Chapter 4
Mycoremediation of Paper, Pulp and Cardboard Industrial Wastes and Pollutants

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4.1 Introduction

The importance of paper is increasing day by day with the literacy and cultural development of countries. Demand of paper has dramatically increased in the past several years. This demand is satisfied by different types of paper and pulp industries existing in different countries. Paper and pulping operations and washout operations in paper and pulp industries have resulted in soil and water contamination with unused and discharged residues and effluents.

Improperly handled and disposed paper and pulp and cardboard industrial wastes imperil both human health and the environment. Paper and pulp (Klekowski et al. 2006) have proved toxic and mutagenic to human beings (Kulshreshtha et al. 2010a). Occupational and nonoccupational exposures of human beings to these industrial wastes have led to various health effects such as headaches, nausea, lung and skin irritations and congenital malformations (Shinka et al. 1991; Morikawa et al. 1997) and cancer (Felton et al. 2002).

Industrial development is pervasively connected with the disposal of number of toxic pollutants. Pollutants present in wastes are the major point of concern in many countries. Some of these pollutants require a high priority of treatment for protecting useful resources such as soil, water, air, etc. A growing concern for continuing deterioration of environment and public health has led to establishment

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of legislation for control of quality of effluents and receiving water. Various government norms have also been made more and more stringent for the sake of nature. The environmental norms are made so strict in the countries like China that there has been a closure of almost 50 % of the papermaking units during the last 10 years (approximately 7,000 units in 1995 and only 3,500 in 2005) due to the use of inefficient procedures, outdated technologies, poor product quality and environmental ill effects. Similarly in India, in 1996, Supreme Court gave as many as 12 verdicts in pollution-related areas. Delhi Government had to shut down over 2,000 small-scale industries as a result of the Supreme Court's verdict in Nov. 2000 that no polluting industry must be allowed to operate in Delhi's residential areas. However, despite the regulations for toxic discharges into the environment, many situations exist in which discharging of paper and cardboard industrial pollutants and wastes to the water body is still a practice in many developing countries. For small-scale industries, environmental concerns are considered as just a good wish better to avoid and the pollution control measures are considered as a luxury which couldn't be afforded by the small-scale sector. However, now the situation is changing even for them, and the implementations of environment protection will have to proceed in a manner that sustains the economic health of the industries, yet ensures human health and environmental quality. Setting up a treatment plant at industrial level may be the ultimate solution for pollution caused by paper and cardboard industries. The common effluent treatment facilities are being created in the different industrial clusters at the small level.

Therefore, it becomes imperative to completely degrade paper and cardboard industrial pollutants which cannot be completely degraded by well-established techniques like conventional wastewater treatment methods. In recent years, there has been increasing interest in developing mycological techniques for remediation of paper and pulp industrial wastes which contaminate soil and water. Fungi can degrade paper and pulp industrial wastes by using their own enzymatic pathways. The main objective of bioremediation processes is to mineralise and degrade organic and inorganic contaminants.

4.2 Overview of Bioremediator Fungi for Remediation of Paper and Cardboard Industrial Wastes

To use fungi as agent of mycoremediation, it is necessary to know what makes paper—a good substrate for fungal growth. Paper is made up of cellulose- and lignin-containing fibres which are obtained from various sources such as agricultural residues, trees, cotton and linen rags, etc. Fungi can use lignin and cellulose for their growth and lead to the remediation of paper and cardboard industrial waste from the environment. This reveals the requirement of fungi that degrade lignin and cellulose by secreting nonspecific extracellular enzymes for remediating paper, cardboard and their industrial discharges. Generally, lignin and cellulose degrading fungi can be divided into two major categories, i.e. white-rot fungi and non-white-rot fungi.

Table 4.1 Comparison of the properties of MnP, LiP and Lac from white-rot fungi (Wesenberg et al. 2003)

Properties	Manganese peroxidase (MnP)	Lignin peroxidase (LiP)	Laccase (Lac)
E.C. no.	1.11.1.13	1.11.1.14	1.10.3.2
Prosthetic group	Mn(II):H_2O_2 oxidoreductases Heme	Diarylpropan O_2, H_2O_2 oxidoreductases Heme	*p*-benzenediol: O_2-oxidoreductases 1 type-1 Cu, 1 type-2 Cu, 2-coupled type-3 Cu
MW(kDa)	32–62	38–47	59–110
Glycosylation	N-	N-	N-
Isoforms	Monomers, up to 11	Monomers, up to 15	Mono-, di-, tetramers; several
C–C cleavage	Present	Present	Absent
H_2O_2—regulated	Regulated	Regulated	Not regulated
Stability	Highly stable	Stable	Highly stable

White-rot fungi have ability to degrade lignin and chlorinated lignin derivatives effectively which are present in pulp, paper and cardboard industries (Eriksson 1991; Bajpai and Bajpai 1994) due to the production of a variety of extracellular ligninolytic enzymes such as lignin peroxidase, manganese peroxidase, laccase and H_2O_2-producing oxidases. Degradation of lignin is apparently a very energy-intensive process (Leisola et al. 1983; Boman et al. 1988) and requires specific cultivation conditions, necessary for the production of ligninolytic enzymes by white-rot fungi to degrade lignin (Boman et al. 1988). Various cultivation methods have been devised to overcome these difficulties. For instance, MyCoR process, MYCOPOR-system (Jaklin-Farcher et al. 1992), continuous-flow systems (Prasad and Joyce 1991) and immobilisation techniques employing white-rot fungi (Livernoche et al. 1983; Kirkpatrick et al. 1990) (Table 4.1).

Non-white-rot fungi also possess extracellular oxidative enzymes, in particular, lignin-degrading enzyme systems (LDS), for remediation of paper and pulp industrial wastes. These fungi may be present in the pulp and paper industrial waste indigenously and possess a well-developed lignin-degrading enzyme system which helps in bioremediation of lignin- and cellulose-containing waste. Autochthonous non-white-rot fungal strains are able to use lignin cellulose as carbon and energy source. Many fungal strains have been reported to be isolated from soils polluted by paper, pulp and cardboard industrial wastes (Table 4.3) belonging to the phycomycetes, zygomycetes and deuteromycetes. Phenotypic and biochemical assays revealed the ability of these filamentous fungi to synthesise extracellular oxidative enzymes and suggested a relationship between the LDS and paper and cardboard waste bioconversion.

