Chapter I

Review of Literature
1.1. ENVIRONMENTAL POLLUTANT AND HUMAN HEALTH

One of the major environmental problems facing the world today is the contamination of soil, water and air by toxic chemicals. Eighty billion pounds of hazardous organopollutants are produced annually in the world and only 10% of these are disposed off safely (Reddy and Mathew, 2001; Husain and Husain, 2008). There are several classes of compounds that have been selected as priority pollutants due to their negative impact on the environment and human health (Dayan, 1993). Persistent organic pollutants (POPs); like polycyclic aromatic hydrocarbons (PAHs), aromatic amines, benzidine, dioxins, bisphenol A (BPA) and other endocrine disrupting compounds (EDCs), pentachlorophenol (PCP), polychlorinated biphenyl (PCBs), 2,4,6 trinitrotoluene (TNT) and phenols are known to have mutagenic/carcinogenic effects. Most of the POPs have been derived from anthropogenic sources and such compounds are a matter of great concern for humans. Halogenated derivatives of biphenyl and diphenyl ether are very toxic substances. These are widespread in industrial applications as heat transfer fluids or flame retardants and serve as the basic structure for many herbicides such as nitrofen (2,4-dichlorophenyl-4′-nitrophenylether) and bifenox (5-(2,4-dichlorophenoxy)-2-nitrobenzoic acid). These compounds showed similarity to lignin which is a highly recalcitrant wood component. Polychlorinated-dibenzo-p-dioxins, dibenzofurans, and biphenyls are part of the larger family of polyhalogenated aromatic hydrocarbons which are well-known environmental pollutants. Colored pollutants such as anthraquinone based dyes were also most resistant to degradation due to their fused aromatic structure. Basic dyes have high brilliance and therefore exhibit higher color intensity which made them more difficult to decolorize. Metal-based complex dyes such as chromium-based dyes can lead to the release of chromium, which was carcinogenic in nature (Hou et al., 2004; Fang et al., 2004). All such compounds pose a serious threat to the health and vitality of the earth system. Recently it has been reported that phenol and their various derivatives have contaminated several industrial wastewater streams particularly those from the oil refining, paper and pulp, dye manufacturing, polymer processing and fiber industries. These compounds represent a potential danger to human health because majority of these have been reported as toxic and some of them
are known or suspected carcinogens (Wright and Nicell, 1999; Sakurai et al., 2003; Wilberg et al., 2006). Some of these aromatic pollutants are highly toxic and causes many problems related to human health. Thus, detailed description of these pollutants became necessary before their remediation from polluted water.

Most toxic chemical compounds among POPs are the dioxins and dioxin like compounds. Dioxin is the term given to a group of chemical compounds with 75 polychlorinated dibenzo-\(p\)-dioxins and 135 polychlorinated dibenzofurans (Baldrian et al., 2000; Cajthaml et al., 2002). Dioxins are present in traces in all matrices in the environment. It has been observed that such compounds were accumulated in environment by binding to the organic matter in sediments and soils and such compounds can be transported over long distances from the source of emission. These compounds can also be quickly transported to great distances through evaporation and condensation cycles in environment. As a result of global circulation patterns and low evaporation rates in cold climates, dioxins tend to accumulate in arctic regions where such types of chemicals bio-accumulate in living organisms and pass to humans through the food chain (Cajthaml et al., 2002; Veitch, 2004). The main source of dioxins in the textile industry are dioxazine and anthraquinone dyes and pigments, produced from chloranil as an intermediate product and chloranil itself used as a catalyst in the production of dyes and pigments (Venkata et al., 2005). \(\alpha\)-Chloranil is formed as the final product of the chlorate HCl oxidation of many aromatic compounds because of its great resistance to further oxidation. Chloranil is also used in tier manufacture. Dibenzo-\(p\)-dioxins and 135 polychlorinated dibenzofurans are formed during the synthesis of \(\alpha\)-chloranil from chlorinated phenols. Exposure to even low doses of dioxins can lead to cancer, damage to the nervous system, disease of immune system and reproductive disorders. Dioxins appear to act like extremely persistent synthetic hormones. They bind effectively to specific aryl hydrocarbon receptors in living cells and trigger a chain of reactions resulting in biochemical and cellular changes. Over recent decades, a great deal of emphasis has been put on evaluating dioxin-like toxicity. As these compounds showed strong affinity to aryl hydrocarbon receptors, 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (Eljarra and Barcelo, 2003).
Exposure to aromatic amines occurs in different industries and agricultural activities. These compounds have been used as an antioxidant in the production of rubber and cutting oils and also act as intermediates in azo dye and pesticides manufacturing (Zhu et al., 2002). These compounds are common contaminants in several working environment including the chemical and mechanical industries. Human exposure to carcinogenic aromatic amines arose from the dye manufacturing industries as early as from late nineteenth century (Weisburger, 1997; Soares et al., 2006). Aromatic amines can enter the environment as contaminants of azo colorants from their own manufacturing processes but there are other significant roots that include their use in subsequent industrial sector such as textile, paper, plastic and lacquers. Therefore, most of the attention concerning the possible hazardous arising from the use of azo dyes has been transferred to the presence of selected aromatic amines (Pinheiro et al., 2004; Hai et al., 2007). Epidemiological evidences on the relationship between aromatic amines and cancer risk has been reviewed (Acquawell et al., 1999). In particular, cancer risk in humans resulting by exposure to aromatic amines from occupational sources and tobacco smoking was assessed with reference to ecologic, cohort and case-control studies (Robinson et al., 2001).

Benzidine is an aromatic amine with a molecular weight, (Mₐ) of 184.2 and it is a serious pollutant. However, accidental releases from closed systems potentially could result in environmental exposure through inhalation, ingestion or dermal contact. It is moderately persistent in the environment, exposure of populations living near benzidine or benzidine dyes manufacturing or waste-disposal sites may be a matter of great concern (England et al., 1998; Bi et al., 2003). It has been used for over a century as an intermediate in the production of azo dyes, sulfur dyes, fast color salts, naphthols and other dyeing compounds. In the past, this aromatic amine also has been used in the manufacturing of rubber, plastic films, for detection of H₂O₂ in milk and for quantitative determination of nicotine in drugs (Stavric, 2000; Hai et al., 2007). It has been listed as a carcinogenic agent for the first time in the First Annual Report on Carcinogens and dyes metabolized to benzidine were listed in the Ninth Report on Carcinogens (Tannenbaum, 2000). Numerous epidemiological studies of workers in various geographical locations have reported a strong association between occupational exposure to benzidine and bladder cancer. Some workers have also
reported an association between benzidine exposure and cancer at other tissue sites; i.e., liver, kidney, central nervous system, oral cavity, larynx, esophagus, bile duct, gallbladder, stomach and pancreas. The evidence for an association with benzidine was more limited for these cancers than for bladder cancer (Pinheiro et al., 2004).

Recently, great attention has been paid to the toxicity caused by environmental BPA. The level of BPA in fresh and sea water samples ranged from 0.010-0.268 μg L⁻¹. It is released by the combustion of polycarbonates or epoxy resin products. BPA from the hazardous waste landfill is one of the most important sources of environmental pollution (Welshons et al., 2006; Vandenbarg et al., 2007). It has also been shown that BPA leached from lacquer coated carpet and baby feeding bottles (vom-Saal et al., 2007). Its presence in environment has been reported due to leakage of the polymer caused by thermal treatment. BPA was also found in saliva collected from patients who were treated with a bisphenol A diglycidyl ether (BPADGE) based dental sealant. It is extensively used in epoxy resins lining food and beverage containers and as a monomer in polycarbonate plastics in many consumer products. Most studies on the health effects of BPA have focused on well documented estrogenic activity (Takeuchi et al, 2004; vom-Saal and Hughes, 2005), liver damage (Elsby et al., 2001; Tyl et al., 2002), disrupted pancreatic β-cell function (Ropero et al., 2008), thyroid hormone disruption and obesity-promoting effects (Newbold et al., 2008). The potential for low dose effects has added to the controversy about possible hazards and whether currently recommended exposure thresholds require revision (Welshons et al., 2006; vom-Saal et al., 2007; Dekant and Volkel, 2008).

Anthracene is an ubiquitous pollutant released into the environment, generated during incomplete combustion of solid and liquid fuels or derived from industrial activities. Thus, a soil from gas works sites and carbochemical plants as well as refineries and filling stations are often contaminated with anthracene. There is toxicological concern about the presence of anthracene in the environment; since some workers have shown that anthracene and other PAHs has mutagenic and carcinogenic properties (Keith and Telliard, 1979). The natural biodegradation of anthracene is mainly restricted due to low water solubility and its hydrophobicity. Therefore, such types of compounds tend to accumulate on the organic matter in soil and thus, it is not easily degraded by microorganisms (Zheng and Obbard, 2002). The
effects of high environmental exposure to PAHs on the thyroid were observed. Thyroid volume, hypoechogenicity and nodules, presence of antithyroid peroxidase antibodies and abnormal thyroid-stimulating hormone levels in serum (by radioimmunoassay) were examined in adults from the PAHs-polluted areas of various countries of the world. Very high levels of PAHs were found in the polluted area (7300 +/- 871 ng g^-1 lipids). In males from the polluted area, the frequencies of thyroid hypoechogenicity, thyroid nodules, positive anti-thyroid peroxidase antibodies and abnormal thyroid-stimulating hormone level were higher as compared to males from the non polluted areas. It showed that increased thyroid volume and indicators of potential thyroid dysfunction were associated with long-term environmental exposure to PAHs (Hofrichter, 2002). The carcinogenicity of PAHs and its derivatives in humans has also been reported by some earlier workers (Culp et al., 1998). The carcinogenicity of PAHs was demonstrated in 1915 (Phillips, 1983), when it was shown that exposure of ears of rabbits to PAHs derivatives caused tumors at the site of application and a few years later such material proved to be tumorigenic in mice by skin painting. In the late 1920s, dibenz[a,h]anthracene and benzo[a]pyrene (B[a]P) were proved to be carcinogenic in mouse (Phillips, 1983; Dipple, 1985). A recent year’s bioassay with mice demonstrated that coal tar from gasification plant waste sites mixed in the feed at 0.01–1.0% induced tumors in the liver, lung, stomach and other organs. A comparison of the results indicated that lung and liver tumors appeared to be due to other genotoxic components in coal tar besides B[a]P (Culp et al., 1998). Many PAHs have been tested by topical application to the skin of mice or by subcutaneous injection to identify relationship between structural characteristics of the compounds, their metabolism and tumorigenic potency. Many PAHs were considered to be complete carcinogens; thus the compounds were both tumor initiators and promoters.

