicals during reaction. Typical active species include the formation of radical cations in the substrate, peroxide and oxygen free radicals. These radicals are able to attack other phenolic and non-phenolic aromatic substrates, many of which occur as industrial pollutants in soils and water columns. Moreover, due to the large size of the polymer, degradation must take place in an extracellular fashion; the carbon–carbon and ether bonds joining subunits together must be cleaved via an oxidative mechanism. Thus, complex but non-specific enzyme systems are needed for lignin to be degraded.

As stated by Kirk (1984), "The [enzyme] system must differ fundamentally from those involved in the biodegradation of all other important biopolymers, and meaningful analogies can not be readily drawn with other systems".

White rot basidiomycetes tend to harbour gene families encoding the key enzymes responsible for depolymerization of lignin: laccase, lignin peroxidase and manganese peroxidase (Hatakka, 1994). Varying combinations of these enzymes are produced by different species of white rot fungi. The existence of gene families for these extracellular enzymes may be a consequence of their diverse roles in fungal physiology. In addition to these key enzymes, veratryl alcohol (VA), a degradation
product of lignin, and glyoxal oxidase (GLOX), an extracellular peroxide-generating enzyme, also have important functions in lignin degradation. Thus, having versatile machinery of enzymes, the white rot fungi are able to attack directly the "lignin barrier." These enzymes may function separately or co-operatively (Andrzej et al., 2001). Substantial variability exists in the levels and types of lignolytic enzymes produced by different white rot fungi.

**Laccase (p-diphenol:oxygen oxidoreductase, EC 1.10.3.2)** is a copper-containing oxidase produced abundantly by many lignin-degrading white rot fungi. Laccase utilizes molecular oxygen as oxidant and also oxidizes phenolic rings to phenoxy radicals (Eriksson et. al., 1990). Among the consequences of the one-electron oxidation in lignin related phenols are Cα-oxidation, limited demethoxylation and aryl-Cα-cleavage. It also has capacity to oxidize non-phenolic compounds under certain conditions e.g. if the reaction mixture is supplemented with 2,2'-azinobis-(3)-ethylbenzothiazoline-6-sulphonate (ABTS) (Bourbonnais and Paice, 1990). The enzyme may also cause cleavage of phenolic rings when substituted with bulky groups (Higuchi, 1989).

Laccase may interact directly with phenolic components of lignin or, in the presence of a 'mediator' compound, react with a broader range of
substrates including non-phenolic compounds. A mediator is a co-substrate, the most often cited is ABTS, which functions as a diffusable lignin-oxidizing agent. The existence of mediators was postulated because of the fact that purified laccases can not react directly with the intact fibrous cell wall. This indicated that another component was necessary and mediators were hypothesized to be low molecular weight 'oxidizing vehicles' secreted by fungi (Camarero et al., 1998; Bourbonnais et al., 1997). A natural mediator, 3-hydroxyanthranilic acid, has been detected in the white rot fungus *Pycnoporus cinnabarinus* (Eggert et al., 1996). The paucity of information on the natural occurrence of laccase mediators has not slowed the development of effective laccase-mediator systems to enhance ligninolytic activity (Archibald et al., 1997; Bourbonnais et al., 1997). For example, several phenothiazines capable of forming cation radicals have been tested; however, to date, molecules in this class appear too unstable to act as effective mediators.

Low concentrations of laccases are produced constitutively but higher concentrations can be induced by addition of aromatic compounds. Carbon source can induce laccase activity and regulation of laccase expression can differ greatly among species (Mansur et al., 1997). Investigators have induced laccase activity by heat and cadmium
exposure and suggested that laccase induction may be part of some stress responses (Fink-Boots et al., 1998).

More than 17 laccase genes from a variety of white rot fungi have been cloned and sequenced (Kirk and Cullen, 1998). Most organisms contain multiple laccase genes, at least five in *Trametes villosa* and four in *Rhizoctania solani*. Mansur et al. (1997) showed that at least seven laccase isozymes occur in a recently characterized white rot isolate, designated I-62. These gene families contain closely related proteins that differ slightly in their substrate spectrum or regulation. Laccase has been cloned and expressed in *Saccharomyces cerevisiae*.

**Lignin peroxidase (LiP., ligninase, EC 1.11.1.14)** are heme-containing peroxidases excreted by some white rot fungi as components of their lignolytic multienzyme complexes. These peroxidases utilize hydrogen peroxide and organic peroxides to catalyze oxidative cleavage of both synthetic non-phenolic lignin models and many other oxidation-proof compounds (chloroorganic pesticides, carcinogenic hydrocarbons, etc.). LiP from *P. chrysosporium* was initially isolated by various chromatographic procedures and was shown to contain one mole of protoheme IX per mole of enzyme, have molecular mass of 41-42 KD
and be glycosylated (Gold et al., 1984; Kuwahara et al., 1984; Tien and Kirk, 1984).