Interest in mycoremediation of paper and pulp industrial waste logically led scientists to evaluate the potential of fungi for degrading cellulose, lignin, AOX, resins and polychlorobiphenyls (PCBs) present in paper and pulp industrial wastes. It is particularly of interest that fungi, applied for mycoremediation, is capable of producing extracellular enzymes in ligninolytic and non-ligninolytic conditions.

4.3 Overview of Fungal Enzymes Involves in Remediation of Paper and Cardboard Industrial Wastes

Fungal species, used for mycoremediation of paper and pulp mill waste and its constituents, are able to produce different enzymes depending on their genetic make-up and growth conditions. Ligninolytic fungal enzymes that degrade lignin also have potential to remediate the paper and pulp industrial waste. Due to non-specificity of enzymes, these can also transform a variety of organic and chloro-organic compounds. Key lignin degradation enzymes are oxidoreductases, i.e. two types of peroxidases, lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), manganese-independent peroxidase (MIP) and a phenoloxidase, laccase. Lignin-degrading enzymes are applicable in the degradation of highly toxic environmental chemicals such as dioxins, polychlorinated biphenyls, various dyes and polyaromatic hydrocarbons and decolourisation of kraft bleach plant effluents.

Fungal enzyme treatments have the major advantage of requiring only a small stream of the process water to be cooled for generation of fungal culture filtrate with most of the degradation of detrimental organics being accomplished by enzyme (fungal culture filtrate) that function well at process water temperatures up to 75 °C. It is probable that enzymatic treatment will be relatively inexpensive and environmental friendly.

4.3.1 Lignin Peroxidase

LiPs are glycosylated heme proteins protoporphyrin IX which is differing in their catalytic mechanisms. LiPs act by abstracting single electron from aromatic rings of lignin and lignin model compounds, leading to the formation of cation radical and subsequent cleavage reactions.

These enzymes are potentially valuable in pulp, paper and cardboard industrial waste disposal because of their ability to degrade not only lignin but also various pollutants. These enzymes are produced by white-rot fungi (WRF) including edible and nonedible mushroom species, during their secondary metabolism. Since lignin oxidation provides no net energy to the fungus, synthesis and secretion of these enzymes are often induced in limited nutrient levels (mostly carbon and nitrogen) along with hydrogen peroxide. LiP interacts with lignin in the presence of a cofactor called veratryl alcohol (a secondary metabolite of white-rot fungi).

4.3.2 Manganese-Dependent Peroxidase

MnP are glycosylated glycoproteins (mol. wt. 32–62.5 kDa) with an iron porphyrin IX (heme prosthetic group) and are secreted in multiple isoforms. MnP

preferentially oxidise Mn^{2+} into Mn^{3+} which is stabilised by chelators (oxalic acids) excreted by the fungi. Generated Mn^{3+}, a highly reactive intermediate, which, when stabilised by chelators, can diffuse from the enzyme active site to attack and oxidise the lignin structure in situ. Thus, MnP are able to oxidise and depolymerise lignin and other compounds present in paper and pulp mill effluent.

4.3.3 Laccases

Fungal laccases (Lacs) are produced by basidiomycetes as part of the ligninolytic enzyme system. This group of N-glycosylated extracellular blue oxidases with molecular masses of 60–390 kDa contains four copper atoms in the active site that are distributed among different binding sites. Laccases catalyse the oxidation of variety of aromatic hydrogen donors with the concomitant reduction of oxygen to water. Moreover, laccases do not only oxidise phenolic and methoxyphenolic acids but also decarboxylate them and attack their methoxy groups.

The most studied laccase-producing fungus is *Trametes versicolor*. *Phanerochaete chrysosporium* do not produce this enzyme (Hattaka 1994; Thurston 1994) or produce in defined culture medium containing cellulose and ammonium tartrate. Two laccase isozymes (I and II) of *Trametes versicolor* were purified by Bourbonnais et al. (1995). The same reactivities of two isozymes were found to be similar on most of the low-molecular-weight substances. However, significantly higher reactivity of laccase-I than laccase-II was exhibited with a polymeric substrate. The two isozymes had similar qualitative effects on kraft lignin and residual lignin in kraft pulp (Bourbonnais et al. 1995).

Laccases can oxidise many recalcitrant substances, such as chlorophenols, lignin-related structures, nonphenolic lignin models (Kawai et al. 1988) and dyes. Thus, these enzymes may be used for the degradation of pulp, paper and cardboard industrial wastes.

4.3.4 Versatile Peroxidase

A third group of peroxidases, versatile peroxidases (VPs), has been recently recognised that can be regarded as hybrid between MnP and LiP. VP has been found to be present in species of *Pleurotus* and *Bjerkandera*. Since they can oxidise not only Mn^{2+} but also phenolic and nonphenolic aromatic compounds including dyes, they may accelerate the bioremediation process of pulp and paper wastes.

4.3.5 H_2O_2-Producing Enzymes

White-rot fungi also have the ability to produce a number of oxidases, such as glucose oxidase (Kelley et al. 1986), glyoxal oxidase (Kersten and Kirk 1987), methanol oxidase (Eriksson and Nishida 1988), and veratryl alcohol oxidase (Bourbonnais and Paice 1988), that are capable of generating H_2O_2, presumably for utilisation by extracellular peroxidases during degradation of lignin.

In the first step of bioremediation, peroxidases and laccases degrade lignin and produce radical substances. These radical substances spontaneously re-polymerise in the absence of quinone oxidoreductase (CBQ) and cellobiose oxidase (CBO). It has also been shown that when *T. versicolor* laccase is incubated with glucose oxidase, improvement in lignin depolymerisation occurs. This is probably due to the action of glucose oxidase in reducing lignin and thereby limiting their repolymerisation. In this way, polymerisation/depolymerisation equilibrium is shifted towards degradation in the presence of other enzyme.

