CHAPTER 2

REVIEW OF LITERATURE
2-1 Overview & Definition

Most of the research carried out in the area of environmental pollution control was by chemical means. Conventional methods for removing organic pollutants, metals and other contaminants from industrial waste solutions (which include chemical precipitation, chemical oxidation or reduction, filtration, electrochemical treatment, application of membrane technology and evaporation recovery) may be ineffective or extremely expensive, especially when the metals are dissolved in large volumes of solutions at relatively low concentrations (around 1-100 mgL⁻¹) [Voletsy, 1987]. To overcome the expenditure incurred in treatment of polluted systems by conventional chemical methodologies, application of biological systems came into practice, which proved to be a potential alternative.

In the wake of this, bioremediation is attempted as a cheaper technology. Bioremediation is the process in which hazardous wastes are biologically degraded to harmless compounds or to such levels, which are below concentration limits dictated by regulatory authorities [Appendix IV & V].

2-2 Microbes employed in environmental pollution control [Metal reduction and biodegradation]

A diverse group of Gram positive bacteria, Gram negative bacteria, yeasts, fungi and algae were found to be employed in environmental pollution control programmes. They can catalyze the degradation of complex organic molecules because of their enzymatic systems. They possess the ability to adsorb metals like Pb, Cd, Cu, Zn and Ag from solutions [Paknikar et al., 1999]. The metal ion uptake by living cells is a function of the cell age, composition of growth media, contact time, pH of metal solution and temperature [Kapoor et al., 1999]. The radius of the metal cation also influences the uptake mechanism of R. arrhizus [Tobin et al., 1984]. The metal uptake by larger particles (0.84 -1.00 mm) was higher than the smaller particles (0.105-0.295 mm) [Andreas et al., 1995]. The adsorption of cadmium on to Streptomyces pimprina was not observed at pH 2, however, as the pH of the solution increased, a rise in the adsorption could be noticed, which reached peak at pH 5.0 [Puranik et al., 1995].
2-2-1 Algae

Marine algae Sargassum fluitans and Ascophyllum nodosum, Vaucheria species, were used for biosorption studies with cadmium, copper, nickel, lead and zinc[Andreas et al., 1995, Ray et al., 1981, Volesky and Holan, 1995]. The mole ratios of H+ displaced/mol of Mz+ adsorbed for Cu²⁺, Zn²⁺, Mg²⁺ and Sr²⁺ were 1.2, 0.66, 0.59 and 0.30, respectively, with Na⁺ showing none[Andreas et al., 1995, Ray et al., 1981]. The algae have a proton equivalence of ~1000 mmol g⁻¹, that metallic ion adsorption range from ~500 mmol g⁻¹ for Cu²⁺ to 100 for Na⁺, that metals displace each other in the order Cu>Sr>Zn>Mg>Na, and they also displace protons, and that Na⁺ decreases adsorption of positive metallic ion complexes and enhances negative complexes[Ray et al., 1981].

A seaweed, Sargassum fluitans was used for modeling of Cd, Cu and Zn systems. It could be predicted that the sorption performance for three-cation systems from equilibrium constants determined with two-cation systems[Silke and Bohumil, 1996]. The Ascophyllum nodosum was employed for performing equilibrium batch sorption studies over either (Cu+Zn), (Cu+Cd) or (Zn+Cd) systems. The uptake of Zn decreased drastically when Cu or Cd was present. The uptake of Cd was much more sensitive to the presence of Cu than to that of Zn[Chong and Volesky, 1995]. The biosorption potential of A.nodosum biomass in the cobalt removal/recovery processes can be established only by thorough study on desorption equilibrium[Kuyucak and Volesky, 1989].

2-2-2 Fungi

Varieties of Fungi were found to show greater applicability in reduction of pollutants present in the contaminated systems. Benzo[a]pyrene, to an extent of 25% was mineralized to CO₂ by the cocultures of bacteria and fungus (Stenotrophomonas maltophilia and Penicillium janthinellum) over 49 days, accompanied by accumulation and disappearance of intermediates[Sudarat et al., 2000, Schwab et al., 1995]. Five fungal species, Cladosporium resinae, Cladosporium sphaerospermum, Exophiala lecanii-corni, Mucor rouxii, and Phanerochaete chrysosporium were tested for ability to degrade nine compounds commonly found in industrial off-gas emissions. Fungal cultures inoculated on ceramic support media were provided with
VOCs via the vapor phase as their sole carbon and energy sources were found to be effective [Qi et al., 2002]. There is a report of using selected microbial consortium, which was able to rapidly and completely degrade 2,4,6-TCP without requiring any supplemental carbon source and/or growth factor in an aerobic fixed-bed bioreactor [Kharoune et al., 2002]. Biosorption of metals by the biomass of filamentous fungi has been mainly associated with the cell wall. The fungal cell wall is thought to have two main components: interwoven skeletal framework microfibrils, usually of chitin, embedded in an amorphous layer of proteins and various polysaccharides (e.g. mannans, glucans and galactans). Reaction in a porous solid in contact with a liquid involves the following steps:

(a) The transport of reactants to the surface.
(b) The sorption of reactant at the surface.
(c) The diffusion of reactant through the solid phase.
(d) Chemical reaction at specific sites.

The slowest process governs the measure of reaction rate and is referred to as the rate-determining step [Devrim and Hayri, 1992].

Fungi show higher tolerance towards heavy metals and hence are potential candidates for efficient metal recovery. They have other advantages like tolerance of low pH and also show good capacities of metal binding to the cell walls. They also exhibit intracellular metal accumulation [Gadd, 1986]. The application of fungi and yeast as biosorbents depends not only on the biosorptive capacity, but also on the ease with which biomass can be regenerated and reused [Kapoor et al., 1999].

Optimum pH range for lead uptake by S. cinnamomeum was 3.5 - 4.5 while for zinc it was 5.0-6.0. The maximum loading capacity of S. cinnamomeum biomass was 57.7 mg/g for lead and 21.3 mg g⁻¹ for zinc with boiling water pretreatment [Paknikar et al., 1999]. Metal uptake was enhanced by 37% both in case of copper and zinc to obtain 75% and 97% removal from solution of pH 4.0 when mycelial pretreatments with dimethyl sulfoxide and boiling water were employed respectively [Paknikar et al., 1993, Ganguli and Tripathi, 2002]. The surface charge density varied with pH, and the concentration of mercury species adsorbed significantly increased from pH
3.0 to maximum levels at pH 8.0. The maximum biosorption capacity ($q_m$) at pH 7.0 was 79 mg for CH$_3$HgCl, 67 mg for C$_2$H$_5$HgCl and 61 mg for Hg (II) per gram of dried fungal biomass [Necdet et al., 1999]. *Cladosporium cladosporioides* showed maximum loading capacities of 40 µmol g$^{-1}$ of tetracyanocuprate and 34 µmol g$^{-1}$ tetracyanonickelate respectively [Patil and Paknikar, 1999]. The uptake of cadmium was found to increase linearly as a function of cadmium concentration up to 500 mg L$^{-1}$ [Puranik et al., 1999]. Biomass from the filamentous fungi took up more thorium than did yeast biomass. Maximum loading (µmol/mg dry wt) on *S. cerevisiae* was found to be 0.265 and for *Penicillium italicum* – 0.490, *P.chrysogenum* – 0.840, *A.niger* – 0.280 and *R.arrhizus* – 0.500 respectively [Gadd and White, 1988]. The fungus *P.chrysogenum* adsorbed 5 $\times$ 10$^4$ nCi g$^{-1}$ radium at pH 7 [Tsezos and Keller, 1983]. The fungus *R.arrhizus* was employed for biosorption of Uranium and Thorium from aqueous solutions and was reported to be taken up uranium from solution to the extent of 180 mg U$^{++}$ g$^{-1}$ [Tsezos and Volesky, 1981 & 1982]. The fungus *Penicillium digitatum* was employed as a potential biosorbents for removal of Ni, Zn, Cd and Pb [Galun et al., 1987, Derek and John, 1997]. Fungi from marine habitats were also employed for bioremediation activities [Chandralata, 2000].