LiPs are strong oxidizers capable of catalyzing the oxidation of phenols and aromatic amines, aromatic ethers and polycyclic aromatic hydrocarbons. LiP is initially oxidized by $\text{H}_2\text{O}_2$ to form a two-electron deficient intermediate termed compound I. Compound I oxidizes substrates by one electron and forms compound II, a more reduced enzyme intermediate. Compound II can in turn oxidize substrates by one electron and return the enzyme to its resting state (Collins et al., 1997).

LiPs are more powerful oxidative agents than other peroxidases with exceptionally high oxidation-reduction potential and this makes them capable of oxidizing the non-phenolic aromatic moieties that can comprise up to 85% of the lignin polymer. Whether an aromatic nucleus is a substrate for LiP depends in part on its oxidation potential (Hammel et al., 1986). Strong electron-withdrawing substituents such as $\text{C}_\alpha$-carbonyl group tend to inactivate aromatic nuclei by LiP, whereas alkoxy groups activate it. The positions of the latter groups also affect oxidation by LiP. In lignin, the positions of alkoxy groups are set, but the number varies from one to three. The nature and pattern of the substituents also affect the subsequent reactions of the cation, including
nucleophilic attack by water or an internal hydroxyl group, loss of the acidic portion at Cα and Cα - Cβ cleavage (Kirk et al., 1986b).

The phenolic substrates are oxidized to yield products similar to those produced by classical peroxidases, while the oxidation of the nonphenolic methoxybenzenes is unique to the lignin peroxidases (Kersten et al., 1985). The oxidation of these substrates to yield aryl cation radicals can result in demethoxylation, Cα-Cβ cleavage of lignin model compounds, benzylic alcohol oxidation and hydroxylation of aromatic rings and side chains (Kirk and Farrell, 1987).

The substrate range for the enzyme is very broad, with reactivity being largely determined by redox potential. Lignin peroxidase can catalyze the oxidation of substrates with a reduction potential greater than 1.3 volts. The enzyme has been shown to oxidize lignin monomers, dimers and trimers as well as polycyclic aromatic compounds such as benzopyrene (Haemmerli et al., 1986).

Manganese dependant peroxidases (MnP, EC 1.11.1.13):
Kuwahara et al. (1984) discovered a peroxidase activity different from LiP in extracellular growth fluid of lignolytic cultures of P. chrysosporium. The isolated 46 KD enzyme exhibited a requirement
for \( \text{H}_2\text{O}_2 \), Mn (II) and lactate. Huynh and Crawford (1985) isolated a similar enzyme of 45-47 KD requiring Mn (II) and \( \text{H}_2\text{O}_2 \) but no lactate. Later on both the enzymes were designated as manganese dependant peroxidases.

MnP are heme containing glycoproteins which require hydrogen peroxide as an oxidant. MnP oxidises Mn (II) to Mn (III) which in turn oxidises phenol rings to phenoxy radicals which leads to decomposition of compounds (Hatakka, 1994). It has also been reported that when Mn (II) is present, MnP will peroxidate unsaturated lipids causing the formation of lipoxyradical intermediates capable of oxidizing non-phenolic model lignin compounds. To date, five isozymes have been detected in \textit{P. chrysosporium} MP-1. \textit{C. subvermispora} appears to produce as many as 11 isozymes of MnP (Kirk and Cullen, 1998).

Manganese-independant peroxidases have also been described in white rot fungi by several workers (Waldner et al., 1988; Lobarzewski, 1990; Nerud et al., 1991; Vares et al., 1992). Lignin-degrading peroxidases are present in nearly all ligninolytic fungi, but may be expressed differentially in different species. Substantial variability exists in the levels and types of ligninolytic enzymes produced by different white not fungi (Orth et al., 1993).
In the past few years, remarkable progress has been achieved in the cloning and sequencing of lip, mnp, and laccase genes from a variety of white rot fungi. Both homologous and heterologous expression of lip and mnp genes has been reported and molecular approaches to study the regulation of their expression have been described. Expression of the Pycnoporus cinnabarinus laccase gene in A. niger and characterization of this recombinant enzyme has been reported (Reddy, 1995).

Several other extracellular enzymes have been implicated as \( \text{H}_2\text{O}_2 \) producing enzymes for lignin degradation. To name a few, glyoxal oxidase, aryl alcohol oxidase, pyranose-2-oxidase, glucose-1-oxidase and glucose-2-oxidase.