4.3.6 Other Enzymes

There is growing evidence for the participation of other important enzymes in the lignin degradation process. Two enzymes cellobiose, quinone oxidoreductase (CBQ) and cellobiose oxidase (CBO), play important role in the lignin degradation by reducing phenoxy radical compounds. MnP requires the supply of Mn(III)-complexing agent such as cellobionic acid, produced during the oxidation of cellobiose to cellobionic acid, for degradation of lignin. Mn (II) is also required by MnP during oxidation of lignin, since Mn (II) is converted to Mn (III) by this enzyme.

CBQ supply cellobionic acid reduces insoluble Mn (IV) to Mn (II), during the oxidation of cellobiose to cellobionic acid and hence recycling these cations. The small size of the organic acid Mn (III)-complexes makes them an important delignification agent due to their diffusability (Roy et al. 1994) in lignocellulosic wall of paper and cardboard. CBQ activity also inhibits the oxidation of veratryl alcohol (Ander et al. 1990).

The necessity of mycelial-bound ligninolytic enzyme or a hydrogen peroxide-producing system in the fungi plays an important role in dye decolourisation. Daniel et al. (1994) suggested a cooperative role between pyranose oxidase (POD) and MnP. Pyranose oxidase enzyme was reported to be a major source of hydrogen peroxide—an agent required for lignin degradation. Furthermore, both POD and hydrogen peroxide-dependent MnP occur in the periplasmic space of hyphae and extracellular medium.

4.4 Overview of Mycoremediation Process

"Myco" stands for fungi and "remediation" refers to removal of waste. Thus, "mycoremediation" refers to removal of waste by fungi. Mycoremediation is the form of bioremediation in which fungi is used to return polluted environment to less polluted state. Remediation of pollutants is possibly due to the presence of many highly active enzymes in the fungi, during which fungi are at their most metabolically active stage. Fungi inhabiting polluted environments are armed with various resistance and catabolic potentials. This catabolic potential of microbes in nature is enormous and is advantageous to mankind for a cleaner and healthier environment through bioremediation.

Mycoremediation is gaining attention as an alternative approach to presently available methods. It is important to understand the variables that control the rate and extent of fungal attack on paper, pulp and cardboard for meaningful exploitation of fungi. Paper and cardboard possess lignin and cellulose as main ingredients which are needed to be degraded by fungi and fungal enzymes. Ligninolytic enzymes degrade not only lignin but also several compounds present in the paper and cardboard industrial waste. The process of lignin degradation is proposed to be completed in two phases. In the initial primary phase, the ligninolytic enzyme system is synthesised, while during the secondary idiophasic metabolism, lignin is degraded. The white-rot fungi, in general, and *P. chrysosporium*, in particular, are by far the most active ligninolytic organisms described to date. These are widely used for the degradation of paper and pulp industrial waste. However, some indigenous fungi and known standard cultures can be used for remediation of paper and pulp industrial wastes.

4.4.1 Source of Fungal Cultures

When using fungi for bioremediation, availability of fungal inoculum is a practical consideration. Fungal cultures can be obtained in many ways for bioremediation of paper and cardboard industrial waste.

4.4.1.1 Standard Cultures

A lot of work has been reported in the literature for the treatment of pulp, paper and cardboard industrial effluents. Most of the work is reported to be based on the use of standard microbial strains. Cultures may be purchased from the well-known centres for fungal culture storage, given in Table 4.2. Isolates may also be purchased from the place reported in literature where the culture is deposited.

Table 4.2 Centres for getting fungal cultures

S. no.	Name of centre	Country
1	International Mycological Institute or Commonwealth Mycological Institute (CMI)	United kingdom
2	United Kingdom National Culture Collection (UKNCC)	United kingdom
3	American Type Collection Centre (ATCC)	United States of America
4	Central Bureau voor Schimmelcultures (CBS)	Netherlands
5	IHEM Culture collection	Belgium
6	MUCL Culture collection	Belgium
7	Herbarium Cryptogame Indiae Orientalis	India
8	Microbial Type Collection Centre, Chandigarh	India

4.4.1.2 Indigenous or Autochthonous Fungi

Indigenous fungi refer to the fungi present in waste itself. There are many fungi occurring naturally in conditions ecologically modified by industrial effluents. They degrade the surrounding substrates and wastes discharged by industries. These primary invaders change substratum conditions by their action making way to secondary invaders. Due to the continuous presence of microorganisms with industrial effluents and recalcitrant compounds, genetic potency develops to degrade these compounds. These isolates are present in low numbers and are not effective in bioremediation. To make them effective in mycoremediation process, these indigenous microbial communities can be enriched in the presence of intermediary metabolites of toxic compounds, and significant strains will be evolved with the process of adaptation which may possibly be employed under controlled conditions to degrade the industrial wastes. The metabolic activity leads to the change in the structure and function of the community. Based on this principle, indigenous fungi can be isolated from paper and cardboard industrial wastes. These can be isolated by using different techniques such as bait technique (using paper and pulp sludge), serial dilution method (using various fungal media) and blotter technique (pieces of rags and paper).

In bait and blotter technique, paper and cardboard pieces are used as bait/blotter which is wetted by industrial effluent and kept in petri dishes. These petri dishes are incubated at 25–35 °C for the particular period of time and observed daily for the growth of fungi. As fungal colonies appeared on bait, these are cultured on the suitable medium for obtaining pure culture which will be later screened for cellulolytic and ligninolytic ability.

In dilution method, initially, serial dilution of waste or contaminated soil/water is prepared in sterile normal saline. Appropriate dilution is inoculated on medium containing Mandel's salts solution with addition of 17.5 and 5.0 g/l phosphoric acid-swollen cellulose and 0.5 % L-sorbose as a colony restrictor and inducer of cellulose production (Wang et al. 1995). Further, all isolated fungi will be screened for their ability to degrade cellulose/lignin, dyes and other compound present in the paper and pulp industrial wastes.