1.2. TREATMENT OF ORGANOPOLLUTANTS BY CLASSICAL METHODS

Several physical and chemical methods; i.e., electrochemical oxidation, membrane filtration, coagulation/flocculation, sorption, ion exchange, electrolysis, adsorption, advanced oxidation processes (chlorination, bleaching, ozonation, fenton
oxidation and photocatalytic oxidation) and chemical reduction have been used for the treatment of aromatic compounds (Hao et al., 2000; So et al., 2002). Such factors, like; pollutant type, wastewater composition, dose, costs of required chemicals, operational costs, environmental fate and handling costs of generated waste products determine the technical and economic feasibility of each single compound removal technique. Above mentioned physico-chemical techniques have their own limitations. The use of one individual process might often not be sufficient to achieve complete oxidation of all types of compounds. One major disadvantage of ion exchange was the high operation cost (Mishra and Tripathy, 1993). The high cost of chemicals for precipitation and coagulation as well as for pH adjustments problems associated with dewatering and disposing of generated sludge and high concentration of residual cation levels which remained in the supernatant (Anjaneyulu et al., 2005). Electrochemical methods are expensive due to large energy requirements and limited stability of the electrodes (Vandevivere et al., 1998). The disadvantage of ozonation is its short half-life (typically being 20 min) demanding continuous application making it a cost intensive process (Xu and Lebrun, 1999). In Fenton oxidation, large volumes of waste sludge are generated at higher pH (Aplin and Waite, 2000). Drawbacks of the photo-catalytic oxidation process are the relatively high costs and the occasional lack of effectiveness (Hao et al., 2000).

1.3. BIOLOGICAL METHODS OF TREATMENT

Recently, biological treatment of aromatic pollutants has attracted much attention. Many researchers have demonstrated partial or complete biodegradation of these chemicals by pure and mixed cultures of bacteria, fungi and algae (Pearce et al., 2003; Soares et al., 2006). An advantage of biological treatment over certain physico-chemical treatment methods was that over 70% of the organic material present in waste might be converted to bio-solids (Anjaneyulu et al., 2005).
1.3.1. Bacterial biodegradation

*Bacillus subtilis* was the first bacterial culture which was used for the degradation of aromatic compounds. Later on several other bacteria were also found to be responsible for the degradation of various types of aromatic pollutants, these included *Pseudomonas* sp, *Escherichia coli* and sulfate reducing bacteria (Anjaneyulu et al., 2005). Mixed bacterial cultures from a wide range of habitats have been exploited for the degradation of aromatic pollutants (Knapp and Newby, 1995; Field et al., 1995a). Metabolic versatility of bacteria allowed them to degrade aromatic pollutants (Eduardo, 2004). Microorganisms that biosynthesize broad-specificity oxygenases initiated metabolism of linear and branched-chain alkanes, nitroalkanes, cyclic ketones, alkenoic acids, chromenes and trichloroethylene (TCE). Bacteria that contained nitropropane dioxygenase, cyclohexanone monooxygenase, cytochrome P-450 monooxygenases, 4-methoxybenzoate monooxygenase and hexane monooxygenase did not degrade TCE. Five mycobacterium strains that were grown on propane as the sole source of carbon and energy degraded TCE. *Mycobacterium vaccae* JOB5 degraded TCE more rapidly and to a greater extent than the four other propane-oxidizing bacteria (Wackett et al., 1989; Caputi et al., 2005).

A *Beijerinckia* sp. and a mutant strain of *Beijerinckia* sp. strain B8/36 were shown to cooxidize the PAHs, acenaphthene and acenaphthylene. Both organisms oxidized acenaphthene to the same spectrum of metabolites, which included 1-acenaphthenol, 1-acenaphthenone, 1,2-acenaphthenediol, acenaphthenequinone and a compound that was tentatively identified as 1,2-dihydroxycacenaphthylene. In contrast, acenaphthylene was oxidized to acenaphthenequinone and the compound identified as 1,2-dihydroxycacenaphthylene by the wild-type strain of *Beijerinckia*. Both of these products were also formed when the organism was incubated with synthetic cis-1,2-acenaphthenediol. A metabolite identified as cis-1,2-acenaphthenediol was formed from acenaphthylene by the mutant *Beijerinckia* sp. strain B8/36 (Schocken et al., 1984). A catabolically diverse microbial community, consisting of bacteria, fungi and algae metabolizes aromatic compounds. Molecular oxygen is essential for the initial hydroxylation of PAHs by microorganisms. In contrast to bacteria, filamentous fungi use hydroxylation as a prelude to detoxification rather than to catabolism and assimilation. Relationship between the chemical structure of the PAHs and the rate of
PAHs biodegradation in aquatic and terrestrial ecosystems were also unique for the degradation of these compounds (Carl, 1992). In another study 46 strains of phenol/benzoate degrading iron reducing bacteria were isolated from long term irrigated tropical paddy soils by enrichment procedures. Pure and some mixed cultures were examined for ferric oxide reduction and phenol/benzoate degradation. All isolates were iron reducers, but only 56.5% iron reduced to phenol and benzoate after one week incubation (Lu et al., 2008). Some early isolated bacterial strains; *Paenibacillus* sp., *Aneurinibacillus aneurinilyticus* and *Bacillus* sp., have been shown to decolorize kraft lignin in 6 d. The release of low Mr aromatic compounds by these bacterial strains during degradation of kraft lignin was analyzed by gas chromatography-mass spectrometry (GC-MS) analysis (Raj et al., 2007). To date, bacterial transformation of organophosphorous pesticides is the main focus of research. *Pseudomonas aeruginosa*, *Clavibacter michiganense* (Singh and Singh, 2003), *Arthrobacter atrocyaneus*, *Bacillus megaterium* and *Pseudomonas mendocina* (Bhadbhide et al., 2002), *Agrobacterium radiobacter* (Horne et al., 2002) and other *Pseudomonas* species (Ramanathan and Lalithakumari, 1999) have been reported to degrade monocrotaphos in solutions and soils.

### 1.3.2. Fungal biodegradation

The white rot fungus (WRF) *Phanerochaete chrysosporium* degraded 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT), 3,4,3′,4′-tetrachlorobiphenyl, 2,4,5,2′,4′,5′-hexachlorobiphenyl, 2,3,7,8-tetrachlorodibenzo- *p*-dioxin, lindane (1,2,3,4,5,6-hexachlorocyclohexane) and benzo[a]pyrene to CO₂. Model studies, based on the use of DDT, have shown that the ability of *Phanerochaete chrysosporium* to metabolize these compounds was dependent on the extracellular lignin degrading enzyme system of the fungus (Bumpus et al, 1985; Barr and Aust, 1992). Many species of WRF were capable of degrading a number of xenobiotic compounds including PAHs, PCBs, insecticides, antibiotics and surfactants through the production of extracellular enzymes (Field et al., 1992; Andersson et al., 2001; Hatakka et al., 2001; Rigas et al., 2005; Kornillowicz-Kowalska et al., 2006; Soares et al., 2006; Cheng et al., 2007). Studies on pesticide metabolism by other fungi such as *Aspergillus niger* have demonstrated, the degradation of pyrethroid (Liang et al., 2005), fenitrothion (Kanaly et al., 2005), carbaryl (Zhang et al., 2003), dimethoate
(Liu et al., 2001) and metolachlor by *Aspergillus flavus* (Sanyal and Kulshrestha, 2004). There are few reports on the degradation of other organophosphorous pesticides such as pyrazophos, dimethoate, malathion, lancer, profenfos derivatives by fungi like *A. flavus* and *Aspergillus sydowii* (Hasan, 1999). All these reports emphasized the enormous potential of soil fungi for bioremediation of persistant aromatic pollutants.

### 1.3.3. Algal biodegradation

Microalgae enhanced the removal of nutrients, organic contaminants, heavy metals and pathogens from domestic wastewater and furnish an interesting raw material for the production of high value chemicals (algae metabolites) or biogas. Photosynthetic O₂ production also reduces the need for external aeration, which is especially advantageous for the treatment of hazardous pollutants that must be biodegraded aerobically but might volatilize during mechanical aeration. Recent studies have shown that the use of microalgae produced O₂ which is required to acclimatize bacteria to biodegrade hazardous pollutants such as PAHs, phenolics and organic solvents. Well-mixed photobioreactors with algal biomass recirculation are recommended to protect microalgae from effluent toxicity and optimize light utilization efficiency (Lu et al., 2007). Limited amount of work is available on the application of algae in the degradation of aromatic pollutants (Jinqui and Houtian, 1992; Balcoglu et al., 2007). Bioconversion and transformation of PAH and benzo [a] pyrene by brown, red, green and chara algae have also been reported earlier (Banat et al., 1996; Kirso and Irsha, 1998).

### 1.3.4. Limitations of biological treatment

The use of microbes in treating pollutants is limited due to high costs of production of microbial culture, limited mobility, time dependent survival of cells in soil, alternative carbon source, completeness of the indigenous populations and metabolic inhibition (Husain and Jan, 2000; Duran and Esposito, 2000; Wagner and Nicell, 2003; Biswas et al., 2007). Since bacterial, fungal and algal degradation of the aromatic compounds was attributed to secondary metabolic pathways, appropriate growth conditions have to be accomplished by additional loads of chemicals.
Moreover, the expression of enzymes involved in phenols, aromatic amines and dyes degradation was not constant with time but dependent on the growth phase of organisms and was influenced by inhibitors that might be present in the effluent (Wessenberg et al., 2003).