2-2-3 Bacteria

Bacteria isolated from soils exposed to bulk drug wastes were used for the aerobic treatment of the drug industrial effluents. Shake flask experiments followed by scale up studies using individual bacterial isolate reduced COD by 75 percent. Supplementation of native bacterial flora with a specific culture helped in efficiently reducing COD of bulk drug effluent [Ajay et al., 1999, Dreyer et al., 1995]. The COD of wastewater was monitored during incubation with biomass; and a 56% COD reduction was observed. Toxicity was also assessed during the treatment of wastewater and a 32% reduction was calculated. However, when toxicity was assayed in synthetic wastewater, a 90% reduction was routinely obtained [Hidalgo et al., 2002]. A Gram negative bacterium, *Sphaerotilus natans* was tested as adsorbent for binary solutions of Cu-Cd, Cu-Pb and Cu-Zn at different equilibrium pH (in the range of 3-5 with molar ratios of 4:1, 2:1, 1:1 and 1:4). The approach could be empirical or developing chemico-physical mechanistic models. The copper uptake was strongly influenced by growing concentrations of lead in solution, while it was less affected by cadmium presence. Cadmium was strongly
Influenced by copper, while lead is weakly sensitive to copper presence [Francesca et al., 2002]. The 3D plot of copper and lead adsorption onto Sphaerotilus natans at pH 5 obtained by equilibrium parameters of Langmuir competitive models reveal that Pb uptake prevails over copper uptake when equimolar feed is considered. The biosorption selectivity expressed in the Langmuir competitive equilibrium parameters are reflected in the permeate concentration curves of copper and lead vs. time obtained by simulation in the membrane reactor. In particular, lead break-through was observable after the copper one, which, being the less retained metal, was released by the system first. According to the experimental data of permeate concentration vs. time, the temporal series of breakthrough curves is Cd, Cu and Pb, and overshoot curves were observed first for Cd and then for Cu [Veglio et al., 2002]. The Pseudomonas soil isolate adsorbed thorium (IV) (430 mg g⁻¹ dry wt) optimally at pH 4, with 91% of equilibrium loading being reached in 1 min [Pinaki and Stainislaus, 2002]. Cell-bound uranium reached a concentration of 10-15% of the dry cell weight, but only 32% and 44% of the Pseudomonas cells within the population possessed visible uranium deposits when examined by electron microscopy [Gerald et al., 1981].

2-3 White rot fungi

White rot fungi, basidiomycetous members are usually found in the woods, mostly degrading the lignin part of the plant. They have been extensively studied highlighting their role in degrading the complex lignin structure with the help of lignolytic enzymes and exploited for the pollution control programmes. They require trace amounts of essential heavy metals such as Cd, Mn or Zn for their growth, but these metals are toxic when present in excess. Toxic heavy metals can inhibit the growth, cause morphological and physiological changes and affect the reproduction of basidiomycetes. Fungal species and strains differ in their sensitivity towards metals and in the protection mechanisms involved. The toxicity of some heavy metals such as Hg, Cu or Ni has been used for the development of antifungal wood preservatives. Extracellular lignolytic and cellulolytic enzymes are regulated by heavy metals on the level of transcription as well as during their action. During the degradation of lignocellulose and xenobiotics by white-rot fungi or isolated enzymes from these fungi heavy metals interfere with both the activity of extracellular enzymes involved in the process of fungal colonization. The ability of
white-rot fungi to adsorb and accumulate metals together with the excellent mechanical properties of fungal mycelial pellets provide an opportunity for application of fungal mycelia in selective sorption of individual metal ions from polluted water [Petr, 2003].

The COD of the pulp and paper mill effluent has declined to 77.7% and 79.4% in presence of T. versicolor and P. chrysosporium, respectively [Prasad and Gupta, 1997]. The wastewaters had a pH of 10 and a phenol content of 370-660 mgL⁻¹, the organic load in terms of COD was 4.7-6.8 gL⁻¹. After inoculation, phenol was completely removed within 48 h at 28 °C, the COD removal ranged from 11 to 56%. Polynuclear aromatic hydrocarbons (PAHs) have not been reported be biodegraded under high pressure and high salt concentrations [Margesin and Schinner, 2001]. About 36% cellulose is consumed accompanied by a crude protein production of 4.45% on a dry weight basis. The most desirable situation in the study, namely, high pellet number and minimum diameter, was obtained at a temperature of 38 °C, an agitation speed of 140 rpm and a pH value of 4.8. Even the pulsation frequency affects the pellet formation [Shubhayu et al., 2002, Moreira et al., 1996, Frederik et al., 2001, Hongzhang et al., 2002]. Twenty white-rot fungi have been checked for their cellular ergosterol content which was found to be in the range of 2380 to 13060 pg g⁻¹ of the fungal biomass. Heavy metals (Cu 80 ppm, Zn 50 ppm or Cd 10 ppm) and fungicides (thiram 3 ppm or pentachlorophenol 1.5 ppm) at concentrations that reduce the metabolic activity between 18% and 53% (Pollutant-stressed cultures) did not affect the ergosterol content [Karlson et al., 1995, Martha et al., 2002]. The biosorption of inorganic mercury (HgCl₂), methyl mercury (CH₃HgCl) and ethyl mercury (C₂H₅HgCl) onto the dry biomass of Phanerochaete chrysosporium was studied from aqueous media concentrations ranging from 5-500 mgL⁻¹. The surface charge density varied with pH, and the concentration of mercury species adsorbed significantly increased from pH 3.0 to maximum levels at pH 8.0. The maximum biosorption capacity (qₑ) at pH 7.0 was 79 mg for CH₃HgCl, 67 mg for C₂H₅HgCl and 61mg for Hg (II) per g of dried fungal biomass [Neddet et al., 1999]. Immobilized inactivated P. chrysosporium showed maximum biosorption of Hg (II) and Cd (II) than immobilized live fungus [Yasemin et al., 2002].

A well-defined PAH-degrading strain (P. sutzeri P-16) and a non-degrading strain (R. rhodochrous RR1) were selected for difference in sorptive affinity. The cultures were grown
individually as described above, washed and combined in known ratios. The rate of fluoranthene and pyrene degradation was reduced from 10.2 µM and 4.2 µM g⁻¹ biomass per day to 3.5 µM and 1.7 µM g⁻¹ biomass per day in the presence of non-degrading system.