Glyoxal oxidase (GLOX) is thought to be a major source of extracellular \( \text{H}_2\text{O}_2 \) and is produced in the culture under the growth conditions that are identical for the production of LiP (Kersten, 1990). GLOX acts by transferring electrons from low molecular weight aldehydes to \( \text{O}_2 \), resulting in the formation of \( \text{H}_2\text{O}_2 \) (Kirk and Cullen, 1998). Substrates for GLOX include glyoxal and glycoaldehyde, extracellular metabolites secreted by \( P. \text{chrysosporium} \). GLOX genes have been cloned from \( P. \text{chrysosporium} \). Kersten et al. (1995) were able to show expression and secretion of GLOX in \( A. \text{nidulans} \), when under control of a maltose-
inducible expression system, at levels 50-fold greater than in
*P. chrysosporium*. Aryl alcohol oxidase is also an $H_2O_2$ generating
enzyme. It catalyzes the conversion of benzyl alcohols to the aldehydes
and in the process transfers electrons to $O_2$ generating $H_2O_2$ (Kirk and
Cullen, 1998).

Veratryl alcohol oxidase, cellobiose quinone oxidoreductase and glucose
quinone oxidoreductase are some of the other enzymes involved in lignin
degradation (Kirk and Farrell, 1987).

As discussed above, white rot fungi secrete multiple and relatively non-
specific enzymes which metabolize lignin as well as environmental
pollutants by both, oxidative and reductive reactions. Moreover, the
ability to generate free radical mechanism mediated by extracellular
peroxidases make the fungi of choice for bioremediation.

**Bioremediation:**

Until the appearance of the human race on Earth, natural processes have
continuously taken place, with microorganisms as the principal agents in
the nitrogen, carbon, phosphorus and sulphur cycles *in situ*. The human
population’s unbalanced activities, along with uncontrolled release of
recalcitrant, xenobiotic and toxic compounds, has raised the need for the development of additional effective remediation processes.

Xenobiotics are widely employed in our day-to-day life. These compounds are either resistant to biodegradation or are metabolized incompletely or slowly and consequently persist and accumulate in the environment. They pose major disposal problem for all industrialized nations and a need exists for effective and permanent solution, particularly since millions of tons of wastes are generated each year that require some degree of detoxification before they can be safely released into the environment. Although physical and chemical methods are available for the treatment of hazardous wastes, bioremediation is accepted as an environmentally sound and economic treatment.

Bioremediation is defined as a technology that exploits microorganisms and its limits in the use of the same as environment clean up tools. It is also a process by which living organisms act to degrade hazardous organic contaminants or transform inorganic contaminants to environmentally safe levels in soils, subsurface materials, water, sludges and residues (Layton et al., 1998). Depending on the situation, laboratory-cultured indigenous microflora (if present) or "superbugs" developed and grown in the laboratory could be used.
The potential of the white rot fungi for *in situ* bioremediation has been attributed to their ability to degrade a variety of xenobiotic chemicals via a free radical mechanism mediated by extracellular peroxidases. Several reasons account for the attractiveness of white rot fungi in the decontamination of pollutant sites: They are capable of mineralizing a wide variety of toxic xenobiotics, ubiquitously present in the natural environment, easily grow on agro-waste, do not require preconditioning to a particular pollutant, oxidize substrates that have low solubility etc. The key enzymes of fungi are expressed under nutrient-deficient conditions, which are prevalent in many soils (Barr and Aust, 1994). The *P. chrysosporium* system has been found active against diverse substrates such as DDT, lindane, PCBs, TNT and crystal violet, with substantial mineralization in many cases (Higson, 1991).

**Bioremediation of heavy metals:**

Heavy metals comprise a group of approximately 65 metallic elements. They have diverse physical, chemical and biological properties but generally, all of them exert toxic effects. Although elevated levels of toxic heavy metals occur in some natural locations, on an average, their presence is generally low. Most of the naturally occurring high concentrations of heavy metals occur in immobilized form in sediments and ores and is biologically unavailable. However, ore mining and many
other modern industries have disrupted natural biogeochemical cycles and have caused increased deposition of heavy metals in the terrestrial and aquatic environment.

Major sources of heavy metal pollution are combustion of fossil fuels, mineral mining and processing, nuclear and other industrial effluents, sludges, brewery and distillery wastes, biocides and preservatives including organometallic compounds. Many industrial activities can lead to the pollution by heavy metal at local sites of environment. Heavy metals which are common pollutants of environment due to industrial activities are copper, zinc, cadmium, iron, mercury, lead, nickel, silver, gold and radioactive elements like uranium, thorium etc.

Although, solvent extraction, hydroxide precipitation, evaporative concentration, electrowining and membrane concentration like technologies are available, bioremediation of heavy metals is more widely used method today.