1.4. ENZYMATIC TREATMENT

In an attempt to overcome the problems associated with the traditional chemical and biological waste treatment procedures, recent research has focused on the applications of enzymes in such fields. Due to variety of chemical transformations catalyzed by enzymes, this enzyme based technology became a focus of attention for the biotechnologist. Recent advances in this direction, through better isolation and purification procedures have allowed the production of cheaper and more readily available enzymes that can be used in many remediation processes to target specific recalcitrant pollutants present in wastewaters (Husain, 2006; Xu and Salmon, 2008; Hamid and Rehman, 2009). Enzymes isolated from their parent organisms have been often preferred over intact organisms due to greater specificity, better optimization, easy handling and store (Wagner and Nicell, 2003). Moreover, unlike chemical catalysts, the enzymatic systems have the potential of accomplishing complex chemical conversions under mild environmental conditions with high efficiency and reaction velocity (Husain and Husain, 2008; Michniewicz et al., 2008). Because of their high specificity to individual species, enzymatic processes have been developed specifically to target selected compounds that cannot be treated effectively using traditional techniques (Ryan et al., 2003; Couto et al., 2005a; Husain et al., 2009). Alternatively, enzymatic treatment has been used as a pretreatment step to remove one or more compounds that interfere with subsequent downstream treatment processes. For example, if inhibitory or toxic compounds can be removed selectively, the bulk of the organic material could be treated biologically, thereby minimizing the cost of treatment (Gianfreda and Rao, 2004). Due to the susceptibility of enzymes to inactivation by the presence of other chemicals, it is likely that enzymatic treatment will be highly suitable in those streams where the highest concentration of target contaminant and lowest concentration of other contaminants that might tend to
interfere in enzymatic treatment (Baldrian et al., 2000; Wagner and Nicell, 2003; Biswas et al., 2007; Matto and Husain, 2009a). Thus, the potential advantages of enzymatic treatment as compared to conventional treatments can be summed up as: application to recalcitrant materials, operation at low and high contaminant concentrations over a wide pH, temperature and salinity range, absence of shock loading effects, delays associated with the acclimatization of biomass, reduction in sludge volume, the ease and simplicity of controlling process, need of bio-acclimatization and remediation of various aromatic compounds under dilute conditions (Haemmerli et al., 1986; Sumathí and Manju, 2000; Borchert and Libra, 2001; Held et al., 2005; Husain, 2006).

Before the full potential of enzymes may be utilized, a number of significant issues must be considered; these included: (i) development of low-cost sources of enzymes in quantities that required at the industrial scale; (ii) demonstration of the feasibility of utilizing the enzymes efficiently under the conditions encountered during wastewater treatment; (iii) characterization of reaction products and assessment of their impact on downstream processes or on the environment into which they are released and identification of methods for the disposal of solid residues, among others. Current research has focused on these issues, particularly the development of enzyme based treatment systems that target removal of aromatic compounds from industrial effluents. The enzymatic transformation of pollutants to less toxic or even innocuous products is an alternative to their complete removal. In this regard, a number of different redox enzymes are able to transform a wide range of toxic pollutants, such as phenols, azo dyes, PAHs, PCBs and heavy metals etc. Peroxidases have already shown their potential in targeting such types of aromatic pollutants (Husain, 2006; Husain and Husain, 2008; Husain et al., 2009).

1.5. Peroxidases

Peroxidases (E.C.1.11.1.7.) are ubiquitous heme containing oxidoreductases having protoporphyrin IX with Fe\(^{3+}\) (Duarte-Vazquez et al., 2003). The iron ion is coordinated to 4 pyrrole nitrogens of the heme and nitrogen of an axial histidine.
Peroxidases have broad substrate specificity; can use various organic and inorganic substrates which act as hydrogen donors *in vitro* in the presence of H$_2$O$_2$ (Vianello et al., 1997). These enzymes are present in animals, plants and microorganisms, having $M_r$ from 30-150 kDa (Regalado et al., 2004). Based on their structural and catalytic properties, these are divided into three superfamilies: (i) peroxidases in animals (glutathione peroxidase, myeloperoxidase and lactoperoxidase); (ii) catalases in animals, plants, bacteria, fungi and yeast; (iii) peroxidases in plants, fungi, bacteria and yeast. The amino acid sequence among members of plant peroxidase superfamily had been found to be highly variable, with less than 20% identity in most of divergent cases. Based on differences in primary structure, the plant peroxidase superfamily is further categorized into three classes (Welinder, 1992). Class I, the intracellular peroxidases, includes yeast cytochrome c peroxidase, ascorbate peroxidase and bacterial peroxidases (Passardi et al., 2007). Class II consists of extracellular fungal peroxidases; lignin peroxidase (LiP) and manganese peroxidase (MnP). These are monomeric glycoproteins involved in the degradation of lignin. Class III contain secretory plant peroxidases, which have multiple tissue specific functions: e.g. removal of H$_2$O$_2$ from chloroplasts and cytosol, oxidation of toxic compounds, biosynthesis of the cell wall, defense responses towards wounding, indole-3-acetic acid catabolism, ethylene biosynthesis, etc. Some of the well known peroxidases of this class are horseradish peroxidase (HRP), turnip peroxidase (TP), bitter gourd peroxidase (BGP) and soybean peroxidase (SBP). Class III peroxidases are also monomeric glycoproteins, having four conserved disulphide bridges and require calcium ions (Conesa et al., 2002).

WRF can oxidize various recalcitrant xenobiotics released into environment by the human activities through the production of lignin modifying enzymes (LMEs). These extracellular LMEs have very low substrate specificity so they are able to mineralize a wide range of highly recalcitrant organopollutants that are structurally similar to lignin (Pointing, 2001; Cajthaml et al., 2002; Mansur et al., 2003; Veignie et al., 2004). These fungal enzymes work extracellularly on many non-polar and non-soluble toxic compounds (Reddy and Mathew, 2001; Levin et al., 2003).

LiP also known as ligninase or diaryl propane oxygenase. It is a heme-containing glycoprotein and has an unusually low pH optimum. It catalyzed oxidation
of non-phenolic aromatic lignin moiety and variety of similar compounds (Tien and Kirk, 1983; Christian et al., 2005). The oxidation of such compounds by LiP resulted in cleavage of \( C_\alpha-C_p \) bond, the aryl–\( C_\alpha \) bond, aromatic ring opening, phenolic oxidation and demethoxylation. Due to its high redox potential and enlarged substrate range in the presence of specific mediators, it has been used to mineralize a variety of xenobiotic compounds including PAH, polychlorinated phenols, nitro aromatics and azo dyes (Krcmar and Ulrich, 1998; Abadulla et al., 2000; Husain, 2006; Jadhav et al., 2009). The main reactions those are catalyzed by LiP include depolymerization, demethoxylation, decarboxylation, hydroxylation and aromatic ring opening. Most of these reactions resulted in \( O_2 \) activation, creating radicals that perpetuate oxidation of organopollutants (Reddy and Mathew, 2001; Kandelbauer et al., 2004).

The reaction mechanism of MnP starts with the enzyme oxidation by \( H_2O_2 \) to an intermediary oxidized state that, in turn, promotes the oxidation of \( Mn^{2+} \) to \( Mn^{3+} \) during the catalytic cycle (Hofrichter, 2002). Afterwards, in a mechanism involving two successive electron transfer reactions, substrates such as azo dyes reduce the enzyme to its original form. \( Mn^{3+} \) is stabilized by organic acids such as oxalic acid and the resulting \( Mn^{3+} \) organic acid complex acts as an active oxidant (Schlosser and Hofer, 2002). Thus, MnP oxidizes its natural substrate, i.e. lignin as well as textile dyes (Heinfling et al., 1998).

Versatile peroxidase (VP) has been recently recognized as a new group of ligninolytic peroxidases, together with LiP and MnP obtained from *Phanerochaete chrysosporium* (Martinez, 2002). VP from *Bjerkandera adusta* was reported to show a hybrid molecular architecture between LiP and MnP. This hybrid enzyme combines the catalytic properties of these two peroxidases, being able to oxidize typical LiP and MnP substrates. The catalytic mechanism of VP is similar to that of classical peroxidases; the substrate oxidation is carried out by two-electron multistep reactions at the expense of \( H_2O_2 \) (Pogni et al., 2005). VP oxidizes \( Mn^{2+} \) to \( Mn^{3+} \) at around pH 5.0 while also oxidizing aromatic compounds at around pH 3.0, regardless of the presence of \( Mn^{2+} \) (Ruiz-Duenas et al., 2001; Tinoco et al., 2007).

Microperoxidases are small heme-peptides obtained by proteolytic digestion of cytochrome c. These enzymes consisted of a short or medium length polypeptide
chain, covalently linked to an iron protoporphyrin IX moiety via two thioether bonds involving Cys residues at the c-porphyrin A and B pyrrole rings (Tullio et al., 2005).

1.5.1. Peroxidase mediated treatment of organopollutants

Plant peroxidases are receiving increasing attention due to their activity on broad spectrum aromatic compounds and potential applications in clinical, analytical, biochemical, biotechnological and other related areas (Okumura et al., 2003; Husain and Husain, 2008; Husain et al., 2009).