The removal of Pb (II) by live, resting and dead cells of a lignolytic white-rot fungus, *Phanerochaete chrysosporium* was investigated. Kinetic studies revealed the fact that adsorption is a two-stage process: a very rapid surface adsorption within the first hour and a slow intracellular diffusion during 2 h of metal exposure. The results showed that the resting were able to uptake up to 80 mg Pb (II)/g dry cell. The saturation sorption capacities of live and dead cells were 9 and 20 mg Pb (II)/g dry cell. It appeared that the young resting cell held higher Pb (II) adsorption capacities than old ones. Alkali pretreatments resulted in enhanced removals in young and old cells [Ulku *et al.*, 2000]. *Phanerochaete chrysosporium* could survive at the concentrations up to 400 µg ml⁻¹ of Cd, Cu, Pb, Mn, Ni and Co. At those concentrations the yield percentages were 33, 35, 30, 27, 18 and 31% in media containing Cd, Cu, Pb, Mn, Ni and Co, respectively [Abdullah, 1997]. The biosorption of Hg (II) ions onto carboxymethylcellulose and both immobilized live and heat-inactivated fungal mycelia of *Phanerochaete chrysosporium* was studied using aqueous solutions in the concentration range 30-700 mg L⁻¹. Maximum biosorption capacity for immobilized live and heat-inactivated fungal mycelia of *Phanerochaete chrysosporium* was found to be 83.10 and 102.15 mg Hg (II)/g, respectively, whereas the amount of Hg(II) ions adsorbed onto plain carboxymethylcellulose beads was 39.42 mg g⁻¹ [Saglam *et al.*, 2002]. The biosorption of UO₂²⁺ ions on CMC and immobilized and heat-killed fungal mycelia of *T. versicolor* and *P. chrysosporium* was studied from the aqueous solutions in the concentration range of 100-1000 mg L⁻¹. Maximum biosorption capacities for immobilized and dried powdered fungal mycelia of *T. versicolor* and *P. chrysosporium* was found as 309.1 mg UO₂²⁺ g⁻¹ and 158.0 mg UO₂²⁺ g⁻¹, respectively; whereas the amount of UO₂²⁺ ions adsorbed on the plain CMC beads was 29.2 mg g⁻¹. Biosorption equilibria were established in about 20 minutes. Optimum biosorption for all the systems was reported at pH 4.5. For the synthetic wastewater samples, it was observed that immobilized and dried powdered fungal mycelia of *T. versicolor* and *P. chrysosporium* removed 91.8% and 66.8% of Uranium ions, respectively [Genc *et al.*, 2003].
2-4 Fungal growth – Substrates - Effect of surfactant

Varieties of substrates have been employed for the bioremediation activities; they include sugarcane bagasse, rice bran, wheat bran apart from effluents of different kinds. Supplementation and enrichment of the media and substrates were proved to enhance the degradation capabilities of the microbes employed. One such method is addition of surfactant to enhance the lignolytic activity of the fungus *Pechysosporium* in bioremediation programmes. When bioremediation of PAH-contaminated soil was studied using Tween 80, approximately 85% (w/w) of a total of nine PAHs in a month old contaminated soil (total PAH concentration = 403.61 μg g⁻¹) could be solubilized in a 2.5% (w/v) at a soil/water ratio of 1:10. The disappearance of most PAHs tested (mol. wt. 178) correlated well with their ionization potentials and 66.4% (w/w) of the total PAHs in washwater with 2.5% (w/v) [Zhongming and Jeffrey, 2000, Tittle et al., 1995]. Tween-80 showed little impact on synthesis of lignolytic enzymes but, addition of 0.4% Tween-80 enhances the elimination of acenaphthene and fluorene by 15-33% [Zhongming and Jeffrey, 2001, You et al., 1995].

2-5 Pretreatment Studies

Materials (specifically, Substrates) employed for remediation treatment with microorganisms cannot be directly used, and they should undergo a prior pretreatment process. It is necessary to remove water and neutralize the pH to ensure best conditions for microbial growth. Bulking agents are also added to increase porosity of the sludge and decrease the bulk density. The increased porosity may help in drainage of water, which can be carried out either by gravity or by exerting pressure on the sludge [Thassitou and Aranitoyannis, 2001]. Another report say that the pretreatment process by unique microbial consortium can depolymerize or desulphurize 40% of organic sulphur from coal, which is the major source of pollution in coal combustion [Naqvi, 1993]. Pretreatment of biomass with boiling water for 15 min increased the biosorption of lead and zinc by 52 and 41% respectively. The optimum pH range for lead uptake was 3.5 – 4.5 while for zinc it was 5.0-6.0. The maximum loading capacity of *S.cinnamoneum* biomass was found to be 57.7 mg g⁻¹ for lead and 21.3 mg g⁻¹ for zinc with boiling water pretreatment [Puranik and Paknikar, 1997]. Metal uptake was enhanced by 37% both in case of copper and zinc to obtain 75% and 97% removal from solution of pH 4.0 when
mycelial pretreatments with dimethyl sulfoxide and boiling water were employed respectively [Paknikar et al., 1993].

The level of inoculation is also a factor that has a major impact on both the efficiency and cost of bioaugmentation. Inoculation at a high level, for example $10^6$ colony-forming units (cfu) g$^{-1}$, would certainly be efficient for the degradation of specific contaminants but the associated cost could become excessive. Inoculation at a low level would be less expensive but could affect the efficiency of biodegradation due to factors retarding or even preventing growth during the acclimation period preceding contaminant removal [Yves, et al., 1993].

Bioresmediation earlier was dealt with application of microbes in removal of colour in textile and dye effluents and also in soil contaminated systems. The understanding of bioremediation goes back with application of microbes like *Pseudomonas*, *Achromobacter*, *Flavobacter* etc. for cleaning up of oil spills and various contaminated soil systems. Then comes the group white-rot fungi, which include the species of *Phanerochaete*, *Pleurotus*, *Coriolus*, *Trametes*, *Bjerkandera*, etc. and their application as bioremediation tools for their capabilities to degrade complex organic natural polymers like lignin, hemicellulose and cellulose [Bisaria, 1998].

2-6 Lignin and its role

Lignin is the second most abundant organic molecule present on earth preceded by cellulose. It forms an integral part of the shoot system of the plants. Naturally, degradation of lignin is a tedious and time consuming process as it is found mostly encrusted in hemicellulose layer, which forms difficult for the biocatalysts to act upon. Lignin is a phenylpropanoid polymer synthesized from coniferyl, synapyl, and $p$-coumaryl alcohols (Figure 2-1). Free-radical condensation results a heterogeneous, amorphous, optically inactive, random, and highly branched polymer with at least 12 different types of linkages such as $\beta$ - aryl ether and carbon-carbon bonds connecting the aromatic nuclei [John and Steven, 1987]. The unique structure requiring depolymerization by extracellular oxidative mechanisms accounts for the recalcitrance of lignin towards degradation by most microorganisms. Lignin is degraded by *Phycomyces* only during secondary (idiophasic) metabolism, whose onset is triggered by depleting nutrient nitrogen, carbon, or sulphur. Since lignin contains a variety of bonds that are commonly present
in aromatic pollutants and since the lignin-degradative system of these fungi is nonspecific and oxidative, several laboratories have examined such fungi as potential bioremediation agents [Tonon et al., 1989, Akihiko et al., 2001].

White rot fungi gained significance in bioremediation because of their intrinsic ability to produce and secrete ligninases, which help in catalysis of complex structures of lignin. In 1982 and 1984, two extracellular enzymes-lignin peroxidase (LiP) (EC 1.11.1.14) and manganese peroxidase (MnP) (EC 1.11.1.13), were discovered in *Pchrysosporium* that were demonstrated to be major components of lignin degradation system [Dongmei et al., 2001]. LiP is present as a series of glycosylated isozymes with pIs ranging from 3.2 to 4.0 and molecular masses ranging from 38 to 43 kDa. MnP has pIs ranging from 4.2 to 4.9 and molecular masses ranging from 45 to 47 kDa [Liesola and Garcia, 1989].

In catalytic role of LiP, the primary reaction product of LiP with H$_2$O$_2$ is the two-electron oxidized state compound I (LiP I). As with HRP, LiP I is reduced back to the native enzyme via two single-electron steps with compound II (LiP II), as an intermediate (Scheme A). In the process, the aromatic reducing substrate is oxidized to an aryl cation radical (Ar$^+$) [Harvey et al., 1989].