Bioremediation of heavy metals could be brought about by employing methods such as bioaccumulation, biosorption, bio-precipitation and uptake by purified biopolymers from microbial cells. (Churchill et al., 1995; Simmons et al., 1995; Naomi, 1991). Recently several plants have
also been tried to remove heavy metals from polluted water (Sulochana et al., 1998). All these methods are alternatives to the conventional physical and chemical methods.

Metabolism independent binding or adsorption of heavy metals to living or dead cells, extracellular polysaccharides, capsules and slime layers, all referred to as "Biosorption". Walls and envelopes of bacteria, yeasts, algae and fungi are very efficient in biosorption due to the charged groups present on them.

Advantages of biosorptive processes over physico-chemical methods in accumulation of heavy metals from environment can be listed as follows:

- High efficiency at low metal concentration.
- Successful operation over a wide range of pH and temperature.
- Calcium and magnesium ions compete for binding sites in ion exchange resin columns, which does not happen for biosorption.
- Ligands of biological origin are soft in comparison to resins.
- Microbial biomass required for biosorption may be available as a fermentation waste product from enzymes, antibiotics, organic acids and vitamins producing units. Application of this biomass as bioadsorbant serves two purposes: (a) Removal of toxic metals
from effluents, (b) Overcoming disposal problem for fungal biomass.

- Microbial biomass may be specifically grown, using cheap substrates.
- Biosorptive processes are cost-effective when compared with other physico-chemical methods.
- Recovery of accumulated metal and regeneration of bioadsorbant is relatively easy. Also, scaling up is possible when high flow rates of effluent are expected.

Microbial systems used in biosorption are given in Table-1.1.

Cell walls of fungi contain many anionic sites such as amines, carboxyl, hydroxyl and phosphate groups. The adsorption is executed by live fungi, dead biomass, isolated fungal walls, chitins, chitosan, fungal glucans and pigments of fungi.

**Bioremediation of PAHs:**

The term 'Polycyclic Aromatic Hydrocarbons' (PAHs) commonly refers to a large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. At ambient temperatures, PAHs are solids. The general characteristics common to the class are high melting and boiling points, low vapour pressure, and very low water solubility that tend to decrease with increasing molecular mass.
Table 1.1
Microorganisms involved in biosorption of heavy metals (Jogdand, 1995).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Metals adsorbed</th>
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<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas fluorescence</em></td>
<td>nitrate, lead, zinc</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>uranium, plutonium</td>
</tr>
<tr>
<td>“Resting cells” of <em>Citrobacter</em></td>
<td>cadmium, lead, copper</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>gold, palladium</td>
</tr>
<tr>
<td><em>Streptomyces phaceromogenes</em></td>
<td>gold, palladium</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus arrhizus</em></td>
<td>uranium, thorium, cadmium</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>silver, zinc</td>
</tr>
<tr>
<td><em>Aspergillus oryzae</em></td>
<td>cadmium</td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>zinc, uranium</td>
</tr>
<tr>
<td><em>Trichoderma viride</em></td>
<td>copper</td>
</tr>
<tr>
<td><strong>Algae</strong></td>
<td></td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>uranium, gold, silver, mercury</td>
</tr>
<tr>
<td><em>Ascophyllum nodosum</em></td>
<td>cadmium, lead</td>
</tr>
<tr>
<td><em>Sargassum natans</em></td>
<td>cadmium</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em></td>
<td>lead</td>
</tr>
<tr>
<td><em>Halimeda opuntia</em></td>
<td>chromium</td>
</tr>
</tbody>
</table>
PAHs are soluble in many organic solvents and are highly lipophilic. They are chemically rather inert. Reactions that are of interest with respect to their environmental fate and possible sources of loss during atmospheric sampling are photodecomposition and reactions with nitrogen oxides, nitric acid, sulfur oxides, sulfuric acid, ozone, and hydroxyl radicals. Structures of some common PAHs are given in Fig. 1.2. Environmental pollution with PAHs has attracted much attention in recent decades. Being hydrophobic in nature they persist within ecosystems and carcinogenic substances may be formed during metabolism of PAHs in humans and microorganisms (Means et al., 1980; Miller and Miller, 1981).

PAHs are degraded by photodegradation, biodegradation by microorganisms, and metabolism in higher biota. In general, they are biodegraded under aerobic conditions, the biodegradation rate decreasing drastically with the increase in number of aromatic rings (Cerniglia and Heitkamp, 1989). Under anaerobic conditions, degradation is much slower.