1.5.1.1. Treatment of Bisphenol A

Degradation of BPA and nonyl phenol (NP) was investigated by MnP, both chemicals disappeared within 1 h treatment from the reaction mixture. The estrogenic activities of BPA and NP still remained 40% and 60% in the reaction mixtures even after 1 h and 3 h treatment, respectively. Extension of the treatment time to 12 h completed the removal of estrogenic activities of BPA and NP. A gel permeation chromatography analysis revealed that main reaction products of BPA and NP may be oligomers which formed by the action of enzymes (Tatsumi et al., 2001). In another study three endocrine disrupters, BPA, p-nonyl phenol (p-NP) and p-octylphenol (p-OP) were oxidized by HRP in the presence of H_2O_2. The optimal pHs for BPA, p-NP, and p-OP were 8.0, 7.0 and 5.0, respectively. The optimal temperature for BPA oxidation was 20 °C. Most of the oxidation products of BPA were polymers, although some 4-isopropenylphenol (4-IPP) was produced. Toxicological study of BPA and HRP catalyzed product was performed in male Japanese medaka (Oryzias latipes) and it showed vitellogenin in the blood increased when exposed to BPA. However, no increased vitellogenin was observed in medaka exposed to HRP-oxidized BPA. The enzymatic oxidation of BPA by HRP has eliminated its estrogen like activity (Sakuyama et al. 2003). Some earlier workers demonstrated that BPA could be effectively converted into precipitable solid products by HRP-mediated oxidative coupling reactions. Total 13 reaction intermediates and products were identified using liquid chromatography-mass spectrometry and GC-MS techniques. It has been suggested that two BPA radicals were coupled primarily by interaction of an oxygen atom on one radical and propyl-substituted aromatic carbon atom on another,
followed by elimination of 4-IPP carbon cation. It was found that catalyzed oxidative coupling reactions may be important natural transformation pathways for estrogenic phenolic compounds and indicated their potential use as an efficient means for removal of estrogenicity from wastewater (Huang and Weber, 2005).

Imanaka et al. (2005) investigated oxidative degradation of BPA by fruit homogenates. These homogenates were incubated with BPA at 25 °C for 0-120 min and acetone extracts were analyzed by high pressure liquid chromatography (HPLC) with a photodiode array detector (200-650 nm). The two degradation products were identified by GC-MS analysis. In other study biocatalytic elimination of EDCs; p-NP, BPA and the personal care product ingredient triclosan by the enzyme from the WRF Coriolopsis polyzona was investigated. Analysis of variance methodology showed that pH and temperature were statistically significant factors in removal of p-NP, BPA and triclosan. The elimination of p-NP and triclosan was best at 50 °C and maximum disappearance of BPA was at 40 °C, The most suitable pH for all three micropollutants maximum removal was pH 5.0. All of the p-NP and BPA (5 mg L⁻¹) were eliminated. In the case of triclosan, 65% was removed after either 4 or 8 h treatment. The elimination of p-NP and BPA was directly associated with the disappearance of estrogenic activity. GC-MS analysis showed that the enzymatic treatment produced high M, metabolites through a radical polymerization mechanism of NP, BPA and triclosan. These oligomers were produced through the formation of C–C or C–O bonds (Hubert et al., 2007).

Peller et al. (2009) studied the oxidation of BPA by MnP with radiolytically generated hydroxyl radicals. In deionized water, BPA reacts with the hydroxyl radical by addition to the aromatic ring, \( k = 6.9 \times 10^9 \) (±0.2) \( M^{-1} S^{-1} \), to eventually form prominent, long lived, hydroxylated intermediate product. In contrast in treated water solutions the initial hydroxyl radical addition reaction occurs, the hydroxylation is averted and a different mechanistic pathway ensues. The removal constant for the hydroxyl radical reaction with BPA was 0.45 ± 0.04 \( \mu \)mol kg⁻¹, corresponding to an overall degradation efficiency of 76%. The oxidative removal and polymerization of BPA via a Coprinus cinereus peroxidase (CCP) was obtained as a dark brown powdery precipitate. The relatively hydrophobic solvent, 2-propanol, gave a better yield (95%) than hydrophilic solvents, such as methanol, ethanol or acetone. CCP
catalyzed product was also charactarized by FT-IR and $^{13}$C NMR spectrum (An et al., 2010).

1.5.1.2. Degradation of dioxins

WRF peroxidases were non-specific in the selection of chemical structures of organic substrates, due to this property of WRF, these enzymes can be applied for the remediation of wide range of organopollutants. Bumpus et al. (1985) investigated degradation of 2,3,7,8-trichlorodiethylidioxane (2,3,7,8-TCDD) by WRF Phanerochaete chrysosporium. The evolution of CO$_2$ from 2,3,7,8-TCDD was less than 2.5% when recovery was calculated with respect to the initial substrate concentration. Hammel et al. (1986) have made more detailed mechanistic study of reaction mechanism for fungal LiP. Takada et al. (1996) have shown that the 2,3,7,8-TCDD and higher halogenated were the target compounds for the action of peroxidase obtained from Phanerochaete sordida strain. These compounds were significantly degraded at the initial concentrations of about 50 ng L$^{-1}$ (10-60%) within 7 d. In case of higher concentrations of 2,3,7,8-TCDD and octachlorobenzo-$p$-dioxin only 10% were degraded under similar experimental conditions. The degradation products in culture medium were identified as 4,5-dichlorocatehol and tetrachlorocatechol by GC-MS analysis.

1.5.1.3. Phenol removal

In recent years, a lot of research has been made to develop processes in which peroxidases were used to remove phenolic contaminants from wastewater (Klibanov et al., 1983; Tatsumi et al., 1996; Duran and Esposito, 2000). Phenols were oxidized by peroxidases to generate phenoxy radicals, which coupled each other to form oligomeric and polymeric products (Ward et al., 2001).

Lignin-degrading MnP purified from the culture of a wood-rotting basidiomycete, Bjerkandera adusta, was used for the polymerization of guaiacol. MnP catalyzed polymerization of guaiacol in 50% aqueous acetone, dimethyl formamide (DMF), methanol, ethanol, dioxane, acetonitrile, ethylene glycol and methylcellulose. Maximum yield of polyguaiacol was achieved in 50% aqueous
acetone. The $M_r$ of the polymer was calculated as 30,300 by gas permeation chromatography. However, matrix-assisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF-MS) analysis gave a more reliable $M_r$ of 1,690. Infrared spectrophotometry, $^{13}$C nuclear magnetic resonance ($^{13}$C-NMR), MALDI-TOF-MS, pyrolysis and GC-MS analyses showed the presence of C-C and C-O linkages and quinone structure in polyguaiacol. It was also observed that polyguaiacol has a methoxy-phenyl group as the terminal moiety. It was shown that polyguaiacol was a branched polymer in which guaiacol units were cross-linked at the phenolic groups. MnP also catalyzed the polymerization of o-cresol, 2,6-dimethoxyphenol (2,6 DMP), other phenolic compounds and aromatic amines. $M_r$ of their polymeric products ranged was from 1000-1500 kDa (Iwahara et al., 2000).

CCP catalyzed oxidation of 1-naphthol, 2-naphthol and 4-hydroxybiphenyl (4-HBP) oxidation was investigated. The initial rates of naphthols and 4-HBP oxidations were linearly dependent on enzyme concentration. The rates depended on substrate concentrations and saturated above 100 $\mu$M of $H_2O_2$, 25-50 $\mu$M of naphthols and 10 $\mu$M of 4-HBP. At the peroxide concentration of 100 $\mu$M, the calculated value of $K_m$ and maximal rate ($V_{max}$) were 74.7 $\mu$M and 0.53 mM sec$^{-1}$ or 175 $\mu$M and 2.0 $\mu$M sec$^{-1}$ for 1 or 2 naphthol, respectively and 29.68 $\mu$M and 0.42 $\mu$M sec$^{-1}$ for 4-HBP (Bratkovskaja et al., 2004). The oxidative polymerization of 1-naphthol was investigated in the presence of HRP. Naphthol polymerization products (NPP) were characterized for their relative polarity using octanol-water partitioning experiments and reverse-phase high pressure liquid chromatography. Their structure was evaluated using gel permeation chromatography and LC-MS. The toxicity of product was measured by bacterial bioluminescence method. Peroxidase reaction resulted in the production of soluble and insoluble NPP. Soluble NPP was predominantly more polar than the parent naphthol. Results from this study showed that HRP-mediated treatment of naphthol-contaminated soils could be achieved through the formation of large hydrophobic oligomers that were immobilized on the soil matrix; and via reduction in aqueous-phase toxicity due to polymer precipitation (Xu et al., 2005).

Kim et al. (2005b) investigated degradation of PCP by electroenzymatic method and this procedure combined enzymatic catalysis and electrogeneration of $H_2O_2$. The experiments were conducted in a two compartment packed-bed reactor
using HRP immobilized electrode. The highest production of H₂O₂ and the current efficiency were observed at 0.4 V vs. Ag/AgCl and a flow rate of 1 mL min⁻¹. The highest initial degradation rate and degradation efficiency of PCP were achieved at pH 5.0 and 25 °C.

Several substrates were compared on their affinity with HRP according to the kinetic parameters of reactions. The results showed that 4-chlorophenol (4-CP) was the easy reactant and followed by phenol and 3-chlorophenol (Bodalo et al., 2008). Maximum removal of phenols and their mixtures by immobilized BGP was found in the buffers of pH 5.0-6.0 and at 40 °C in the presence of 0.75 mM H₂O₂. Immobilized BGP removed remarkably very high concentration of phenols from their mixtures. 2,4 DCP and a phenolic mixture were also treated in a stirred batch reactor with fixed quantity of enzyme for longer duration. Immobilized enzyme removed higher concentration of phenolic compounds from the reaction mixture (Akhtar and Husain, 2006). The degradation of phenol by HRP in the presence of talc, a natural abundant and low cost mineral was investigated. Adsorption of the reaction products on the talc effectively protected the biocatalyst against inactivation caused by oxidative products, thereby prolonging its catalytic action and leading to almost complete elimination of phenols from the aqueous media. Suitable conditions for the depollution of water containing phenolic substances were developed after optimization of various reaction conditions (Didier, 2006).