Scheme A:

\[
\begin{align*}
\text{LiP} + \text{H}_2\text{O}_2 & \rightarrow \text{LiP I} + \text{H}_2\text{O} \\
\text{LiP I} + \text{Ar} & \rightarrow \text{LiP II} + \text{Ar}^+ \\
\text{LiP II} + \text{Ar} & \rightarrow \text{Ar}^+ + \text{H}_2\text{O}
\end{align*}
\]

The important difference between the peroxidases is in the nature of the reducing substrate. LiP catalyzes the oxidation of nonphenolic lignin model compound such as veratryl alcohol to veratryl aldehyde. Kinetic results also indicate a 'Ping-Pong' mechanism in which H$_2$O$_2$ first oxidized the enzyme and the oxidized enzyme intermediate (Compound I) reacts with veratryl alcohol.

The primary reducing substrate in the MnP catalytic cycle is Mn(II), which efficiently reduces both MnP I and MnP II, generating Mn (III), which subsequently oxidizes the organic substrate.
Organic acids such as oxalate and malonate, which are secreted by *P.chrysosporium*, stimulate the MnP reaction stabilizing the Mn (III) so that it can diffuse from substrate, lignin.

Scheme B:

\[
\begin{align*}
\text{MnP} + H_2O_2 & \rightarrow \text{MnP I} + H_2O \\
\text{MnP I} + \text{Mn (II)} & \rightarrow \text{MnP II} + \text{Mn (III)} \\
\text{MnP II} + \text{Mn (II)} & \rightarrow \text{MnP} + \text{Mn (III)} + H_2O
\end{align*}
\]

The mycelium of *P.chrysosporium* is coenocytic, with few septa and as many as 15 randomly dispersed nuclei per cell. The septa lack clamp connections. Although the multinucleate conidia are generally heterokaryotic, the basidiospores contain two-identical nuclei arising from a postmeiotic mitotic division. Evidence has been presented for both homothallic and heterothallic mating systems in various strains of *P.chrysosporium* [Michael and Margaret, 1993].

![Figure 2-1: Typical SEM micrograph of P.chrysosporium mycelia. Source: Downloaded from www.sciencedirect.com. (Nathan et al., 1995)](image-url)
2-7 Modeling and its application

Langmuir, Freundlich and Redlich-Peterson isotherms were attempted to fit the obtained experimental data. In both single and two metal systems, a simple procedure of model discrimination was performed to establish which of the tested models better represents the experimental behaviour [Pagnanelli et al., 2001]. The applicability of mono-component Langmuir and Freundlich models at both the studied pH values (1.0 and 4.0) indicated that the individual biosorption of Cr (VI) and Ni (II) ions are favorable and could be characterized as a monolayer, single-site-type phenomenon with no interaction between sorbed components and the microbial surface [Aksu et al., 2002]. The Rarrhizus and activated sludge, a complex consortium of microorganisms mainly containing bacteria were employed for kinetic studies and found to be reasonably fast. The rate of lindane, 2-PCB and diazinon biosorption by activated sludge, for the rapid first stage, can be described by second order kinetics. The biosorption of lindane on both biomass types is reversible, and the desorption process is rapid and could be described by zero order kinetics when activated sludge used as adsorbent [Marios and Wang, 1991].
Equilibrium metal uptake performance of *Rhizopus arrhizus* was studied for Cr (VI), Fe (III) and Cu (II) ions with equal initial molar concentrations, the relative surface coverage of three metals on the biomass was 45-55%, 36-41% and 8-14%, respectively [Yesim et al., 2001].

The metal uptake was found to be rapid in the first 10 min, reaching equilibrium within 30 min of contact in *S. cinnamomeum, P. chrysogenum* and *Citrobacter* sp. The rate of metal uptake was independent of initial metal concentration [Puranik et al., 1999].

At pH 2, the chitin uptake of Uranium (ca. 1mg g⁻¹) was significantly lower than the uptake of uranium at pH 4 (9mg g⁻¹) under similar experimental conditions [Marios, 1983].

Unconditioned activated sludge (UAS) bound up to 35 mg Cu g⁻¹, although there was a significant leaching of organic material [Paul Chen et al., 2002]. A new biosorbent, PFB1, had a specific surface area of 256.8 m² g⁻¹ and showed high equilibrium capacities for cobalt uptake, the highest being 190 mg g⁻¹, in the range of concentration employed. The equilibrium uptake decreased with increasing temperature in the range 30-45 °C. The desorption efficiency with 0.1N HCl was 92.3% at the end of the first cycle and reduced to 70.5% at the end of the third cycle. The desorption efficiency with 0.1N H₂SO₄ was 90.5% at the end of the first cycle and reduced to 72.4% at the end of the third cycle. The re-adsorption efficiencies for cobalt biosorption were 89.6 and 72% of the initial adsorption efficiency after the first and third cycles, respectively, when 0.1 N HCl was used as the desorvent. The corresponding values when 0.1 N H₂SO₄ was used were 65.4 and 47%, respectively [Suhasini et al., 1999]. Two biosorbents, PFB1 and PFB2, have been developed for high uptakes for nickel 214 mg g⁻¹ for PFB1 and 110 mg g⁻¹ for PFB2 respectively. The average efficiency for nickel removal was 84.5% (PFB1) and 60.8% (PFB2) [Suhasini et al., 1999]. The fungus *Aspergillus carbonarius* NRC 401121, adsorbed chromium and copper from their solutions. The amount of the adsorbed metal per unit of biomass increased with a decrease in the biomass concentration. Pre-incubation of the biomass with glucose enhanced the metal adsorption. The optimum glucose concentration in the process was 0.1% and uptake of Cu²⁺ was found to be 9.43 (µg mg⁻¹) when Cu²⁺ was added after shaking the suspension with glucose for 2 h [Sameer and Zdravko, 1995].
The Advection-Dispersion-Reaction (ADR) equation has been applied as the basic modeling equation for the special case of Local Equilibrium (LE) is applied. An apparent axial dispersion coefficient was used as the key parameter of the model. The other main assumption, that biosorption equilibrium is rapid, the use of an apparent overall dispersion coefficient makes the model applicable for the cases where mass transfer resistances are present in the liquid and solid phases [Hatzikioseyian et al., 2001]. The sorption of metals is accompanied by displacement of other cations. Langmuir treatment is not appropriate whereas a Scatchard plot will give a clear idea, when observed carefully the chemical sorption is one of the method of ion-exchange, not simple adsorption phenomenon [Ray et al., 1994].

The exchange between the metal and protons was the major binding mechanism in biosorption by protonated biomass. It is recommended to use two-site model instead of Langmuir or Freundlich models in order to accommodate the significance of ion exchange on biosorption [Silke and Bohumil, 1995]. The presence of Zn tends to lower the total metal uptake in two-metal systems (with Cu or Cd). Its own uptake cannot compensate for the uptake inhibition of the other metal [de Carvalho, 1995].

2-8 Veratryl Alcohol – its role

During the growth of *Rhzosporium*, veratryl alcohol is synthesized in the idiophasic stage and is known to be a key and determining intermediate as it triggers the production of ligninases. Contrasting results are also available stating that veratryl alcohol, a secondary metabolite of *Rhzosporium* growth cycle, acts only as a stabilizer of lignin peroxidase activity and not as an inducer of lignin peroxidase synthesis [Aida et al., 1993]. The recovered yield of veratryl alcohol by reduction of veratraldehyde was between 60-75% (w/w) of the starting material, 0.4 to 0.2 mM veratryl alcohol. The ratio of veratryl alcohol to veratraldehyde in the final product was 99:1 as determined by the peak areas of the chromatogram [Kanyawim et al., 1995]. Substitution of micromolar concentrations of Azure A, B and C for the millimolar concentrations of VA in the LP assay gave reaction rates that were constant over many minutes and that were equal or to a greater than observed VA oxidation rates under the same conditions [Frederick, 1992].