After isolation of indigenous fungi, growth of the fungal strain is analysed for initially phenotypic characterisation of fungi. A scale based on colony growth is used for interpreting the results: nongrowth, residual growth, weak growth, moderate growth and good growth. Classic and molecular identification of fungal strain can be done by macro- and microscopic studies of morphological characters (hyphae, conidia, chlamydospores, conidiogenous cells and conidiophores). The information was compiled in a taxonomic description for comparison with specialised literature. For molecular identification of autochthonous paper and cardboard degrading fungal strains, PCR amplifications of 28S rRNA gene should be performed using the following specific primers: NL1 (forward), 5′-GCATATCAATAAGCGGAGGAAA AG-3′, and NL4 (reverse), 5′-GGTCCG-TGTTTC AAGACGG-3′. PCR reactions can be performed in thermocycler using deoxyribonucleotide triphosphate and fungal genomic DNA as template. PCR conditions consisted of an initial denaturation at 95 °C for 5 min, 40 cycles of amplification at 95 °C for 35 s, annealing at 52 °C for 30 s, extension at 72 °C for 20 s and final extension at 72 °C for 10 min. The PCR product can be purified using the DNA purification kit and sequenced. Sequence analysis of nucleotide sequences can be performed using the Lasergene software package DNASTAR Programs, BLAST (Altschul et al. 1997) and FASTA (Pearson and Lipman 1988).

The ability of the fungal strains to produce extracellular oxidoreductases of lignin-degrading system (LDS) can be performed by the 2, 2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) test as described previously by Saparrat et al. (2000). The chromogen ABTS is a very sensitive substrate that allows rapid screening of fungal strains producing the extracellular oxidative enzymes by means of a colourimetric assay at 420 nm (Saparrat et al. 2000). Each strain can be processed under controlled conditions at 30 °C in darkness, for definite period of time. A scale based on the colour intensity was used for interpreting the results: colourless, indicates no ABTS-oxidising activity; low colour intensity (+); moderate colour intensity (++); and high colour intensity (+++) indicating high ABTS-oxidising activity.

Mostly, indigenous fungi belong to phycomycetes, ascomycetes and deuteromycetes and are isolated from soil and water contaminated by paper and cardboard discharges. List of some of these fungi is given in Table 4.3.

4.4.1.3 Fungal Culture from Previously Inoculated Waste

It could be advantageous to use fungal culture procured from previously inoculated waste (Buswell 1994). Previously, inoculated waste is used here for waste treatment plant which is inoculated with fungal culture for treatment purpose. In the case of mushroom culture, culture can be collected from spent mushroom compost. Spent mushroom culture (i.e. *Pleurotus ostreatus*, oyster mushroom; *Lentinula edodes*, shiitake mushroom) is a by-product from commercial mushroom growers.

As with spent mushroom culture, pre-fruit body fungi can be obtained from commercial mushroom growers. It is an alternative to use colonised mushroom substrate before mushroom (fruit body) production.

Table 4.3 List of fungi distributed and isolated from water and soil samples of paper and pulp industrial effluent (Wahegaonkar and Sahasrabudhe 2005)

S. no.	Isolated fungi	Occurrence in water sample	Occurrence in soil sample
1	*Mucor* spp.	*	***
2	*Rhizopus* spp.	***	***
3	*Syncephalastrum racemosum*	***	***
4	*Chaetomium indicum*	*	*
5	*Corynascus spedonium*	00	*
6	*Eurotium herbariorum*	00	*
7	*Eupenicillium javanicum var. levitum*	00	*
8	*Pseudoeurotium multisporum*	00	*
9	*Acremonium* sp.	*	***
10	*Alternaria* spp.	*	***
11	*Aspergillus* spp.	00	***
12	*Aureobasidium indicum*	*	00
13	*Cephalosporium curtipes*	*	00
14	*Cephalosporium* sp.	*	00
15	*Cladosporium macrosporium*	*	00
16	*Curvularia* spp.	*	***
17	*Dendrostilbella indica*	00	*
18	*Drechslera* spp.	***	***
19	*Cunninghamella* spp.	*	***

"*" indicates the frequency of occurrence and "00" indicates non-occurring of fungi in the sample

4.4.2 Inoculation of Waste with Fungi

Another concern when using fungi for bioremediation of pulp and paper industrial wastes is how the inoculum is applied? Inocula of fungi can be applied in different ways depending upon the form of waste (liquid effluent/solid sludge) to be treated. Fungal inoculum can be applied by making the layers or in the form of homogenous mixture, if waste is present in sludge/solid form.

4.4.2.1 Inoculation of Sludge

Layering the Fungi with the Sludge/Soil

In this technique, fungi are applied in the form of layers in between the layers of sludge and soil. Layering the fungi with the waste would be easier and therefore more economical rather than mixing the fungi and waste.

Homogenised Mixture of Sludge or Soil with Fungi

In this technique, sludge, soil or solid waste is mixed with fungi in the form of homogenised mixture that degrades paper and pulp mill waste more effectively than soil and substrate layers.

4.4.2.2 Inoculation of Effluent

Fungus can be applied in five different ways in the effluent, if effluent is to be treated for remediation.

Agar Blocks

Fungi can be cultivated as monocultures on different media such as malt extract agar, potato dextrose agar, Czapek's agar and incubated for the definite period of time at the appropriate temperature. Then, agar blocks of equal size can be cut from the zone of active fungal growth and used for inoculation of paper, pulp and cardboard industrial effluent.

Spores Inoculation

Fungi can be grown on malt agar (2 %) medium in petri dishes for 72 h at the appropriate temperature. As sporulation occurs, spores can be separated from mycelium by filtration process and then suspended in sterile water. Paper and cardboard industrial effluent containing each flask can be inoculated with the equivalent of amount of spores/ml culture medium.

A novel method of using spores for bioremediation was developed by Childress et al. (1998). In this technique, spores can be encapsulated into alginate granules (1–3 mm in diameter) with Pyrex™ (non-nutritive filler such as saw dust, corn cob grits) for mycoremediation purpose. Encapsulated fungal spores can be stored in sterile petri dishes sealed with parafilm in refrigerator and should be checked regularly for the spore viability. The viability of spores is reported to be good in refrigerating condition as well as at room temperature. In future, this technique may be used for the mycoremediation of paper, pulp and cardboard industrial wastes contaminating soil and water.

Mycelium Inoculation

The strains can be grown in agitated cultures (200 rpm) in 100 ml flasks containing appropriate medium, for 3 days at 25–37 °C. Then the cultures can be centrifuged, and the mycelium can be ground in sterile distilled water. This mycelium can be

used for the inoculation of flask containing pulp and paper mill effluent with equivalent amount of dry weight of mycelium per litre of effluent.