1.5.1.4. Oxidation of polycyclic aromatic hydrocarbons

PAHs are a class of organic compounds that have accumulated in 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 on human health. This concern has attracted researchers to develop methods to detoxify/remove such types of organic compounds from the natural environment. Bioremediation is one approach that has been used to remediate contaminated land, waters and promotes the natural attenuation of the contaminants using in situ microbial community at site. Variety of fungi and bacteria that are capable of these transformations, described the major aerobic and anaerobic breakdown pathways (Selina et al., 1999). WRF extracellular
peroxidases such as LiP and MnP have degraded dimeric lignin model compounds (Leontevskii et al. and Golorlera, 1990; Maltseva et al., 1991) and in some studies their involvement in initial oxidation of lignin polymers has been demonstrated (Wariishi et al., 1991; Kocan et al., 2003). LiP and MnP are extracellular peroxidases produced by WRF and the onset of their production is associated with secondary metabolic conditions in response to nutrient depletion (Bumpus and Steven, 1987). The ligninolytic system is non selective, consequently aromatic substrates are potentially oxidized and biodegraded by WRF (Vazquez-Duhalt et al., 1994; Field et al., 1995b; Field et al., 1996).

Cajthaml et al. (2002) studied the ability of purified enzymes to degrade several PAHs. Levin et al. (2003) investigated biodegradation of two PAHs, nitrobenzene and anthracene, by a WRF, *Trametes versicolor*. They found high activity of MnP and laccase in the fungal cultures, which were responsible for PAH degradation. MnP are thought to predominantly achieve the degradation of PAHs with lower ionization potentials and higher Mr. This has been demonstrated by the conversion of compounds such as benzo[a]pyrene, benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene to larger extents than the higher ionization potential lower molecular mass PAHs such as phenanthrene and fluoranthene (Steffen et al., 2003).

Weber and Huang (2003) have shown participation of phenanthrene in phenol-coupling reactions mediated by HRP. Aqueous-phase concentrations of phenanthrene were observed to decrease dramatically with phenol as a result of formation of precipitated products, suggesting a potential means for simultaneous treatment of phenolic contaminants and PAHs using peroxidase-mediated oxidative coupling processes. It has been shown that phenanthrene removal from aqueous phase occurred by a combination of sorption by and chemical bonding to precipitated reaction products. In that the oxidative coupling reactions of phenolic compounds comprised of an important step in the initiation of humification processes. The development of an efficient degradation system for polyaromatics based on the use of peroxidases *in vitro* required their increased bioavailability by using co-solvents. The action of MnP in the presence of water miscible organic solvent, acetone (36%, v/v) was evaluated as a feasible system for *in vitro* degradation of three PAHs: anthracene, dibenzothiophene and pyrene. These compounds were degraded to a large extent after
a short period of time at conditions maximizing MnP oxidative system. The initial amount of enzyme present in reaction medium was determinant for the kinetics of the process. The order of degradability, in terms of degradation rates was as follows: anthracene > dibenzothiophene > pyrene. The intermediate compounds were determined using GC-MS and degradation mechanisms were proposed. Anthracene was degraded to phthalic acid. A ring cleavage product of the oxidation of dibenzothiophene, 4-methoxybenzoic acid was also observed (Eibes et al., 2006). Eibes et al. (2005) have shown complete degradation of anthracene by MnP from *Phanerochaete chrysosporium* and *Bjerkandera* sp. BOS55 in organic solvent mixtures. Due to maximum solubilization of anthracene and minimum loss of MnP activity, acetone was selected as the optimal co-solvent, which enhanced anthracene solubility to 140% in the presence of 36% (v/v) acetone. *In vitro* degradation of anthracene by MnP was investigated for different concentrations of the main cofactors and substrates that affect the catalytic cycle of MnP (Mn^{2+}, H_{2}O_{2} and organic acids) as well as for other environmental parameters; temperature, air/oxygen atmosphere and light source. Another study was conducted to determine the potential for the treatment of a poorly soluble compound, anthracene by MnP from fungus *Bjerkandera* sp. BOS55. silicone oil was used as the water immiscible organic solvent, which contained anthracene at high concentrations. The optimization of the oxidation process was conducted taking into account the factors which might directly affect the MnP catalytic cycle and those that affected the mass transfer of anthracene between the organic and the aqueous phase. The complete oxidation of anthracene in water immiscible solvent showed the application of enzyme for the removal of poorly soluble compounds (Eibes et al., 2007). MnP purified from WRF *Irpex lacteus* was used for degradation of four representatives of PAHs; phenanthrene, anthracene, fluoranthene and pyrene and the enzyme showed the ability to degrade them *in vitro*. The results confirm the role of MnP in PAH degradation by *Irpex lacteus*, including cleavage of the aromatic ring (Baborrova et al., 2006).

1.5.1.5. Oxidation of aromatic amines

Aromatic amines are great concern due to their high toxicity and many negative impacts on animals, birds and humans (Biswas et al., 2007). LiP from the WRF *Phanerochaete chrysosporium* was chemically modified by reductive alkylation
with benzyl, naphthyl and anthracyl moieties, thereby increasing its superficial hydrophobicity. The three chemical modifications altered the kinetic behavior of the enzyme in 10% acetonitrile with four different substrates: carbazole, pinacyanol, pyrene and VA. Benzyl modification of LiP increased the catalytic efficiency ($k_{cat}/K_{m, app}$) 2.7 times for carbazole oxidation. Thirteen N-containing compounds, including pyrroles, pyridines and aromatic amines, were tested to determine whether they could be oxidized by LiP in 10% acetonitrile. All pyrrole analogues and amines tested were oxidized, but none of the pyridine analogous reacted. Some products were isolated and analyzed by high-resolution mass spectrometry. Most were dimers or polymers and in some cases, these contained oxygen atoms (Vazquez-Duhalt et al., 1995).

The influence of phenol and its derivatives on the kinetics of oxidation of arylamines catalyzed by novel plant peroxidase, cationic peanut peroxidase was studied. The kinetics of enzymatic oxidation of benzidine, $o$-dianisidine, and 3,3’,5,5’-tetramethylbenzidine with $H_2O_2$ was found to depend on a correlation between redox properties of phenols and the indicator-substrate of peroxidase. Thus, the catalytic activity of peanut peroxidase is inhibited by phenols with redox potentials higher than that of arylamines mentioned above, whereas phenols with potentials below those of arylamines, play a role of second substrate of enzyme (Nailya et al., 2001). In another study microsomal fraction from tulip bulbs (Tulipa fosteriana, L.) contains cytochrome P 450 (EC 1.14.14.1) and peroxidase catalyzed the NADPH and $H_2O_2$ dependent oxidation of the xenobiotic substrates, N-nitrosodimethylamine, N-nitrosomethylaniline, aminopyrine and 1-phenylazo 2-hydroxynaphthalene. Oxidation of these model xenobiotics has also been assessed in a reconstituted electron-transport chain with a partially purified cytochrome P 450 fraction, phospholipid and isolated tulip NADPH- cytochrome P 450 reductase (EC 1.6.2.4.). Peroxidase isolated from tulip bulbs (isoenzyme C) also oxidized these xenobiotics (Stiborova et al., 2000).

Goerlewska-Roberts et al. (2004) evaluated the role of lactoperoxidase (LPO) catalyzed activation of most commonly studied arylamine carcinogens: 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP), benzidine, 4-aminobiphenyl (ABP), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). Partially purified LPO from bovine or
human milk in the presence of H₂O₂ and calf thymus DNA was used for the activation study in vitro. Products formed during enzymatic activation were monitored by HPLC with UV and radiometric detection. The DNA binding level of ³H and ¹⁴C radio-labeled amines after peroxidase catalyzed activation was dependent on the H₂O₂ concentration and the highest levels of carcinogen binding to DNA. Carcinogen activation and the level of binding to DNA were in the order of benzidine > ABP > IQ > MeIQx > PhIP. The susceptibility of aromatic and heterocyclic amines for LPO catalyzed activation and the binding levels of activated products to DNA suggested a significant role of LPO catalyzed activation of carcinogens in the etiology of breast cancer. Anaerobic degradation, an effective treatment process of textile industry effluent, generates sulfonated aromatic amines, which are carcinogenic, mutagenic and resistant to microbial degradation. These aromatic amines could be effectively removed by oxidative polymerization catalyzed by peroxidase. The amines, generated from the anaerobic reduction by zero valent iron of two reactive azo dyes; Reactive Red 2 and Reactive Black 5 were successfully removed (90%) by ARP. Enzymatic treatment of two model compounds; diphenylamine (DPA) and 2-amino-8-naphthol-3,6-disulfonic acid (ANDSA) was also studied for better understanding of the process. DPA has a similar diarylamine bond as Reactive Red 2. The ANDSA has a similar structure as the dye reduction products. The secondary amine bond in DPA and Reactive Red 2 were oxidized by ARP. Enzymatic reaction of sulfonated aromatic amines generated soluble colored compounds, which were removed by adding a coagulant (Biswas et al., 2007).

1.5.1.6. Transformation of pesticides

Purified VP from the WRF Bjerkandera adusta UAMH 8258 was used to study transformation of several pesticides, including some as highly halogenated wood preservative PCP. From the 13 pesticides assayed, dichlorophen, bromoxynil and PCP were transformed by VP in the presence and absence of manganese (II). For all the pesticides transformed, the activity was higher in the absence of Mn(II) at pH 3.0 than in the presence of Mn(II) at pH 4.0. Catalytic constants (k_cat) in the absence of Mn(II) at pH 4.0 were 194 and 409 min⁻¹ for dichlorophen and bromoxynil, respectively. The K_M values were 32 and 31 µM for the pesticides and 26 and 19 µM for the H₂O₂, respectively. Analysis of reaction products by GC-MS showed the
presence of 2,3,5,6-tetrachloroquinone among the products from PCP oxidation, while the main product from dichlorophenol was 4-chlorophenol-2,2'-methylenequinone. Several polymers were obtained from the peroxidase catalyzed oxidation of bromoxynil (Davila-Vazquez et al., 2005). Pizzul et al. (2009) exploited the ability of pure MnP, laccase, LiP and HRP to degrade the widely used herbicide glyphosate and other pesticides. Complete degradation of glyphosate was obtained by MnP, MnSO₄ and Tween 80, with or without H₂O₂. In the presence of MnSO₄, with or without H₂O₂, MnP also transformed the herbicide, but to a lower rate. Laccase degraded glyphosate in the presence of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), MnSO₄ and Tween 80. The metabolites were detected in all cases where degradation of glyphosate occurred by GC-MS. The LiP was tested alone or with MnSO₄, Tween 80, VA or H₂O₂ and in the HRP assay the enzyme was added alone or with H₂O₂ in the reaction mixture. However, these enzymes did not degrade glyphosate. MnP together with MnSO₄ and Tween 80 degraded glyphosate in its commercial formulation Roundup® Bio. The same enzyme mixture was tested for degradation of 22 other pesticides and degradation products present in a mixture and all compounds were transformed, with degradation ranged from 20-100%.