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Bioremediation activities have been focused mainly on soil contaminations with petroleum products spillage. Variety of microorganisms were tried and tested for efficient degradation of complex organic molecules, which include aliphatic, aromatic hydrocarbons, PAHs, PCBs and PCDDs etc. Aliphatic hydrocarbons are prone easily to microbial degradation when compared to aromatic compounds. Many PAHs are widespread environmental pollutants and have toxic, mutagenic and carcinogenic properties. PAHs are formed during pyrolytic processes or incomplete combustion of organic substrates. These are highly lipid soluble and thus readily absorbed from the gastro-intestinal tract of mammals [Sudip et al., 2002]. Moreover, the hydrophobic nature of most of the PAHs makes them less likely to be affected by natural elimination processes such as volatilization, photolysis and biodegradation. Generally, two mechanisms are available for biological fission of the aromatic nucleus. Depending on the type of cleavage, the mechanisms have been designated as ortho- and meta- forms. In the former, cleavage occurs between the hydroxylated carbon atoms, while in case of the latter the cleavage takes place adjacent to the hydroxylated carbon atoms. Catabolic enzymes have broadly been grouped into peripheral and ring-cleavage enzymes. The ring-cleavage enzymes from a variety of microbes exhibit significant functional similarity. The peripheral enzymes, however, convert a xenobiotic compound into metabolites, which are degradable. The aromatic ring-oxygenases add molecular dioxygen into the aromatic ring and need cofactors such as NADH, NADPH during this process. These play a significant role in catabolism of naturally occurring and xenobiotic compounds. Di-oxygenases increase the reactivity of aromatic rings by catalyzing the incorporation of two hydroxyl groups. Dehalogenases are the key enzymes catalyzing dehalogenation of aromatic hydrocarbons by cleaving the carbon-halogen bond [Vachaspati et al., 2001]. Aromatic compounds become resistant to electrophilic attack by oxygenases with the presence of electron-withdrawing substituents, such as halo, azo, or nitro groups. Theoretically, these compounds are more likely to be reduced by the enzymes rather than oxidized, particularly in anoxic microbial systems, which are capable of catalyzing a wide range of reductive reactions. Reductive dechlorination, hydrogenolytic denitrations, and reduction of nitro groups decrease the redox potential of the xenobiotic compounds to such an extent that the metabolites generated are now susceptible to oxidative treatment process. Increased
reactivity of the reduced products with molecular oxygen can facilitate a subsequent oxidative treatment process [Parales et al., 2002]. Degradation of benzene, toluene, ethyl-benzene and xylenes (BTEX), a group of common organopollutants derived from gasoline and aviation fuels, was observed in work with Pchrysosporium [Andrzej and Ronald, 1995]. Biodegradation occurred over a broad range of temperature (45-77°C) [Margesin and Schinner, 2001].

Xenobiotic-degrading microorganisms have tremendous potential for bioremediation but new modifications are required to make such microorganisms effective and efficient in removing these compounds [Sudip et al., 2002]. Mineralization of naphthalene in polluted soil, determined at 8°C, was 4.02% and 1.42% CO₂ day⁻¹ with and without N fertilization, respectively, and was considerably higher than degradation of hexadecane. Bacillus strains degraded about 80-89% of the crude oil (5gL⁻¹) within 5 days at their optimum growth temperature of 60°C, biodegradation was 3-to-4-fold lower at 40°C. Resting strains, Thermus aquaticus and Thermus sp. cell suspensions degraded 10-40% of low BTEX concentrations (initial total concentrations were 2 and 7 mgL⁻¹) within 45 days at 60°C and 70°C. Only small fractions of benzene and toluene were metabolized to CO₂ [Brigita et al., 1999]. Biomass formation (2.5g cell dry weight L⁻¹) and BTEX degradation (Sp. Activity of 2-10 nmol degraded BTEX mg/cell dw/min) by Thermus sp. were higher in fed-batch culture than in batch culture. A slow and moderate specific growth rate of 0.02-0.07 h⁻¹ favored BTEX degradation, while a high growth rate, such as 0.16 h⁻¹, was inhibiting. Biodegradation occurred over a broad range of temperature (45-77 °C) [Margesin and Schinner, 2001].

Over 87% of phenanthrene was degraded at 40% water content after 7 days culture at drum bioreactor rotation speeds (0,1,3 and 6 rpm). While 70% of fluorene was degraded in the initial short period of 3 days. Significant degradation of pyrene, a tetracyclic compound, could be observed after 10 days when most of the fluorene and the phenanthrene had been exhausted. Similar results showing the relationship between bioavailability and degree of condensation have been reported. Anthracene, in contrast to phenanthrene, which has the same degree of condensation, began to be degraded after about 7 days but very slowly and steadily. This could be related to low water solubility of anthracene as well as its ring structure [Seung and Jong, 1999, Christopher, Article in press (2003)]. Laccases (EC 1.10.3.2) of Trametes versicolor could
2-10 Ligninolytic Enzymes

The major components in the bioremediation activities include the performance significance of the extracellular enzymes being secreted by the fungus, *P. chrysosporium*. LiP, MnP and Laccase are the three common lignolytic enzymes being produced by the white rot fungi employed for biodegradation activities [Ming and Kant, 1983]. LiP is characterized by oxidation of high redox-potential aromatic compounds (including veratryl alcohol) whereas MnP requires Mn$^{2+}$ to complete the catalytic cycle and forms Mn$^{3+}$ chelates acting as diffusing oxidizers. *Pleurotus* and *Bjerkandera* versatile peroxidase (VP) is able to oxidize Mn$^{2+}$ as well as non-phenolic aromatic compounds, phenols and dyes [Angel, 2002, Fiechter *et al.*, 1989]. Experiments conducted reveal that lignin degradation was at least 50% more rapid in cultures under 100% O$_2$, 1800 UL$^{-1}$ LiP than those under air, 1200 UL$^{-1}$. Addition of 0.12% nutrient N increased two to five fold in rate of lignin degradation over a two-week incubation, or approximately 2.9 mg mg$^{-1}$ fungal cell protein day$^{-1}$ [Yang *et al.*, 1980, Nathan *et al.*, 1995]. It is evident that *Oxysporus* sp. was most active in terms of detectable laccase activity and degradation (ca. 70%) of the lignified material in pre-treated olive pomace, with *P. chrysosporium* achieving ca. 60% when grown at 37 °C [Haddadin *et al.*, 2002].