Enzymes Inoculation

Enzymes can be use directly, without immobilisation on different substrates, for bioremediation of pulp, paper and cardboard industrial effluent. However, these can treat a small quantity of waste effluents and sludge due to nonrecyclability of enzyme. In contrast to immobilised enzymes, these enzymes cannot be recycled and treat comparatively low amount of effluent.

The use of the purified enzymes showed limited activity in bioremediation. For example, MnP and LiP from *P. chrysosporium* can be used for bioremediation purpose. It was shown that only MnP had about 25 % decolourisation activity. Therefore, in vivo decolourisation, which attained more than 80 %, could depend on the production of other enzyme (Jaspers and Penninckx 1996). Due to this drawback, use of fungi is more beneficial for the mycoremediation purpose because the inoculated and adapted fungi can produce and secrete various enzymes as per the requirement for waste degradation.

Immobilised Mycelium or Fungal Enzymes

Fungal mycelium can be immobilised on various substrates such as polyurethane foam, agar gel, agarose gel, calcium alginate gel beads, magnetite, chitosan, etc. Thereafter, immobilised enzymes can be used for inoculation and remediation of pulp and paper mill effluents. Depending upon the requirement of bioremediation, substrate may vary. Immobilised enzymes could treat a large quantity of wastewater compared to soluble enzymes. Sometimes, degradation products absorb on the material used for immobilisation and accelerate the remediation process. For example, when phenols are oxidised by tyrosinases, quinones are obtained which adsorb rapidly and strongly onto chitosan beads (Muzarelli et al. 1994) and accelerate the degradation process.

Fungal enzyme such as laccase can be immobilised on carbon fibre electrodes using classical methods: physical adsorption, glutaraldehyde, carboimide and carbodiimde and glutaraldehyde for coupling laccase to carboxyl groups on carbon fibres. *P. chrysosporium* immobilised on cubes of polyurethane sponge of 1.5-cm sides or cylinders of polyurethane foam was found to produce ligninase under nitrogen sufficient condition (Chen et al. 1991).

Fungal enzymes LiP and MnP can be coated on nanoparticles for bioremediation purpose. Nano-assemblies of LiP and MnP are successfully fabricated and characterised on a flat surface as well as colloidal particles. During the assembly of enzymes on nanoparticles, a unique dynamic adsorption–desorption of enzyme layer occurs. Time, number of runs, nonaqueous media and drying of the enzyme layers have significant effect on the activity of assembled enzymes. A novel

concept of using of silica nanoparticles can improve bio-catalysis. Formation of silica nanoparticles is based on electrostatic interaction between oppositely charged species. This may be successful strategy for treating the paper and cardboard industrial effluent in the coming future.

4.5 Bioreactors as Mycoremediation System

The pragmatic approach of biotreatment is analysed by way of using chemostat for enrichment and bioreactor for testing the relevance of the microorganisms in the treatment process. Various parameters and cultivation strategies need to be considered, for successful bioremediation of paper and pulp industrial wastes. In this context, various bioreactors have been tested for bioremediation of mill-made paper and pulp plants effluents and are discussed below.

4.5.1 Batch Treatment Process

Generally, for batch treatment, effluent treatment process is carried out in erlenmeyer flasks. Nagarathnamma and Bajpai (1999) conducted experiments in shake flasks and evaluated different fungi for bleach plant effluent treatment. They selected *Rhizopus oryzae* due to having high decolourisation efficiency and low sugar requirements during colour removal. During treatment, 92–95 % of the colour, 50 % of the COD and 72 % of the AOX were removed in 24 h at 25–45 °C and a pH of 3.5.

In a batch experiment, *Coriolus versicolor* was used to decolourise lignin-containing kraft E1-stage effluent, and it was found that both adsorption and oxidation proceeded best (Royer et al. 1985). Hence, the batch treatment was found to be successful in remediating the waste of paper, pulp and cardboard industries.

4.5.2 Continuous-Flow Systems

The results of using bench-scale bioreactor, in which aeration and mixing can be achieved with a diffuser that is placed at the side of the bottom of the reactor, provided a reasonable basis for setting out continuous-flow process consisting of a mixing tank, aeration basin and clarifier. In mixing tank, effluent would first be adjusted to the proper pH and mix with nutrient solution. The effluent would then go through a sedimentation basin where fungal solids would be removed and recycled to the head of the aeration unit. On the basis of this hypothesis, three continuous-flow laboratory-scale reactors have tested for bioremediation of pulp and paper mill waste (Prasad and Joyce 1991):

(a) System I consisted of a conventional oxidation basin for fungal cultivation. Mycelial mats of fungal species can be transferred to the reactor for decolourisation of paper mill effluent without aeration. This system could reduce effluent colour by at least 50 % for the first 6 days.
(b) System II consists of a vessel with increased height and decreased surface area. It possesses four baffles which divide the vessel into four compartments. Fungus culture, packed in wire bags, can be suspended in the middle of each zone with the supply of continuous aeration. Again, this system could reduce effluent colour by at least 50 % for the first 6 days.
(c) System III is similar to MyCoR reactor in which fungal mats can be clasped between circular wires, supported by outer central rings and securely fixed in a metallic frame, possessing the continuously rotating discs. In contrast to system I and II, system III has given the best results with a greater than 78 % total colour removal and 25 % COD reduction from extraction-stage paper mill effluent.

4.5.3 MyCoR Reactor

MyCoR reactor was described by Eaton et al. (1982), in which fungi are immobilised on the surface of rotating discs which are partially submerged during operation. These discs could be enclosed for additional oxygen supply and steam sterilisation.

In this reactor, at first growth of fungi has occurred till the nutrient is present, and then fungi become ligninolytic in nutrient-limiting condition. Simultaneously, decolourisation of the effluent occurs. The MyCoR process reduced colour in an alkaline stage spent liquor of pulp and paper industry by 80 % in less than 24 h in the laboratory.

In MyCoR process, only 40 % of the mycelium is in contact with substrate which seems to be the poor surface to volume ratio. Moreover, the mycelium is present in a thick layer that could result in deficient oxygen and nutrient supply and a lower general productivity.