1.6. APPLICATIONS OF IMMOBILIZED ENZYMES

A number of oxidative enzymes from bacteria, fungi and plants have been employed in numerous waste treatment applications. Peroxidases act on specific recalcitrant pollutants by producing oxidized products which change into polymeric products and permitting a better final treatment of the waste (Fatima and Husain, 2007a; 2007b; 2008). The important reason that enzymatic procedures have not been exploited at an industrial level is the presence of large volume of wastewater which demands remediation. Soluble enzymes suffer from certain drawbacks such as the stability against physical and chemical denaturants, susceptibility to attack by proteases, activity inhibition and reusability (Husain and Jan, 2000, Husain and Husain, 2008). An important disadvantage of using soluble enzymes in the detoxification and remediation of hazardous aromatic pollutants was that the free enzyme cannot be used in continuous reactors. In order to overcome all the above
listed limitations, the use of enzymes in their immobilized form is the best alternative to employ them at industrial level. The immobilization of enzymes allowed the reuse of the enzymes and thus reduced the cost for such treatments (Akhtar et al., 2005c; Khan et al., 2005; Kulshrestha and Husain, 2006a; 2006b; Matto and Husain, 2006). Improvement in the useful life and thereby a reduction in treatment cost has been accomplished through enzyme immobilization. HRP, LiP and MnP mineralized a variety of recalcitrant aromatic compounds.

1.6.1. Removal of phenol and phenolic compounds by immobilized enzymes

Tatsumi et al. (1996) immobilized HRP on magnetite and used this enzyme preparation for the removal of chlorophenols. Magnetite adsorbed HRP could remove almost 100% chlorophenols. The effect of HRP immobilized on activated alumina on the removal efficiency of phenol exhibited that one molecule of HRP was needed to remove approximately 1100 molecules of phenol when the reaction was carried at pH 8.0 and room temperature (Vasudevan and Li, 1996). Singh and Singh (2002) immobilized HRP onto aminopropyl glass (APG) with glutaraldehyde, which binds specific activity of 50.7 U g\(^{-1}\) APG. HRP immobilized on APG was used for the removal of 4-CP from water. The polymerization of 4-CP into insoluble precipitate was completed within 3 h after the the addition of H\(_2\)O\(_2\) with a maximal removal efficiency of 25% at pH 7.5. Regalado et al. (2004) have reported removal of more than 90% phenol by using alginate entrapped TP in 3 h. Immobilized turnip peroxidase (TP) was used to remove phenol by oxidative polymerization. Alginate beads entrapped enzyme removed more than 90% phenol after 3 h of reaction. Microporous polypropylene hollow fiber membranes impregnated with HRP were considered for the degradation of selected hydroxylated aromatic compounds; 3,4-dimethylphenols, 4-ethylphenol, 2-hydroxy-1,2,3,4-tetrahydronaphthalene, 2-hydroxy-decahydronaphthalene and 4-hydroxy-biphenyl. All these substrates except 2-hydroxy-decahydronaphthalene were efficiently degraded by 50-100% within 48 h. In these experiments, the transport behavior of the analytes through the membranes before and after enzyme immobilization was studied in batch experiments. In addition, a continuous-flow hollow fiber membrane device was used to evaluate the degradation of substrates by immobilized enzymes (Moeder et al., 2004). Degradation of 2,6-dichlorophenol (2,6-DCP) was carried by CCP. Polyacrylamide matrix
entrapped enzyme enhanced 2,6-DCP oxidation (Pezzotti et al., 2004). Phenol and BPA dissolved in water was also polymerized by HRP immobilized on the fiber-forming polymeric materials such as cellulose, chitosan and ethylene-vinyl alcohol copolymer (Maki et al., 2006).

An electroenzymatic method that uses an immobilized HRP was investigated within a two compartment packed-bed flow reactor. To evaluate the electroenzymatic degradation of phenolic compounds present in wastewater, electrolytic experiments were carried out with 0.42 U mL⁻¹ HRP at -0.5 V. The overall application of the electroenzymatic method led to a greater degradation rate than the use of electrolysis alone. The byproducts formed during reaction were an aromatic amine, sulfanilic acid and unknown compounds (Kim et al, 2005a). HRP was successfully immobilized on aluminum-pillared interlayered clay to obtain enzyme-clay complex for the treatment of water polluted with phenolic compounds. The immobilized HRP exerted a perfect phenol removal by precipitation or transformation into insoluble products over a broader range of pH from 4.5 to 9.3 (Cheng et al., 2006).

Trivedi et al. (2006) have demonstrated the removal of phenol by immobilized soybean seed hull peroxidase. SBP from the raw soybean seed hulls entrapped within hybrid (silica sol-gel/alginate) gel. Dalal and Gupta (2007) immobilized HRP by bioaffinity layering and used for the treatment of water contaminated with 4-CP. Con A bound Sephadex was used for preparing alternative layers of HRP and Con A. The most efficient design consisted of three layers of Con A and peroxidase each. This immobilized enzyme preparation could retain 80% of the initial activity. In another study HRP and SBP were covalently immobilized onto aldehyde glass through their amino groups. The removal 4-CP by immobilized HRP was significantly higher as compared to free enzyme. However, immobilized SBP needed more to reach the same transformation as by the free enzyme. It was noticed that at an immobilized enzyme concentration in the reactor of 15 mg L⁻¹, SBP removed 5% more 4-CP than HRP. Since immobilized SBP was less susceptible to inactivation than HRP and provided higher 4-CP elimination (Bodalo et al., 2008).

Quintanilla-Guerrero et al. (2008a) have isolated a peroxidase from turnip roots by modification with methoxypolyethylene glycol. The catalytic activity of the
modified TP on ABTS increased 2.5 times after 80 min of reaction. modified TP was immobilized in calcium alginate beads and considered for the oxidative polymerization of concentrated phenolic solutions and it retained more than 65% phenols removing efficiency even after 17th repeated use. In a further study purified peroxidase from turnip (Brassica napus L. var. esculenta D.C.) was immobilized by entrapment in the spheres of calcium alginate and by covalent binding to Affi-Gel. Both immobilized TP preparations were compared for the detoxification of phenolic compounds polluted water and an industrial effluent from a local paints factory. The effectiveness of phenolic compounds removal by oxidative polymerization was evaluated using batch and recycling processes in the presence and absence of polyethylene glycol (PEG). The presence of PEG enhanced the TP stability. In addition, reaction times were reduced from 3 h to 10 min and more effective phenol removals were achieved when PEG was added. TP was able to perform 15 reaction cycles with an industrial effluent containing phenolic compounds removals >90% during the first 10 reaction cycles (Quintanilla-Guerrero et al., 2008b).

Peroxidase from Caldariomyces fumago catalyzed the oxidation of several chlorinated phenols commonly found in industrial wastewaters in the presence of H₂O₂. This study compared the direct addition of H₂O₂ with its continuous electrogeneration during the enzymatic oxidation of chlorinated phenols. Reaction mixtures were studied containing chemically modified peroxidase, H₂O₂ and the phenolic substrates: phenol, 4-CP, 2,4-DCP, 2,4,6-TCP and PCP prepared in 100 mM sodium-potassium phosphate buffer, pH 6.0 at 25 °C (Camilo et al., 2007). Some workers immobilized peroxidase on the CNBr activated rodlike cellulose nanocrystals and this immobilized peroxidase was employed for the treatment of phenolic compounds in solutions. Immobilized peroxidase exhibited significantly higher removal of phenolic compounds from solution as compared to free enzyme (Yang et al., 2008).

Glutaraldehyde-activated APG beads immobilized SBP and HRP were employed for the removal of phenol from aqueous solutions in the presence of H₂O₂. The reaction with immobilized HRP was faster than SBP at all the enzyme concentrations. The immobilization had a beneficial effect on the stability of HRP and the phenol conversions, which were higher than those obtained by free enzyme.
However, at the enzyme concentrations lower than 0.028 mg mL\(^{-1}\), 7\% and 20\% more phenol was removed by SBP than HRP (Gomez et al., 2006). Further same workers have immobilized SBP on glass supports with different surface areas by covalent attachment and these immobilized preparations were used for phenol removal. The influence of different operational variables on the process was also studied. Enzyme immobilized on supports with the highest surface area had successfully removed 80\% phenol from polluted water (Gomez et al. (2007). Gomez et al. (2008) have developed an expanded version of Dunford mechanism, which extended the initial peroxidase cycle to the reaction products and it was employed for the kinetic analysis of the immobilized SBP/phenol/H\(_2\)O\(_2\) system. At the same time, an enzyme deactivation model, based on the gradual covering of the catalytic particles by the products originated during the reaction has also been described.

SBP immobilized on graphite power was used to treat 4-CP at concentrations range from 50-500 mgL\(^{-1}\). It has been reported that the excilamp can facilitate higher removal efficiency in all cases with complete 4-CP removal within 5 and 90 min. Approximately 80\% of the 4-CP was removed by both free and immobilized enzyme up to concentrations of 250 mg L\(^{-1}\). Immobilized system showed much higher removal efficiency at 500 mg L\(^{-1}\) due to increased enzyme stability in the presence of formation of higher by-products (Gomez et al., 2009).