Studies on the role of microorganisms in PAH degradation revealed that two main groups of microorganisms are involved in the oxidation and subsequent mineralization of these compounds: soil bacteria and white
Fig. 1.2
Structural formula, solubilities and carcinogenicities of model PAHs

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>Solubility (mg/l)</th>
<th>Carcinogenicity</th>
</tr>
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<tbody>
<tr>
<td>Naphthalene</td>
<td>128.2</td>
<td>31.7</td>
<td>Non-carcinogen</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>154.2</td>
<td>3.9</td>
<td>Non-carcinogen</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178.2</td>
<td>0.07</td>
<td>Non-carcinogen</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178.2</td>
<td>1.3</td>
<td>Non-carcinogen</td>
</tr>
<tr>
<td>Pyrene</td>
<td>202.3</td>
<td>0.14</td>
<td>Non-carcinogen</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>228.3</td>
<td>0.002</td>
<td>Carcinogen</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>252.3</td>
<td>0.003</td>
<td>Carcinogen</td>
</tr>
</tbody>
</table>

Source: Cerniglia (1992)
rot fungi. The degradation of PAHs is limited by their low water solubility (Means et al., 1980). Whereas soil bacteria were found to effectively degrade low molecular weight PAHs, white-rot fungi can also oxidize more condensed PAH molecules with up to six aromatic rings and therefore decrease their toxicity (Muncnerova and Augustin, 1994).

Pathways for microbial catabolism of PAHs are given in Fig. 1.3. PAHs oxidized by different species of bacteria, fungi and algae are illustrated in Table-1.2, 1.3 and 1.4 respectively.

The initial reactions of PAH degradation by white rot fungi are usually ascribed to their extracellular ligninolytic enzymes, i.e., laccase, lignin peroxidase, and Mn-dependent peroxidase (MnP) (Paszczynski & Crawford, 1995; Collins et al., 1996). Under natural conditions, these enzymes attack the polyphenolic molecule of lignin, the principal component of wood. However, due to their low specificity, ligninolytic enzymes can also attack molecules structurally similar to lignin, including halogenated organic compounds and PAHs (Shah et al., 1992). Purified enzymes are able to transform PAHs in vitro and attempts have therefore been made to apply these fungi for the bioremediation of soils contaminated with compounds not sufficiently degradable by other soil microorganisms (Collins et al., 1996; Lang et al., 1997).
**Fig. 1.3**
Pathways for microbial catabolism of PAHs

Source: Cerniglia (1992, 1997)
Table 1.2
PAHs oxidized by different species of bacteria.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td><em>Acinetobacter calcoaceticus</em>, <em>Alcaligenes denitrificans</em>, <em>Corynebacterium renale</em>, <em>Mycobacterium sp.</em>, <em>Pseudomonas sp.</em>, <em>Rhodococcus sp.</em>, <em>Moraxella sp.</em>, <em>Streptomyces sp.</em>, <em>Bacillus cereus</em></td>
</tr>
<tr>
<td>Acenaphthene</td>
<td><em>Beijerinckia sp.</em>, <em>Pseudomonas sp.</em></td>
</tr>
<tr>
<td>Anthracene</td>
<td><em>Beijerinckia sp.</em>, <em>Mycobacterium sp.</em>, <em>P. putida</em>, <em>P. cepacia</em>, <em>Rhodococcus sp.</em>, <em>Flavobacterium sp.</em>, <em>Arthrobacter sp.</em></td>
</tr>
<tr>
<td>Benz[a]Anthracene</td>
<td><em>Alcaligenes denitrificans</em>, <em>Beijerinckia sp.</em>, <em>P. putida</em></td>
</tr>
<tr>
<td>Benzo[a] pyrene</td>
<td><em>Beijerinckia sp.</em>, <em>Mycobacterium sp.</em></td>
</tr>
</tbody>
</table>

### Table-1.3

PAHs oxidized by different species of fungi.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>Aspergillus niger, Basidiobolus ranarum, candida utilis, claviceps paspali, Cunninghamella elegans, C. japonica, Gliocladium sp., Neurospora crassa, P. chrysogenum, Rhizopus oryzae, R. stolonifer, Zygorhynchus moelleri</td>
</tr>
<tr>
<td>Anthracene</td>
<td>Bjerkandera sp., C. elegans, P. chrysosporium, Rhizoctonia solani, T. versicolor</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>C. elegans</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>C. elegans, P. chrysosporium, T. versicolor, P. ostreatus</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>C. elegans</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>Aspergillus ochraceus, Bjerkandera sp., Candida maltosa, C. elegans, Neurospora crassa, Penicillium sp., P. chrysosporium, T. versicolor, Trichoderma viride</td>
</tr>
</tbody>
</table>

Table-1.4

PAHs oxidized by different species of algae.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td><em>Oscillatoria</em> sp.(strain JCM and strain MEV), <em>Nostoc</em> sp., <em>Agmenellum quadruplicatum</em>, <em>Anabaena</em> sp., <em>Aphanocapsa</em> sp., <em>Chlorella autotrophica</em>, <em>Chlamydomonas angulosa</em>, <em>Amphora</em> sp., <em>Synedra</em> sp., <em>Navicula</em> sp., <em>Porphyridium cruentum</em></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td><em>Oscillatoria</em> sp.(strain JCM), <em>A. quadruplicatum</em></td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td><em>Selenastrum capricornutum</em></td>
</tr>
</tbody>
</table>