MnP activity was around 62 UL$^{-1}$ on first day and reached maximum 154 UL$^{-1}$ on 9th day, whereas LiP was 26, 39 and 194 UL$^{-1}$ on 4th, 6th and 13th day respectively when checked in static-bed bioreactor. MnP activity started on 6th day (254 UL$^{-1}$) and increased to 1477 UL$^{-1}$ on 11th day and LiP activity began on 6th day (61 UL$^{-1}$) and increased after short gap to 277 UL$^{-1}$ on the 10th day when checked with Immersion Bioreactor [Susana *et al.*, 2001, Gloria *et al.*, 1997, Susana *et al.*, 2002, Nathan *et al.*, 2002]. When grown in liquid cultures, *P. flavido-alba* secretes two families of MnPs (MnP 1 and MnP 2), LiP and Laccase. In carbon-limited media, the two MnP families were detected, but the main peak coincides with MnP 1. On the other hand, in nitrogen limited and high Mn$^{2+}$ media the MnP activity was distributed into two peaks. The MnP 2 remains stable during the entire biodegradation course, probably plays an important role in Olive Mill Waste depolination [Teresa *et al.*, 2002, Yasumi and Mikio, 1996]. MnP supported the slow oxidation of phenanthrene to 2,2'-diphenic acid in a reaction that required Mn(II), oxygen and unsaturated lipids [Mark and Kenneth, 1994]. MnP from *Phanerochaete chrysosporium*
oxidizes bromide and iodide forming tribromide and triiodide complexes. Transient-state kinetic studies reveal that the reaction between MnP compound I and bromide or iodide occurs via a single two-electron step process obeying second-order kinetics [Dawei and Michael, 1997, Serguei and Steven, 1997, Gerin et al., 1997]. The highest 4-CP degradation was observed in medium with high glucose/low nitrogen, which produced high level of MnP [Dolarice and Regina, 2001, Sigoillot et al., 2001, Shinichi et al., 1997]. Total degradation of 4-CP occurred within 4 days of incubation while only 60% of 4-CP was degraded without Mn II in the medium [Hela et al., 2002, Rubio et al., 1997]. MnP activity probably decreased due to proteases present in the culture. Otherwise, LiP activity was observed in the exponential growth phase, in the first days of fermentation. However, maximum activity was obtained at 16 days of incubation (8.1 x 10^3 Uml^1) [Cruz-Curdova, 1999, Orly et al., 1998]. A novel mannose-6-phosphatase (M6Pase) from the extracellular culture fluid filtrate of *Phanerochaete chrysosporium*, shown to be responsible for the extracellular post-translational modification of LiP was purified and characterized. The enzyme displayed a molecular mass of 82 kDa by gel filtration and 40.4 and 39.1 kDa on SDS-PAGE, suggesting the native form is a dimer. The uniqueness of the M6Pase in LiP dephosphorylation is emphasized by the inability of acid or alkaline phosphatases to dephosphorylate LiP [Nathan et al., 1999, Martins et al., 2002, Raghukumar et al., 1999].

Maximum activities of MnP and LiP in free cultures of *Phanerochaete chrysosporium* were 258 UL^1 and 103 UL^1, respectively, in an airlift bioreactor [Dominguez et al., 2001]. Immobilization of the fungus on an inert carrier as well as several design modifications of the bioreactor employed gave MnP activities around 500-600 UL^1 during 9 days’ operation. The continuous operation of the latter led to MnP and LiP activities about 140 UL^1 and 100 UL^1, respectively, for two months, without operational problems [Dominguez et al., 2001, Toshiya et al., 2001]. Maximum MnP, Laccase and LiP activities observed in a batch rotating drum bioreactor were 1350, 56 and 364 UL^1, respectively [Dominguez et al., 2001]. A maximum of LiP activities achieved in a continuous FBR was around 700 UL^1 [Rodriguez et al., 2002].

2-11 Radioactive materials and their mineralization

Most of the laboratory scale bioremediation experiments were carried out radioactive labeling to track down the degradation patterns and pathways of xenobiotic compounds. Mineralization
of 2,4-[\(^{14}\)C] dinitrotoluene by \textit{Phanerochaete chrysosporium}, approximately 34% of the substrate was degraded to \(^{14}\)CO\(_2\) in nitrogen-limited (1.2 mM ammonium tartrate) cultures, as after 24-hours of incubation\cite{1992}. The radiolabeling study conducted for 14 days on the reactor biomass showed a very reasonable mass balance of \(^{14}\)C\textsuperscript{TNT} with recovery of 95% of the \(^{14}\)C\textsuperscript{TNT}. Of the original \(^{14}\)C\textsuperscript{CO}, 27% was used in making cellular materials. The rest of the TNT was accounted for as intermediates. In the insitu bioremediation study conducted, the original TNT concentration of 3839 mg kg\(^{-1}\) of soil dropped steadily and reached 0.5 mg kg\(^{-1}\) of soil on day 305 of the experiment\cite{2000,1995}. Decomposition of \(^{14}\)C-labeled lignin to \(^{14}\)CO\(_2\) by the lignin-decomposing fungi \textit{Phanerochaete chrysosporium} and \textit{Coriolus versicolor} required a growth substrate such as cellulose or glucose. Growth with lignin as sole carbon addition to an otherwise complete medium was negligible\cite{1976}.

2-12 Polychloro Biphenyls and PCDDs

The priority chemicals included in the USEPA list are mostly pesticides and rodenticides, which are highly complex organic molecules. Study on their structural complexity and mode of action and mechanism of their degradation is worthwhile. The degradation of Aroclors 1242, 1254 and 1260 was reported about 60.9, 30.5 and 17.6% respectively with lignolytic systems\cite{1995}. The highest PCB transformation (70%) was obtained with \textit{T. versicolor} at an initial PCB concentration of 1800 mgL\(^{-1}\), whereas \textit{P. chrysosporium} could modify 73% at 600 mgL\(^{-1}\). Interestingly, \textit{P. chrysosporium} was the most effective for PCB metabolization at an initial concentration of 3000 mgL\(^{-1}\), and it reduced up to 34% of the PCB mixture\cite{2002,1995}. Dichlorodibenzo-\(p\)-Dioxin was slowly oxidized by LiP to yield 4-chloro1,2-benzoquinone and 2-hydroxy-1,4-benzoquinone was identified as its phenazine derivative, after reductive acetylation. When veratryl alcohol was added to the reaction mixture, the product yields increased but no additional products from DCDD were observed\cite{1992,1996}. 

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Decolorization – Pragmatic Shift in Bioremediation

Earlier, bioremediation work was focused on decolorization of textile effluents, where application of white rot fungi for such decolorization may be due to either adsorption on the fungal biomass or biodegradation. The extent of color removal by adsorption alone was always limited (generally <50%), and strong decolorization (>90%) was always associated with primary degradation, although it is uncertain whether mineralization has occurred in the process[Knapp et al., 1995, Jagroop et al., 2001]. P.chrysosporium totally decolorized amaranth, new coccine and orange G and 60% tartrazine. Pleurotus sajor-caju totally decolorized amaranth and new coccine, 50% orange G and a maximum of 20% tartrazine[Eliana and Lucia, 2001]. Clitocybula dusenii, a white-rot fungus had higher MnP and laccase activities when grown with dye effluent than in control cultures. Flavodon flavus, a basidiomycete isolated from a marine habitat decolorized pigments in molasses spent wash (MSW) by 80% after 8 days of incubation[Chandralata and Gauri, 2001, Raghukumar et al., 1999]. The activity of MnP increased commensurately with the proportion of the raw dye wastewater in the medium (control: 20 UL⁻¹; 10% v/v effluent: 67 UL⁻¹; 25% v/v effluent: 130 UL⁻¹; and 33% v/v effluent: 180 UL⁻¹)[Dirk et al., 2002, Helena et al., 1999, Andrez and Ronald, 1995]. Three copper phthalocyanine dyes, Remazol TB, Everzol TB and Heligon B were found to be biosorbed by P.chrysosporium and also metabolized by its lignolytic extracellular enzymes resulting in dye decolorization, formation of free copper ions and organic metabolites with ultimate extensive phthalocyanine ring breakdown[Annette et al., 1998, Conneely et al., 2002, Robinson et al., 2001]. During decolorization of Indigo carmine lignolytic enzymes of Phanerochaete chrysosporium, the intensive blue colour of the solution was converted into light. Growth medium with high MnP activity showed intensively red coloured product formation during decolorization. This colour was observed with LiP decolorization[Helena et al., 2001, Ursa et al., 2003]. Immobilization of P.chrysosporium has carried out in a continuous packed bed bioreactor for periods longer than 30 days for degradation of azo-dye (Orange II). Nearly, 95% decolorization was achieved when working at a high dye load rate of 0.2 gL⁻¹d⁻¹, a temperature of 37 °C, a hydraulic retention time of 24h and oxygen in a pulsed flow. These conditions favored MnP production and also the decolorization of Orange II. The operation was started initially by applying an Orange II loading rate 0.1gL⁻¹d⁻¹, these conditions when
maintained for 12 days, the efficient decolorization higher than 98% was observed. MnP activities were in the range of 110 and 130 UL⁻¹. A maximum of 175 UL⁻¹ of MnP activity was observed [Mielgo et al., 2001, Kubatova et al., 2001, Mahnaz et al., 2001, Martins et al., 2001]. Decolorization of Vat textile dye like Indigo present in the liquid medium was checked. Decolorization has started in a few hours and after 4 days the removal of dye by Phellinus gilvus culture was in 100%, by Pleurotus sajor-caju 94%, by Pycnoporus sanguineus 91% and by Phanerochaete chrysosporium 75% [Dolarice and Regina, 2001, Martins et al., 2001]. The efficiency of Pflavidio-alba for the decolorization of OMW (Oil Mill Waste) was checked and was found to be 70% after treatment. The reduction of aromatic compounds obtained was 51%, and the toxicity of the culture medium was reduced by up to 70%. We can therefore state that Pflavidio-alba is capable of reducing important environmental parameters of industrial effluents and that prospects are positive for the use of this process at a larger scale, even when working at room temperature [Blanquez et al., 2002].