Peroxidase from chayote (Sechium edule) was immobilized onto the organic support polystyrene-divinylbenzene copolymer functionalized with triglycine and activated with 1-1’carbonyldimidazol. This immobilized enzyme was used for the decontamination of synthetic wastewater containing phenolic compounds. Synthetic waters containing phenol, 2-methoxyphenol or 3-CP were oxidized and converted into polymer by peroxidase in the presence of H\(_2\)O\(_2\). The removal of phenolic compounds was from 75-100\%, while the removal of polymers using filtration was from 65-80\% in the case of phenol and 2-methoxyphenol, respectively (Villegas-Rosas et al., 2003).
1.6.2. Decolorization of dyes and effluents

Hydrophobic matrix bound *Saccharum* peroxidase was employed for the degradation of four textile dyes, Procion Navy Blue HER, Procion Brilliant Blue H-7G, Procion Green HE-4 BD and Supranol Green (Shaffiq et al., 2002). These dyes at an initial concentration of 50 mg L⁻¹ were completely degraded within 8 h by the enzyme immobilized on the modified polyethylene matrix. The immobilized enzyme was also employed in a batch reactor for the degradation of Procion Green HE-4BD and the reusability was studied for 15 cycles and the half-life was found to be 60 h. Fruhwirth et al. (2002) have used a catalase-peroxidase from the newly isolated *Bacillus* SF to treat textile bleaching effluents. The enzyme was immobilized on various alumina based supports with different shapes. Bleaching effluent was treated in a horizontal packed-bed reactor containing 10 kg of the immobilized enzyme at a textile-finishing company. The treated liquid (500 L) was reused within the company for dyeing fabrics with various dyes, resulting in acceptable colour differences for all dyes. Acrylamide gel entrapped HRP showed effective performance in acid dye decolorization as compared to free and alginate entrapped enzyme. Alginate entrapped HRP showed inferior performance over the free enzyme due to consequence of non availability of the enzyme to the dye molecule due to diffusion problem of substrate (Venkata et al., 2005). BGP immobilized on a bioaffinity support; Con A Sephadex and this immobilized preparation has been exploited for the decolorization of industrially important dyes from polluted water. Dye decolorization was maximum in the buffer of pH 3.0 and at 40 °C. This immobilized enzyme was repeatedly used for the decolorization of eight reactive textile dyes from fresh batch of dye solutions and after 10th repeated uses it retained nearly 50% of the initial activity. Mixtures of dyes were decolorized more than 80% by immobilized BGP. Immobilized BGP could remove significantly high percentage of color and TOC from individual dyes, mixtures of dyes and dyeing effluent (Akhtar et al., 2005b). Con A-cellulose-bound tomato peroxidase (TMP) was exploited for the decolorization of recalcitrant dyes in the presence of redox mediators. Immobilized peroxidase decolorized dyes to different extent in the presence of investigated redox mediators. However, HOBT was found to be most suitable redox mediator for TMP catalyzed decolorization of direct dyes. These dyes were maximally decolorized at pH 6.0 and
40 °C by soluble and immobilized TMP. The absorption spectra of the untreated and treated dyes exhibited a marked difference in the absorption at various wavelengths (Matto and Husain, 2008). Further Con A-cellulose bound TP and TMP were used for the decolorization of two textile carpet industrial effluents. Both effluents were recalcitrant to the action of peroxidases. However, the decolorization of effluents was enhanced in the presence of 2.0 mM HOBT. Both effluents were maximally decolorized at pH 5.0 at 40 °C by soluble and immobilized TP whereas the maximum decolorization by soluble and immobilized TMP was found at pH 6.0 at 40 °C. The immobilized TP and TMP treated effluent exhibited significant loss of TOC from the solution (Kulshrestha and Husain, 2010).

An inexpensive immobilized TP preparation was employed for the decolorization and removal of some direct dyes in batch as well as in continuous reactors. Wood shaving was used as an inexpensive material for the preparation of bioaffinity support. Con A-wood shaving adsorbed TP showed 67% of the original enzyme activity. Both soluble and immobilized TP could effectively remove more than 50% color from dyes in the presence of metals/salt and 0.6 mM HOBT after 1 h of incubation. The columns containing immobilized peroxidase could decolorize 64% Direct Red and mixtures of dyes upto 50% at 3 months of continuous operation (Matto and Husain, 2009a). Calcium alginate–starch entrapped and Con A layered calcium alginate-starch beads immobilized BGP has been employed for the treatment of a textile industrial effluent in batch as well as in continuous reactor. The textile effluent was recalcitrant to the decolorization by BGP; thus, its decolorization was examined in the presence of a redox mediator, HOBT. Entrapped enzyme could remove more than 70% of effluent color in a stirred batch process after 3 h of incubation whereas surface immobilized BGP decolorized more than 90% color under similar experimental conditions. Entrapped BGP retained 59% effluent decolorization reusability even after its 10th repeated use. The two-reactor systems containing both types of immobilized enzyme preparations retained more than 40% effluent decolorization capacity even after 2 months of their continuous operations (Matto and Husain, 2009b; Matto et al., 2009; Matto and Husain, 2009c).
1.6.3. Polymerization of PAHs

Edwards et al. (2002) have investigated the immobilization of laccase and MnP from *Trametes versicolor* onto polysulphone UFMs, in order to facilitate enzyme-substrate contact. The activity of immobilized enzymes and duration of their activity was measured with respect to the enzymatic degradation of aromatics within effluent. During application of membrane, fouling is a big problem and this fouling was reduced by action of layer of immobilized enzymes. The system was also employed to an industrial petrochemical-based effluent and compared with the synthetic make-up effluent in terms of ‘defouling’ efficiency. The presence of high concentrations of fluoride at the membrane interface during ultrafiltration of the petrochemical-based effluent contributed significantly to the inhibition of immobilized enzyme suite and thus manifested as a significant decrease in ‘defouling’ potential in comparison with the system being operated using the synthetic make-up effluent. The spent mushroom compost (SMC) of *Pleurotus pulmonarius* immobilized laccase (0.88 mmoles min\(^{-1}\) g\(^{-1}\) and MnP (0.58 mmoles min\(^{-1}\) g\(^{-1}\)) of which the optimal temperatures were 45 and 75 °C, respectively. In laboratory test, complete degradative removal of individual naphthalene, phenanthrene, benzo[a]pyrene and benzo[g,h,i]perylene (200 mg PAH kg\(^{-1}\)) sandy-loam soil) by 5% SMC was obtained in 2 d under continuous shaking at 80 °C. The SMC-treated PAH samples had significantly reduced or removed their toxicities as revealed by the Microtox bioassay. These results were confirmed by GC-MS analysis on the breakdown products. A phthalic derivative, which is reported as a degradative product of PAHs by ozonation or ligninolysis was also detected in the SMC-treated samples. The results demonstrate the potential in employing SMC *in ex situ* bioremediation (Laus et al., 2003)

1.6.4. Transformation of other aromatic compounds

Lee et al. (2003) have demonstrated HRP-catalyzed removal of 2,4,6-TNT by an electrochemical method operated in a circulating batch mode with the help of *in situ* generated H\(_2\)O\(_2\). HRP immobilized on the reticulated vitreous carbon electrode was prepared for the cyclic voltammetry of 2,4,6-TNT. The effects of pH and temperature on the 2,4,6-TNT electroreduction in 0.2 M phosphate buffer saturated with oxygen were evaluated. HRP immobilized on carbon electrode could catalyze
oxidation and detoxification of 44 μM 2,4,6-TNT in aqueous solution under optimized conditions. The removal rate of 2,4,6-TNT by electroenzymatic method was much greater as compared to electrochemical and biochemical methods.

An electro-enzymatic method was employed to degrade 2-chlorobiphenyl (2-CB) by HRP immobilized on glassy carbon electrode. Cyclic voltammetry was used to understand the participation of redox reactions and an applied potential of -0.5 V was found suitable for the degradation of 2-CB. During degradation, dechlorination of 2-CB was confirmed by measurement of chloride ion concentration in the reaction mixture and was found almost equimolar to the initial 2-CB concentration. Chloride concentration was 0.53 ppm at the end of reaction while the initial 2-CB concentration was 3.15 ppm. Almost 97% of the chlorine from 2-CB sample was liberated. TOC measurements showed that almost 83% 2-CB was mineralized. Degraded products were analyzed by GC-MS to find reaction intermediates. However, no peak could be detected except 2-CB. Therefore, the most probable route of degradation is through dechlorination followed by biphenyl ring cleavage and mineralization (Khan et al., 2007).

The adsorption of HRP onto silicon wafers was performed by means of in situ ellipsometry, atomic force microscopy and contact angle measurements. A smooth HRP layer adsorbed onto Si wafers. The activity of free or adsorbed HRP was determined by oxidation of ABTS and by the emulsion polymerization of ethylene glycol dimethacrylate. However, same HRP-covered Si wafer could be reused three times for the polymerization of ethylene glycol dimethacrylate (Naves et al., 2007).

1.7. APPLICATION OF REDOX MEDIATOR

Redox mediators were first described by Bourbonnais and Paice (1990). Redox mediators are compounds that speed up reaction rate by shuttling electrons from biological oxidation of primary electron donors or from bulk electron donors to electron-accepting aromatic compounds (Fabbrini et al., 2002). Redox mediators provide high redox potentials (>900 mV) to attack recalcitrant structural analogs and
are able to migrate into aromatic structure of the compounds and accelerate reactions by lowering the activation energy of the total reaction. In some cases, the presence of these mediators might even be a prerequisite for the initiation of reaction (van der Zee and Cervantes, 2009). The catalytic effect of such organic molecules with redox mediating properties on the bio-transformation of a wide variety of organic and inorganic compounds has been extensively explored (Dos-Santos et al., 2004; Guo et al., 2008; Husain and Husain, 2008; Jing et al., 2009). The role and mechanism of action of laccase-mediator system is well known and it has been applied to other enzymes (Couto et al., 2005b). When a substrate is oxidized by laccase, the redox mediator forms cation radicals; short-lived intermediates that co-oxidize non-substrates. These cation radicals formed by two mechanisms: either redox mediator oxidized substrate to a radical cation via a one electron (Xu et al., 2000; 2001), or the redox mediator can abstract a proton from the substrate, converting it into a radical (Fabbrini et al., 2002). However, ABTS acted by first mechanism whereas HOBT employed second procedure (Fabbbrini et al., 2002; Hirai et al., 2006).