Biobleaching:

In pulp and paper industries, mechanical, semi chemical and chemical processes are frequently used methods for bleaching of wood. Many chemical pulping processes use sulfur-based compounds and during pulping, environmentally troublesome sulfur containing lignin waste products are produced. These waste products must be further processed to meet water and air quality requirements and sludge must be properly disposed of (Smook, 1982). The processing of these waste products is costly even when chemical recovery processes are employed. Ways are needed to overcome these problems. One potential route is to use biological pulping agents.

White rot basidiomycetes are important in this regard because of their unique capacity to degrade all wood components. Many of these fungi possess the ability to selectively degrade lignin, leaving a significant portion of the cellulose intact (Otjen et al., 1987). Cellulose fibers left by these fungi after wood decay could be advantageously used as an animal feed. Biological pretreatment of wood chips before mechanical pulping has resulted in improved strength properties of the paper produced from the pulp while decreasing the refining energy requirements (Myers et al., 1988). In addition, enzymes from white rot fungi may be used for
biological bleaching of crude pulp and for treatment of waste effluents generated in pulp and paper production. Other lignocellulosic materials (e.g., sugarcane bagasse and straw) can also be advantageously treated with white rot fungi to improve digestibility or produce pulp fiber suitable for paper production (Zadrazil et al., 1980).

**Bioremediation of dyes:**

Gujarat is one of the most leading industrial states with remarkable growth of chemical and dyestuff industries. The dye manufacturing units are in different industrial estates of the state. Vatva, Naroda and Odhav near Ahmedabad, Nandesari and Makarpura near Baroda, Ankleshwar near Surat and Atul, and Vapi near Valsad are the major industrial areas where small, medium and large scale industries are located. A survey carried by Sudarshan Chemical Industries, Pune for Green Environment Service Co-operative Society Limited (GESCSL), a society established by Gujarat Dyestuff Manufacturing Association, Ahmedabad, revealed that major problem causing pollutants are vinyl sulphone, H-acid, J-acid, direct dyes, reactive dyes, acid dyes and other intermediates. For all these industries the major problem is effluent treatment. Quantity of effluent generated by various units varies from 2 to 20 m³/day. Common Effluent Treatment Plant (CETP) at Vatva receives 16,000 m³/day influent from...
industries of Vatva estate only. (CETP-Vatva: A Step Towards Better Environment, GESCSL).

Approximately 10,000 different dyes and pigments are used industrially and over $7 \times 10^5$ tons of these dyes are produced annually worldwide (Young and Yu, 1997). Based on the chemical structure of the chromophoric group, synthetic dyes are classified as azo dyes, anthraquinone dyes, triarylmethane dyes etc. Azo dyes constitute the largest of these groups with the greatest variety of colors used in industry (Anliker, 1979; Meyer, 1981). They are a group of compounds characterized by the presence of one or more azo bonds in association with one or more aromatic systems (So, 1989).

**Basic structure of azo dyes:**

All azo dyes contain at least one, but more usually two, aromatic residues attached to the azo group. They exist in a more stable *trans* rather than *cis* form (Fig. 1.4).

**Types of azo dyes:**

Azo dyes can be classified on the basis of their applications (Chatwal, 1997). They are-

- Acidic azo dyes- Methyl orange, Methyl red, Acid red, Fast red A, Ponceau 2R, Orange G, Naphthol blueblack 6B, Carbolan dyes etc.
Fig. 1.4

Basic structure of azo dyes

\[
\begin{align*}
R & \quad N = N \quad R' \\
\text{Trans-form} & \quad \text{Cis-form}
\end{align*}
\]
- Basic azo dyes-Aniline yellow, Butter yellow, Chrysodine G, Bismark brown etc.
- Direct or substantive azo dyes-Congo red, Direct deep black, Benzopurpurin, Rosanthrene, Procion dyes etc.
- Ingrain azo dyes- Para red and Nitraniine red
- Mordant azo dyes-Diamond black F, Chromotrope 2B, Eriochrome black T, Eriochrome red B etc.
- Synthetic fibre dyes
- Stilbene azo dyes

They also exhibit great structural variety and so as a group, they are not uniformly susceptible to microbial attack (Meyer, 1981). Several amino-substituted azo dyes including 4-phenyl azoaniline and N-methyl- and N,N-dimethyl-4-phenylazoanilines are mutagenic as well carcinogenic (McCann and Ames, 1975).