4.5.4 MYCOPOR Process

In the MYCOPOR process, fungi are immobilised in polyurethane foam and used in a continuously trickling filter reactor to treat bleach plant effluent. This process lead to maximum decolouration, 50 % COD reduction and 80 % toxicity elimination of sulphate-based kraft pulp and paper mill effluent.

4.5.5 Fluidised-Bed Bioreactor

A fluidised-bed bioreactor operated in a continuous mode to treat and remediate bleach paper and pulp effluent. In this reactor, fungi can be immobilised on calcium alginate and other beads. This reactor has been tested and found to decolourise caustic stage effluent of pulp and paper industry by 69 % and reduce AOX of this effluent by 58 % at a retention time of 1 day.

4.5.6 Suspended Carrier Technology

This has been in use for a number of years for biological treatment of pulp and paper industrial waste and its bioremediation. Carrier particles such as polypropylene mats, polyester sponge-foam cubes, porous plastic foam cubes and ionically modified porous polyurethane granules have density close to that of water. These are used to minimise the energy required to keep the carriers suspended during treatment. Carrier particles should be engineered with more surface roughness and with surface charge as well as hydrophilicity to better accommodate fungi. Support matrixes are composed of particles that are kept in suspension by aeration. This has major advantages when compared to static biofilm systems that the risk for clogging of the stationary biomass support material with fines and fibres is eliminated.

This technology is used for treatment of bleached kraft pulp and paper mill effluent. At pH 7, 37 °C temperature and with hydraulic retention times of longer than 3.5 h, 55 % of COD removal was achieved. The suspended carrier treatment was also operated at pH 9 and 45 °C as well as at pH 7.0 and 50 °C, and 50 % reduction in COD in each case at a hydraulic retention time of 4 h was reported.

4.5.7 Moving Bed Biofilm

A full-scale treatment plant, based on moving bed biofilm system, is under construction in neutral sulphite paper mill for treating effluent. During a pilot-plant trial, 70 % COD reduction, 96 % BOD reduction and 98 % toxicity reduction were obtained at an organic load of about 25 kg COD/m^3 day. In this case, no clogging was found during the pilot-plant run.

4.5.8 Airlift Reactors

In airlift reactors, the fungi can be inoculated in the pelleted form. This strategy would facilitate recycling and the use of large amounts of fungal biomass. Mehna

et al. (1995) used this reactor and a colour reduction of 92 % with a COD elimination of 69 % using pellets of *T. versicolor* strain in optimum conditions such as pH 4.5, temperature 30 °C and sucrose 7.5 g/l.

4.6 Factor Affecting Mycoremediation

There are specific conditions that need to be maintained for mycoremediation of paper and pulp waste and to operate the process at optimal efficiency. Any serious deviations could result in the complete shutdown of the system. In flask cultures, 80 % reduction in colour of paper and pulp mill effluent was observed after 6 days with *Trametes versicolor*. However, the same fungus, cultivated under optimal aeration in a laboratory fermentor with 0.8 % glucose plus 12 mM ammonium sulphate and at a controlled pH level of 5.0, reported to reduce 88 % colour units within 3 days. This emphasises the importance of culture conditions on the efficiency of decolourisation of effluent (Bergbauer et al. 1991) and simultaneously waste treatment. However, an effective biotreatment system must lead to the degradation of both high and low molecular mass compounds. White-rot fungi can achieve this effectively but possess complicated physiological demands for degradation of lignin-containing waste.

In shake flask, under optimal conditions (pH 4.0 and glucose as carbon source) *Trichoderma* sp. decreased the colour of kraft bleach plant effluent by 85 % and reduced the COD by 25 % after 3 days incubation in shake flasks. The maximum total decolourisation at pH 4.0 without an additional carbon source was 68.6 % after 3 days cultivation and therefore glucose stimulated decolourisation. Other carbon sources such as pulp and pith that are abundant and inexpensive increased the decolourisation after 6 days, since they were not metabolised immediately. The results of these studies again emphasised the importance of carrying out the treatments under strictly defined conditions (Prasad and Joyce 1991). However, there is not sufficient information about the nutritional, physiological and environmental parameters that influence the fungal degradation of lignin, cellulose, toxicants, dyes, etc.

Paper, pulp and cardboard industrial effluent possess highly coloured compounds, generally appeared due to the presence of lignin and lignin degradation compounds. Mycoremediation strategy requires the following factors that are well known to effect the delignification of paper and cardboard industrial effluent and hence decolourise the effluent.

4.6.1 Nitrogen

The onset of lignin degradation is triggered by nitrogen depletion in the medium. Lignin degradation is suppressed in nitrogen-rich medium possibly due to (1) high

nitrogen content promotes rapid depletion of energy sources which are essential for lignin metabolism. (2) Nitrogen metabolism competes with lignin metabolism for fulfilling the requirement of same cofactors. (3) Nitrogen regulates the synthesis of one or more components of the lignin-degrading system. (4) Increased formation of biomass speeds up the rate of respiration and suppress lignin metabolism.

The increased nitrogen inhibits lignin degradation, while the increased carbohydrate supply stimulates. A medium containing an unlimited level of glucose and a limiting dose of nitrogen stopped primary growth and stimulated onset of ligninolytic activity during late stationary phase. In contrast to this, the ligninolytic activity considerably delayed in nitrogen-rich glucose-limited medium without adversely affecting the extent of fungal growth. The most effective nitrogenous suppressors of ligninolytic activity are ammonium chloride, glutamine, glutamate and histidine.

Mostly, white-rot fungi degrade lignin in better way in the presence of carbon to nitrogen ratio rather than the absolute levels of carbohydrates and nitrogen. Degradation of lignin by *Phanerochaete chrysosporium* and *Phlebia radiata* is dependent of C to N ratio. *Dichomitus squalens* and *Lentinus edodes* do not show any effect of C to N ratio. Thus, the effect of carbon nitrogen ratio is variable among the members of white-rot fungi.

In contrast to the physiological model proposed for *P. chrysosporium*, several commercially important and commonly occurring white-rot fungi produce higher ligninolytic enzyme activities in response to a nitrogen-rich medium. Moreover, laccase activities in *Lentinula edodes* and *Pleurotus ostreatus* and manganese-independent peroxidase (MiP) activities of *Bjerkandera* spp. were found to be enhanced by peptone (Kaal et al. 1995). Hence, mycoremediation of paper and pulp industrial waste requires appropriate amount of nitrogen depending on the type of fungi used.