A correlation between the enzyme redox potential and its activity toward substrates has been earlier described (Hirai et al., 2006). The driving force for the redox reaction catalyzed by oxidoreductive enzymes is expected to be proportional to the difference between redox potentials of the oxidizing enzyme and reducing substrate (Zille et al., 2004; Sadhasivam et al., 2009). Among the mediators, those presenting the >N-OH moiety; HOBT, N-hydroxynaphthimid (NHP) and violuric acid (VLA) have shown their potential towards benzylic substrates, through a radical H-abstraction route of oxidation involving an aminoxyl radical (>N-O) intermediate (d’Acunzo et al., 2006).

Further the use of redox mediator 2-aminothiazole and melamine was investigated in the oxidation of aromatic amines. Thirteen N-containing compounds, including pyrroles, pyridines and aromatic amines, were considered to determine whether they could be oxidized by LiP in 10% acetonitrile. All pyrrole analogues and amines tested were oxidized, but none of the pyridine analogous reacted. Some products were isolated and analyzed by high resolution mass spectrometry. Most of these products were dimers or polymers (Vazquez-Duhal et al., 1995). MnP H5 from WRF Phanerochaete chrysosporium, in presence of either Mn(II) (10 mM) or GSH
(10 mM) was able to mineralize $^{14}$C U-ring labeled 2-amino-4,6-dinitrotoluene up to 29% in 12 d. The mineralization extent reached to 82% in the presence of both Mn(II) and GSH. However, there was no significant mineralization observed in the absence of both Mn(II) and GSH (Aken et al., 2000). Karaseva et al. (2002) carried a comparative study on the kinetics of peroxidase catalyzed oxidation of tetramethylbromide (TMB) in presence of 2,4-dinitrosoresorcinol, its polydisulfide derivative and resorcinol polydisulfide substances that competitively inhibited conversion of TMB. The kinetic parameters showed that it was the most efficient inhibitor of peroxidase oxidation of TMB. Weber and Huang (2003) have shown oxidation of phenanthrene with phenol (mediator) coupling reactions mediated by HRP. Aqueous phase concentrations of phenanthrene were observed to decrease dramatically with phenol as a result of the formation of precipitated products. It exhibited that the peroxidase catalyzed oxidative coupling process was an important step in simultaneous treatment of phenolic contaminants and PAHs.

Karasyova et al. (2003) have shown that peroxidase catalyzed oxidation of TMB, o-phenylenediamine and 5-aminosalicylic acid was significantly enhanced in the presence some redox mediators, an increase in their concentrations was associated with a parallel increase in the $k_{cat}$ and $K_m$ values for TMB and o-phenylenediamine. The activation of peroxidase catalyzed oxidation of TMB and o-phenylenediamine was quantitatively characterized by a coefficient $\alpha$, which significantly depends on pH. Huang et al. (2003) have shown that VA at higher concentrations remarkably stimulated LiP catalyzed oxidation of phenolic compounds. This novel phenomenon was due to its competition with phenols for the active site of the enzyme and to the high reactivity of the formed cation radical of VA$^+$, which resulted in an additional oxidation of phenols. Some earlier investigator employed mediator, 2-chloro1,4-dimethoxybenzene for the oxidation of phenolic compounds. Thus indicated a more efficient degradation resulted from the transfer of an electron from the polymer to the radical cation of the mediator (d’Acunzo and Lanzalunga, 2004). Won et al. (2004) described the HRP catalyzed polymerization of cardanol in aqueous organic solvent in the presence of redox mediators. VA and $N$-ethyl phenothiazine were examined as mediators. It was surprising that HRP catalyzed polymerization of cardanol in presence of $N$-ethyl phenothiazine. These findings demonstrated that oxidative
polymerization of a poor substrate, for which the enzyme was not active, could take place in the presence of an appropriate mediator. It would provide more opportunities for the application of enzyme catalyzed polymerization of various compounds.

Bioelectrocatalytic reduction of H$_2$O$_2$ by LiP from *Phanerochaete chrysosporium* was investigated with LiP modified graphite electrodes to elucidate the ability of LiP to electroenzymatically oxidize phenols and catechols, as well as VA and some other high redox potential lignin model compounds. The obtained results have demonstrated different mechanisms for the bioelectrocatalysis of LiP depending on the chemical nature of the mediators and are of a special interest both for fundamental science and for application of LiP in biotechnological processes as solid-phase bioelectrocatalyst for decomposition/detection of recalcitrant aromatic compounds (Ferapontova et al., 2006). In another study oxidation of VA to veratryl aldehyde by LiP was inhibited by PCP. Inhibition was characterized by lag period followed by same rate of VA oxidation. The lag period before VA oxidation was increased by increasing concentration of PCP. During the lag period, PCP was oxidized and the extent of PCP oxidation increased with increasing concentrations of VA. The enzyme stayed as compound II during PCP oxidation in the presence of VA, it showed that VA had a protective role in the LiP catalysis. The kinetics of PCP oxidation in the presence of VA was similar to those of VA oxidation. All these results indicated that PCP was oxidized indirectly via VA$^+$ radical. 2,3,5,6-tetrachloro-p-benzoquinone was a product during PCP oxidation in the presence and absence of VA. An equivalent amount of inorganic chloride was formed by oxidative 4-dechlorination during PCP oxidation in the presence of VA. The increase in the rate and extent of PCP oxidation by VA resulted from mediation of PCP oxidation and reversion of inactive compound III to native enzyme by the VA$^+$ radical. A quinone produced from VA by LiP from the WRF *Phanerochaete chrysosporium* was studied for its ability to mediate reduction (Biswas et al., 2007).

Some workers investigated the treatment of wastewater contaminated with reactive and acid dyes by BGP and TP in the presence of HOBT in a buffer of pH 5.6 and 40 °C. Various complex mixtures of dyes were also successfully decolorized by BGP and TP in the presence of HOBT (Akhtar et al., 2005a; Kulshrestha and Husain, 2007). Matto and Husain (2007) demonstrated the effect of various redox mediators
on TP catalyzed decolorization of direct dyes, used in textile industry. The rate and extent of decolorization of dyes were significantly enhanced by the presence of different types of redox mediators. Six out of 10 investigated compounds have shown their potential in enhancing the decolorization of direct dyes. The performance was evaluated at different concentrations of mediator and enzyme. The efficiency of each natural mediator depends on the type of dye treated. The decolorization of all tested direct dyes and their mixture was maximum in the presence of 0.6 mM redox mediator at pH 5.5 and 30 °C. Reports demonstrated that the use of redox mediators increased the rate of oxidation of aromatic compounds. Both unsaturated fatty acids (UFAs) and HOBT enhanced the enzymatic activity of MnP in an organic medium. The effects of hydrophobic UFAs directly dissolved in an organic medium and hydrophilic HOBT encapsulated in reverse micelles on the oxidation activity of a surfactant MnP complex were investigated. The addition of UFAs or HOBT (mediator) using reverse micelles improved oxidation of 2,4 DCP in toluene up to 3-fold, the oxidation without each mediator. This study presents, for the first time, the possibility of MnP catalyzed oxidation coupled with mediators in organic media. The capability of latter system using HOBT was further assessed by the oxidative conversion of environmental pollutants, i.e. 2,4 DCP, 2,4,5-TCP, 2,4,6- TCP and BPA (Junji et al., 2008).
OBJECTIVES OF PRESENT WORK

- Peroxidases are an important group of enzymes which have an important role in remediation of aromatic pollutants from soil and water. Our objectives revolved around the study of the bitter gourd (*Momordica charantia*) peroxidase, its immobilization and applications in the treatment of various types of organic pollutants present in wastewater.

- In chapter II, peroxidase has been immobilized on a cost free support, fly ash (FA) directly from salt fractionated proteins of bitter gourd. This physically adsorbed enzyme preparation was crosslinked by using glutaraldehyde. The stability of immobilized BGP has been examined against heat, pH, detergent, water-miscible organic solvents and proteolytic enzyme; trypsin.

- In chapter III, the oxidation of various aromatic amines by BGP has been optimized under various experimental parameters such as the effect of pH, temperature, time, concentration of $\text{H}_2\text{O}_2$ and redox mediator $o$-dianisidine HCl. The mixtures of these aromatic amines were also treated by BGP in the presence of $o$-dianisidine HCl.

- In chapter IV, the oxidative degradation and polymerization of BPA by BGP has been investigated in the presence of various redox mediators. Various experimental parameters were also standardized in order to obtain maximum removal of BPA from polluted water. BGP catalyzed BPA oxidized product was also characterized by $^1$H-NMR, FT-IR and GC-MS analysis.

- In chapter V, FA adsorbed BGP has been employed for the removal of BPA from polluted water in the presence of a redox mediator, guaiacol. The experimental conditions have been optimized for the maximum removal of BPA from polluted water by BGP. Immobilized BGP has been employed for the removal of BPA in batch as well as in continuous bed-reactor. Toxicity assays were also performed in order to determine the genotoxicity of the product, 4-IPP by plasmid nicking and comet assays.
In chapter VI, Con A layered calcium alginate-starch beads bound BGP was used to treat anthracene in presence of various mixtures of organic solvents. The effect of different redox mediators on the degradation of anthracene was also evaluated in order to achieve maximum polymerization and removal of anthracene. Immobilized peroxidase was used to remove anthracene in batch as well as continuous spiral bed-reactor.