Inefficiency in the dyeing process, poor handling of spent effluent and insufficient treatment of wastes of the dye stuff industries lead to contamination of environment including soil and water bodies (Nigam et al, 1996). Considerable concern exists over the release of synthetic dyes and / or colored effluents into the environment from textile and dyestuff industries due to aesthetic reasons and potential toxicity and
carcinogenicity of chromophore or its degradation products. Moreover, environmental legislation is being imposed to control the release of dyes, in particular azo-based compounds, into the environment and therefore, remediation of these compounds is of prime importance.

Despite the existence of a variety of chemical and physical treatment processes, such as coagulation, flocculation, precipitation membrane filtration, ion-exchange, electrochemical destruction, photochemical destruction, ozonation and adsorption; bioremediation of textile effluent and contaminated soil is still seen as an attractive solution due to its reputation as a low cost, environmentally friendly and publicly acceptable treatment technology (Banat et al., 1996).

Microorganisms involved in decolorization and degradation of dyes:

Bacteria:

Blhmel et al. (1998) reported the isolation of an unidentified bacterial strain S5 capable of utilizing the model sulphonated azo compound 4-carboxy-4'-sulfoazobenzene (CSAB) as sole carbon and energy source.

The initial step in bacterial azo dye metabolism under anaerobic conditions involves the reductive cleavage of the azo linkage. This process is catalysed by a variety of soluble cytoplasmic enzymes with
low-substrates specificity, which are known trivially as “azoreductases”. Under anoxic conditions, these enzymes facilitate the transfer of electrons via soluble flavins to the azo dye, which is then reduced (McMullan, 2001).

So far, it is believed that azo dyes are not typically degraded under aerobic conditions (Anliker, 1979). Several combined anaerobic and aerobic microbial treatments have been suggested to enhance the degradation of dyes (Bortone, 1995). However, under anaerobic conditions, the azo linkage can be cleaved by azo-reductases to form aromatic amines which are colorless but can be toxic and carcinogenic (Meyer, 1981). Furthermore, azo reductases have been shown to be very specific enzymes, thus cleaving only azo bonds of selected dyes (Zimmermann, 1984).

Chung et al. (1992) demonstrated anaerobic metabolism of azo dyes with the formation of aromatic amines. But, further anaerobic degradation of these compounds has not been reported (Pasti-Grigsby et al., 1992). Stolz et al. (2000) determined localization of the enzyme system involved in anaerobic reduction of azo dyes by Sphingomonas sp. and recently named this species Sphingomonas xenophaga after its ability to “eat foreign compounds”. Haug et al. (1991) observed the mineralization of azo dye
Mordent yellow 3 by a bacterial consortium. Total degradation of the sulfonated azo dyes was achieved by using an alternating anaerobic-aerobic treatment. Some examples of bacteria capable of dye decolorization are given in Table-1.5.

**Actinomycetes:**

Actinomycetes, particularly *Streptomyces* spp., are known to produce extracellular peroxidases that have a role in biodegradation of lignin. The ability of actinomycetes to decolorize and mineralise textile dyes was initially investigated by three groups (McMullan et al., 2001). In 1989, Ball et al. screened 20 strains of actinomycetes, representing wide genera, for their ability to decolorise the polymeric dye Poly R. Only three of the 20 strains were observed to significantly decolorise the dye: *Streptomyces badius* 252, *Streptomyces* sp. strain EC22 and *Thermomonospora fusca* MT800.

Pasti and Crawford (1991) investigated the mechanism by which *Streptomyces chromofuscus* A11 decolorized and mineralized azo dyes. Initial work confirmed that decolorization was related to the ligninolytic capabilities of the isolate but the bacterial enzymatic systems responsible for degradation of azo dyes had a different specificity than those of white rot fungus *P. chrysosporium* (Paszczynski et al., 1992).
Table-1.5

Bacteria capable of dye decolorization.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em> Var 24B</td>
<td>azoreductase (cell free extract)</td>
<td>Yatome et al., 1987</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>azoreductase (in growing cells)</td>
<td>Yatome et al., 1991</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> RS-13</td>
<td>azoreductase</td>
<td>Wong and Yuen, 1996</td>
</tr>
<tr>
<td><em>Pseudomonas cepacia</em> 13NA</td>
<td>azoreductase (cell free extract and growing cells)</td>
<td>Yatome et al., 1991</td>
</tr>
<tr>
<td><em>Pseudomonas luteola</em></td>
<td>azoreductase</td>
<td>Hu, 1954</td>
</tr>
<tr>
<td><em>Pseudomonas stutzeri</em> IAM 12097</td>
<td>azoreductase (cell free extract)</td>
<td>Yatome et al., 1991</td>
</tr>
</tbody>
</table>
Algae:
Several species of *Chlorella* and *Oscillatoria* are capable of degrading azo dyes to their aromatic amines and to further metabolize these amines to simpler organic compounds or CO₂. Some were even capable of utilizing few azo dyes as their sole source of carbon and nitrogen. (Banat et al., 1996). Jinqi and Houtian (1992) have reported algae capable of degrading azo dyes through an induced form of an azoreductase.