2-14 Significance of H₂O₂

The MnP, which catalyzes the oxidation of Mn²⁺ to Mn³⁺, is easily inactivated by hydrogen peroxide. Increase in H₂O₂ resistance was attempted by conformational stabilization around the H₂O₂ binding pocket. At least four amino acid residues, Ser78, Ala79, Asn80 and Asn81, are located near the H₂O₂-binding site. Among them, Ser78 and Ala79 are non-oxidizable and non-bulky amino acids, Asn80 is one of the conserved amino acids, which comprise the hydrogen bond network (His46-Asn80) of MnP. The effects of engineering of oxidizable Met facing the pocket to non-oxidizable residues, also the conformational stabilization around the pocket was proven to be very effective in improvement of H₂O₂ resistance [Chie and Haruo, 2001, Maria et al., 2002].

2-15 Bioreactors in Bioremediation

Several bioreactor configurations were investigated in order to determine the most suitable one for ligninolytic enzyme production: a 1-dm⁻³ -static bed bioreactor, a 1-dm⁻³ -static bed bioreactor with air diffusers into a bed, a 0.5-dm⁻³ -static bed bioreactor with air diffusers into the bed and a tray bioreactor. Although the static-bed bioreactor configurations produced
maximum individual lignin peroxidase (LiP) activities about 400 Udm⁻³ (1.0dm⁻³ bioreactor) and about 700 Udm⁻³ (0.5 - dm⁻³ bioreactor), manganese-dependent peroxidase (MnP) was not detected throughout the cultures. Nevertheless, the tray configuration led to maximum individual MnP and LiP activities of around 200 Udm⁻³ and 300 Udm⁻³, respectively. Therefore, this configuration is the most adequate of the tested different bioreactor configurations [Susana et al., 2001, Daljit et al., 2002, Hickey et al., 1995, Francesca et al., 1996]. Observations suggest that rotating biological contactor could be a very effective approach to chromium bioremediation from industrial effluents provided that methods are developed for making stable biofilms containing the chromate-reducing strain. Cr detoxification in industrial effluents can be economically achieved if mixed with a nutrient-rich wastewater (sewage) which may provide necessary energy and other nutrients to the bacteria in order to carry out Cr(VI) reduction in the mixed effluent [Ganguli and Tripathi, 2002].

*Pchrysosporium*, cultured using nylon sponge as inert support and corncob as a support substrate has shown enhanced MnP and Protease activities. *Pchrysosporium* when cultivated on nylon support showed around 510 Udm⁻³ on sixth day and increased gradually and protease production was a maximum of 20 Ucm⁻². When grown on corncob support *Pchrysosporium* MnP production reached 662 Udm⁻³ on 4th day, whereas protease production was 35 Ucm⁻³. No LiP and Laccase activities were noticed with *Pchrysosporium* [David et al., 2002]. Immobilization with polyurethane foam and polyethylene were used. 100 mgL⁻¹ of 4-CP was used for study, at the end of each culture, the medium was replaced with activation medium. 4-CP disappeared within 4 and 6 days for the first and second batches, respectively and 90% within 12 days for the third batch [Hela et al., 2002, Karlson et al., 1995]. The experiments with suspended and immobilized biomass showed that at steady state by immobilization mineralization was observed to be 96.5% at 19.6 h. In shake flask the degradation was observed till first 170 h and later at 242 h upon additional supply of glucose to the medium [Nirupam et al., 1995, Bosco et al., 1999]. Immobilization of protected cells against phenol and resulted in a remarkable enhancement of their respiratory activity and a short lag phase preceding active phenol degradation. Under optimum operation conditions in a laboratory-scale air-stirred reactor, the immobilized cells were able to completely degrade phenol in synthetic wastewater at a volumetric productivity of 11.5 kg phenol m⁻³ day⁻¹ [Prieto et al., 2002, Soon-Seop and Katsuya, 2002].
Phenol-acclimated cells were adsorbed on the diatomaceous earth, where they grew actively forming a biofilm of short filaments [Prieto et al., 2002]. Operation of solid-state immersion bioreactor, employing cubes of nylon sponge as a support showed the MnP and LiP activities of 574 and 116 UL⁻¹ respectively with aeration level of 0.5 vvm. In batch mode the activity of MnP was 239 U day⁻¹ and in continuous mode it was 150 U day⁻¹. LiP activity for the same was ten-fold higher and can be attributed to influence of operation pH on lignolytic activities [Susana et al., 2002]. MnP production in semi-solid-state cultures of *Pchrysosporium* on nylon sponge show that the onset of enzyme production began between the 3rd and 4th day, reaching to a peak value on the 6th day (500 U dm⁻³). From day six onwards, a quick loss of activity was detected, reaching very low values from the 9th day to the end of cultivation. The pH value, initially 4.8, increased on the first day to a value of 6.2, and then remained at about 5.5 until the end of the cultivation period. The stability of the MnP increased with the addition of HgCl₂ to 4-day-old cultures. There is slight increase in stability in MnP observed when hydrogen peroxide was added to the medium [David et al., 2001, Zohar and Yitzak, 1993]. *Pseudomonas* F1 was employed for TCE degradation in a fibrous bed bioreactor and was operated in a batch mode. 98.5% TCE was removed over 4 h. TCE degradation with addition of H₂O₂ followed by a pseudo-first-order rate, and this rate constant was 1.4 h⁻¹ for 2.4 to 100 mg TCE L⁻¹. Moreover 90% TCE was removed when 95 mg toluene L⁻¹ was presented simultaneously [Gia-Luen et al., 2001].