4.6.2 Carbon Co-substrate

White-rot fungi cannot degrade lignin unless the co-substrate is supplemented simultaneously in the growth medium. Lignin is unable to serve as a growth substrate; the co-substrate is presumably the source of energy required for biosynthesis of individual components of ligninolytic system. Lignin degradation by white-rot fungi and *Aspergillus fumigatus* is dependent on the presence of readily metabolisable co-substrate such as glucose. The growth substrate is required because (1) energy recovered in lignin metabolism is too little to support growth and is at best only a marginal carbon and energy source for maintenance metabolism, (2) extent of ligninolytic activity is very low for fungal growth and (3) nature of carbon source also influences the ligninase activity by affecting the rate of H_2O_2 production.

An additional carbon source donates electrons which are cascaded down to the final electron acceptor. Sometimes the dye, present in the effluent, functions as final electron acceptor which helps in dye decolourisation of paper and cardboard industrial effluent. However, price of glucose may be a limiting factor in scaling-up projects. Addition of simple sugar to the wastewater source converts at least some of the biomass to glucose by brown-rot fungi. Simple sugar accelerates the production of phenoloxidases from the white-rot fungi which involve in breakdown a portion of biomass. As a result of the breaking down of biomass, thereby decreasing amount of phosphorous colour, odour, ammonia, suspended solids and sludge that are difficult to separate from paper and cardboard industrial effluent.

4.6.3 Oxygen or Air Tension

Lignin degradation is an oxidative process and needs the presence of oxygen at a partial pressure equal to that in the natural atmosphere. Increasing the O_2 levels in the culture has a strong activating effect on the rate of lignin degradation and production of LiP and MnP, which is generally optimal at high oxygen tension. Lignin is degraded much faster in the presence of oxygen than air. For example, *P. chrysosporium* reduced lignin of pulp when it is maintained in 100 % oxygen instead of air. The oxygen partial pressure has a profound effect on the rate and extent of lignin degradation by *P. chrysosporium*, but not on the growth of fungi.

4.6.4 Culture Agitation and Supplementation

Agitation is generally used to increase the rate of gas exchange between the atmosphere and culture medium. An initial period without agitation is needed to avoid severe inhibition of onset of degradation activities. Production of LiP and MnP is generally repressed by agitation in submerged liquid culture, while Lac production is often enhanced by agitation.

In agitated cultures, Tween 80 is essential for ligninase production. The supplementation of *P. chrysosporium* growth medium with emulsified vegetable oils, fatty acids and phospholipids sources enhanced the lignin peroxidase activity required for lignin, paper and pulp waste degradation. The extent of growth is good in both agitated and still fungal cultures.

Culture agitation results in pellet formation and strongly suppresses ligninolytic activity and slowing the waste bioremediation process. Agitation of cultures to increase the oxygen supply leads to increased formation of pellet due to the enclosing of fungi with pulp fibres which prevents optimal degradation possibly due to (1) the disturbance of the physiological state of cells on the pellet surface and (2) lower oxygen partial pressure which results in decomposition of pulp and paper fibres only on the exposed outer surface. Furthermore, a close physical association

between fungi and paper, pulp and cardboard fibres is necessary. Fungi associated with paper, pulp and cardboard fibres are incubated in both agitated and still cultures during initial days. In contrast, if the pre-grown mycelial mat is agitated, it does not seem to affect lignin degradation.

In agitated submerged culture, the production of lignin peroxidases can be increased with various detergents and veratryl alcohol. This shows the effect of different supplementation on the production of LiP in agitated cultures.

4.6.5 Micronutrients

4.6.5.1 Manganese

The presence of manganese is necessary for degradation lignin-containing paper and cardboard fibres by white-rot fungi. Manganese is required for the functioning of an extracellular manganese peroxidase enzyme, first discovered in *P. chrysosporium*. Manganese peroxidase is present in numerous cultures of white-rot fungi. Mn regulates the production of LiPs and MnPs and ligninolytic activity of fungi. Ligninolytic activity increases with decreasing Mn(II) concentration.

4.6.5.2 Magnesium

Magnesium supplementation in the form of $MgCl_2 \cdot 6H_2O$ to bleach plant effluent increased the net consumption of chromophores by *C. versicolor* because these ions are known activators of many oxidases that play a role in delignification of paper, pulp and cardboard effluents by ligninases. Besides this, magnesium ions increased the degradation of chromophores indicating the involvement of Mg ions. For instance, addition of magnesium ions initiates the removal of paper mill effluent colour from its initial level.

4.6.5.3 Sulphur

Addition of sulphur also induces ligninolytic activity in *P. chrysosporium*. This shows the positive effect of sulphur on the remediation of paper and cardboard industrial waste.

4.6.6 Hydrogen Ion Concentration

Paper and cardboard waste degradation is based on lignin degradation which is quite sensitive to pH. Hence, adequate buffering is essentially required to control

pH during lignin decomposition, consequently paper and cardboard waste degradation. The medium pH was found to be critical for decomposition of paper and cardboard industrial effluent. The optimum culture pH for delignification by *P. chrysosporium* is observed in the range of 4.0–4.5, with marked suppression above 5.5 and below 3.5. The pH requirement varies for growth and waste degradation. The optimum pH, however, for the growth was somewhat higher than that for lignin degradation. When pH is drifted from the optimum 3.5–5.5 range, the rate of CO_2 released is increased in *P. chrysosporium*.

4.6.7 Toxic Substances

The presence of toxic substances inhibits the mycoremediation process. Sometimes, toxic compound is generated during the treatment with fungi. For example, the toxic amines are generated when azo- and nitro compounds are reduced by fungi which are difficult to degrade. Further, these compounds create many difficulties in paper and pulp mill waste degradation.

4.7 Mycoremediation of Paper and Cardboard Industrial Wastes

Characteristics of waste must be considered for mycoremediation of different types of paper, pulp and cardboard industrial wastes. Wastes generated by these industries depend on the technique of making paper. Generally, all pulp and paper industries can be divided into three main categories on the basis of processing of raw material:

1. Handmade paper industries, process paper by hand or simple machinery
2. Kraft paper industries, process paper mechanical or chemical method
3. Cardboard industries, recycle wastepaper

These can be further divided into small-scale and large-scale categories on the basis of their production capacity.