Fungi:
Over the past decade many studies have demonstrated that, due to their non-specific lignin degrading system, white rot fungi are able to degrade a broad spectrum of structurally diverse organopollutants including synthetic dyes (Paszczynski & Crawford, 1995; Swamy and Ramsay, 1999).

The decolorization of dyes by white rot fungi was first reported by Glenn and Gold (1983). The aerobic degradation of the three azo dyes Congo red, orange II and tropaeolin O by the fungus *P. chrysosporium* was described for the first time by Cripps et al. (1990). Whilst *P. chrysosporium* remains the most widely studied of white rot fungi, *Trametes (Coriolus) versicolor*, *Bjerkandera adusta*, *Pleurotus* and *Phlebia* species, as well as a variety of other isolates are increasingly
being studied (Heinfling et al., 1998; Conneely et al., 1999; Swamy and Ramsay, 1999; Kirby et al., 2000; Pointing et al., 2000). Unfortunately, due to the inherent complexity of both, the dye molecules themselves and the enzymatic mechanisms involved, the degradative pathway utilised by white rot fungi other than \textit{P. chrysosporium} remain unstudied, despite the exploitation of powerful analytical techniques (Smyth et al., 1999). Some examples of fungi and the mechanisms used by them for dye decolorization are given in Table-1.6.

White rot fungi are also being utilized for soil bioremediation. Besides the transformation capabilities of the fungi, the following factors will be indispensible for the utilization of white rot fungi for soil bioremediation:

1. Long-lasting growth and survival of the fungus (under remediation condition).

2. Production of degradative enzymes under remediation conditions, ie. during growth on solid substrates like straw or wood shavings and under competition with the microbiota.

3. Productions of the degradative enzymes in the soil or transport in to soil (so that the catalytic protein and the pollutants come in contact).
**Table-1.6**

Fungi capable of dye decolorization.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Myrothecum verrucaria</em></td>
<td>adsorption</td>
<td>Brahimi-Horn et al., 1992</td>
</tr>
<tr>
<td><em>Myrothecum sp.</em></td>
<td>adsorption</td>
<td>Mou et al., 1991</td>
</tr>
<tr>
<td><em>Neurospora crassa</em></td>
<td>adsorption</td>
<td>Corso et al., 1981</td>
</tr>
<tr>
<td><em>Pycnoporus cinnabarinus</em></td>
<td>extracellular oxidases</td>
<td>Schliephake et al., 1993</td>
</tr>
<tr>
<td><em>Trichordma sp.</em></td>
<td>lignolytic enzymes</td>
<td>Prasad and Joyce, 1991</td>
</tr>
<tr>
<td><em>Candida sp.</em></td>
<td>adsorption</td>
<td>De Angelis and Rodrigues, 1987</td>
</tr>
<tr>
<td><em>P. chrysosporium</em></td>
<td>lignolytic enzymes</td>
<td>Glenn and Gold, 1983</td>
</tr>
<tr>
<td><em>Geotrichum candidum</em></td>
<td>glycosylated heme based peroxidase</td>
<td>Kim and Shoda, 1999</td>
</tr>
</tbody>
</table>
A range of reactor systems aimed at optimising production of lignolytic enzymes has been developed, including stirred tank reactors (Linko, 1988) and rotating disc reactors (Kirk et al., 1986a). Kirby (1999) utilized the rotating tube bioreactor system to investigate the remediation of actual textile effluent by *P. chrysosporium*.

**Present study:**

Xenobiotics are widely employed in our day-to-day life. These compounds are recalcitrant compounds. Owing to toxic, mutagenic and/or carcinogenic in nature, disposal is of prime importance for eco-friendly and clean environment.

White rot fungi and their lignolytic systems (being the non-specific in nature) are successfully employed in bioremediation system.

In present study, we have isolated a white rot fungus from local environment and checked for lignolytic enzyme production. Studies on optimization of environmental conditions and nutritional requirements were carried out for maximum enzyme production. Enzymes were purified and characterized for further applications in bioremediation.
Since, our plan was to exploit the isolated fungus in bioremediation, we checked the ability of the fungus to grow on agro-waste-an economical way of bioremediation.

Attempts have been made to mineralize PAHs, to decolorize dyes, to biosorb heavy metals and to biobleach the wood pulp utilizing lignolytic enzymes and the fungus itself.