A new, potentially attractive application of two-phase partitioning bioreactors is in environmental biotechnology. This bioreactor configuration has been shown to be effective in a treatment of highly concentrated xenobiotics such as phenol, benzene, toluene, *p*-xylene, which was successfully demonstrated by laboratory scale experiments of bioremediation of soil contaminated with these compounds. This may open new opportunities for application of two-phase partitioning bioreactors [Janusz, 2001, Liao and Dyi-Hwa, 1996, Folsom et al., 1995, Vanneck et al., 1995]. The steam–exploded wheat straw had replaced the expensive veratryl alcohol as substrate. It not only acts as a nutrient, but also as an inducer of lignolytic enzymes. By SSF with *Pchrysosporium* ME-446 (ATCC 34541), the activities of lignolytic enzymes were found to be more than in submerged fermentation. Under optimal conditions of SSF, the maximum activities of the enzymes LiP and MnP were 2600 and 1375 UL⁻¹. The optimum starting pH for LiP and MnP was 4.0 and 5.5, respectively. Optimal temperature for enzyme
production was 39 °C [Xu et al., 2001]. The bioreactor operated with partial mixing (2:1 R/F) displayed the best performance as it shows the highest productivity, as well as a relatively stable production. Overall LiP activities of 700, 1462 and 400 U were obtained for the reactors with no recycling, 2:1 and 12:1 recycling modes of continuous operation at HRT of 4 h, respectively. The fungus *Pchryosporium* was immobilized on polyurethane foam [Feijoo et al., 1995; Zohar and Yitzak, 1992]. PAH-degradation could be detected in the reactors containing autoclaved soil inoculated with *Rostreatus* and *Pchryosporium* (by estimating the amount PLFA 18:2ω6,9, to measure the level of fungal growth in a PAH-contaminated soil). In using these treatments even PAHs of up to 5 rings were degraded to some extent and the total PAH concentration decreased from 209 ± 35 to 149 ± 6 from 186 ± 2 to 109 ± 6 mg/kg dw soil for *Rostreatus* and *Pchryosporium* respectively [Lea et al., 1997, Andersson et al., 2000, William and Lisa, 1999]. Bifunctional peroxidases may prove to be valuable in providing information on the structural basis for catalysis and on role of LiP and MnP in lignin degradation. They may also provide information on the structural basis for the factors influencing the pH properties of catalysis [Tunde and Ming, 2001, Barry and Michael, 1996].

The stirred tank contactor, fixed packed bed contactor is used for biosorption studies [Volesky, 1987, Melin et al., 1995]. The finely powdered biomass, entrapped in five different polymeric matrices viz. Calcium alginate, polyvinyl alcohol, polyacrylamide, polyisoprenne and polysulfone was compared for biosorption efficiency and stability to desorbents. The Cr sorption capacity mg Cr g⁻¹ sorbent) of all immobilized biomass was lesser than the native, powdered biomass. The Cr sorption capacity decreased in the order of free biomass (119.2) > Polysulfone entrapped (101.5) > Polysoprene immobilized (98.76) > Polyvinyl alcohol immobilized (96.69) > calcium alginate entrapped (84.29) > Polyacrylamide (45.56) at 500 mgL⁻¹ concentration of Cr (VI). The successive sorption-desorption studies employing polysulfone-entrapped biomass indicated that the biomass beads could be regenerated and reused in more than 25 cycles and the regeneration efficiency was 75-78% [Sudha and Emilia, 2003]. Immobilized biomass of *Rhizopus arrhizus* was used for removal of Cd²⁺, Cu²⁺, Fe³⁺, Mn³⁺, Pb²⁺, and Zn²⁺ from solution [Lewis and Kiff, 1988]. The immobilized biomass particle size significantly affects the overall rate of biosorption uptake. Diffusion of the adsorbate (Uranium) in the particle interior is as significant.
as the mass transfer resistance in the nonbiomass layer [Tzezos et al., 1988]. The immobilized biomass of R. arrhizus significantly affects the overall rate of biosorption uptake [Marios and Ake, 1992]. Castor oil was used as a protector to prevent the toxic effects of o-isopropylphenol and o-tert-butylphenol when yeast cells were immobilized within alginate gel [Janusz, 2001].

Polysulphone beads are mechanically stable and chemically resistant than the beads prepared by using all other matrices. The maximum binding capacity of the beads of Cr(VI) was determined as 101.5 mg g⁻¹ from 500 mg L⁻¹ solution [Sudha and Emilia, 2003].

Metal removal by biological solubilization in three strongly contaminated sediments was carried out in a 2 L stirred bioreactor. Biological treatment yielded metal removal efficiencies in the range of 11-30%, 43-57%, 60-79%, 61-90%, 0-10% for Pb, Cu, Zn, Cd, Ni and Cr respectively. Treated sediments when rinsed with 0.5 N NaCl enhanced 47% Pb removal in all sediments [Chartier et al., 2001]

The free radical present constantly in the biosorbents is not taking part in the metal uptake. However, the cell wall matrix, which has encompassed and trapped this free radical, opens up upon metal uptake, indicate its role in metal uptake. The exposed cell wall matrix thus freely interacts with the metal, resulting in high removal rates [Muraleedharan and Venkobachar, 1990].

A continuous EMB-SRB system was operated and more than 90% (w/v) of the Zn²⁺ present in a wastewater was removed. The most effective precipitant is sulfide, because it precipitates complex metals almost regardless of wastewater characteristics and is far less pH-dependant than hydroxide [Sinsupha et al., 2001].

It was determined that the CO₂ generated by the microbial reaction causes a great deal of back-mixing in the top portion of the reactor. Also, since the bed is tapered, much of the biomass is located in the top of the bed where the cross-sectional area is largest [James and Brian, 1991]. The model predicts that, on a volumetric basis, the total conversion in the tapered bed will be approximately the same as that which would be obtained for a straight reaction bed. This indicates that, from the conversion standpoint, there is no reason that one configuration is preferred to the other. However, experience has shown that the tapered-bed reactor is considerably easier to operate [James and Brian, 1995].
2-16 Limitations of Bioremediation

As a biological process, bioremediation also has limitations inherent in any biological system, so it may not always be an appropriate technology for treating a contaminated site.

a) For a site to be treatable by bioremediation, environmental conditions must support biological activity because extremes of pH, temperature, radioactivity or redox potential may not be tolerated by degradative organisms.

b) Essential nutrients such as nitrogen, phosphorus, sulphur, and trace elements must be present and available.

c) The soil or water to be treated by bioremediation must be free of extremely high concentrations of certain toxic chemicals (e.g., the heavy metals mercury, lead and zinc) and other antimicrobial substances.

d) The concentrations of contaminants may be too low to provide sufficient energy for microbial growth, and cometabolism may not be an option.

e) Contaminants may not be bioavailable, or accessible to microorganisms, but they may still be subject to remediation standards required by regulatory agencies. In complex mixtures of contaminants, the feasible bioremediation processes may be unable to remove all the components.

f) The site to be treated, a vast, deep aquifer, for example, may not be that accessible to engineers.

g) Some xenobiotic compounds simply are not biodegradable.

Thus, bioremediation must be viewed as only a single tool in the environmental restoration toolbox, one that will be used frequently but not for all sites or situations [Andrez and Ronald, 1995].
2-17 Basis for selection

On surfing through the exhaustive literature in the field of bioremediation, as mentioned briefly in earlier sections-

- It was felt that there is a dire need to attend the increasing problem of environmental pollution.

- Most of the earlier research, referred to the treatment processes was related to individual industrial effluents, which forms a limitation for treatment at a CETP.

- As an alternative, we have opted for treating the combined industrial effluent, a composite sample – where about 35 industrial units discharge their effluents at a common place with a potential bioremediation tool [Appendix VIII].

- *Phanerochaete chrysosporium*, a white rot fungus was selected for the treatability studies based on the literature projecting it as a potential lignin degrader. It was thought to apply the same fungus for the treatment of recalcitrant and xenobiotic compounds present in the CIE, which are close to lignin in structural complexity.

- Though, extreme - the situation is - the selected white rot fungus, *Phanerochaete chrysosporium* has shown good levels of treatability in CIE as discussed in next sections.

Based on the above-mentioned points:-

- The specific objectives of the work have been streamlined.

- Valid experimentation protocols were drawn.

- Protocols were applied for logical methods and materials were procured.

- Methodology was adopted for effective bioremediation studies as mentioned in the succeeding section.