4.7.1 Handmade Paper and Pulp Industries

Handmade paper industry is not the topic of discussion of this millennium but is an ancient art of making paper by hand still exist in many part of the world. According to American Paper and Pulp Association “Handmade Paper is a layer of entwined fibres, held together by the natural internal bonding properties of cellulose fibres lifted by hand, sheet by sheet on moulds in suspension of fibres in water with or without sizing.”

Table 4.4 Difference in handmade paper and mill-made paper industries

S. no.	Characteristics	Handmade paper	Mill-made paper
1	Investment on plant on per ton per annum production	12,450	24,850
2	Employment generation on invest of each Rs. one crore	980	130
3	Employment generation of each 1,000 ton of production	1,050	25
4	Import of machinery	NIL	35 %
5	Resource input of production per each ton		
(a)	Forest based raw materials like bamboo and wood	NIL	2.5–3 ton
(b)	Coal	0.085 ton	1.50 ton
(c)	Electricity	150 kwh	2,000 kwh
(d)	Water	38 cu mts	300 cu mts
(e)	Chemicals	0.0051 ton	0.8 ton

Source: Khadi and Village Industries Commission, India

This ancient art of making paper by hand or simple machinery, i.e. handmade paper was discovered in China. Later it spread in Korea, Japan, Asia and Persia. In India, the first handmade paper was manufactured in the year 1159 AD. The first paper mill was set up at Serampur, West Bengal, in the year 1812 which used grass and jute as raw material for making paper. It still exists in India, many European and American companies, contributing in production of high-grade export quality paper.

As there has been phenomenal growth in the export market for Indian handmade paper and its products, especially in the developed countries like the United States of America, West Germany, European Countries, Australia, etc., India is being looked upon as the country with the maximum growth potential in handmade industry. In India, there are about 685 units of large-scale and small-scale handmade paper working all over the country. There are around 300 kagzi units still working in Sanganer (Jaipur) both large scale and small scale. These produce around Rs 21 crore worth of papers, providing full-time employment to 10,000 persons in the rural areas (Khadi Industries Village Commission report, Rajasthan, India). In spite of having competition in the global market, there are definite opportunities for smaller, local firms satisfying specific needs of paper. Differences in handmade paper and mill-made paper industries are depicted in Table 4.4.

The finest handmade papers are made from pure rag pulp, usually linen and cotton, which are washed, boiled and beaten to macerate the fibres. These fibres are then suspended in water where they can be lifted out by the papermaker using a mould and deckle. A mould is a screen of some sort, supported by a frame, which allows the surplus water to drain after dipping the fibres from the vat. A deckle is another frame on top of the mould which keeps the fibres from washing over the edges.

Handmade paper industries (HMP) utilise various lignocellulosic and cellulosic raw materials. On the basis of using raw material, these are further divided into two categories: (1) agro-residue-based HMPs (these industries use agroresidues for

making paper such as hemp fibre, banana pulp, jute pulp, etc.) and (2) textile waste-based HMPs (these industries use cotton rags, hosiery rags, procured as tailor cuttings from textile industries for making paper). For processing of paper, these industries also use a large amount of water and discharge it into the nearby water bodies or in the agricultural fields.

4.7.1.1 Wastes of Handmade Paper Industries and Pollution

Depending on the type of raw material, handmade paper industries generate waste of different characteristics. These industries which use linen or cotton rags or mill broke as raw material having different characteristics compared to those that use agricultural residues and mill broke. Cotton rags are rich in cellulose; however, agro-residues were found to possess lignin and cellulose. Therefore, the processing of these raw materials differs from each other which lead to the generation of effluent with different characteristics. Water used in several steps of making paper is discharged as effluent-possessing chemicals used for the processing of raw materials.

Agro-based handmade paper and pulp industries adopt the procedure of processing the lignocellulosic pulp residues similar to mill-made paper industries. Hence, effluent characteristics are similar to the characteristics of kraft pulp and paper industries. However, the amount of effluent generated by these industries is low because all processes are done by hand or simple machinery.

Cotton- and hosiery rag-based handmade paper industries utilise various chemicals and huge amount of water which is discharged in the form of effluent. Earlier handmade paper used to come only in white colour, but now available in different colours and designs. Many small-scale industries are emerging in India, which are using various chemicals and chemical dyes for making paper.

Generally, these are considered as ecofriendly industries only on the basis of physicochemical characteristics. Although these industries use dyes and chemicals, there are few reports available on the effluent discharge and pollution-causing potential of these industries. These industrial discharges were found to possess effluent BOD, COD, pH and other parameters much above the discharging limit and having mutagenic potential possibly due to the use of dyes for colouring paper (Kulshreshtha et al. 2011).

These industries discharge two types of wastes: (1) effluent and (2) sludge. After making paper dyes, pulp residues containing effluent are discharged to nearby fields or in the water bodies which impart colour to the water body. The effluent colour may increase water temperature and decrease photosynthesis, both of which probably lead to a decreased concentration of dissolved oxygen.

Leftover or unused pulp residues are generated in the form of sludge and accumulated in the close proximity of industries or in the industrial drain and cause soil or water pollution.

4.7.1.2 Effluent Treatment

These are exempted from the list of pollution-causing industries, and therefore, effluent treatment of these industries is meagerly reported. Pulp fibres from handmade paper and pulp industries were separated out by filteration process and used for the cultivation of mushroom along with the sludge of unused pulp residues (Kulshreshtha et al. 2010b).

4.7.1.3 Sludge Treatment

Recently, sludge of these industries is utilised for mushroom cultivation, a macrofungi, for its bioremediation from the environment (Kulshreshtha et al. 2010b). Mushrooms are higher fungi capable of degrading lignin and cellulose efficiently due to the production of cellulolytic and ligninolytic enzymes. Mushroom is not only capable of bioremediation of waste but also provides a highly proteinaceous food. *Pleurotus florida* is a mushroom species which is recently used for the bioremediation of sludge generated by handmade paper and pulp industries.

The pulp and paper industrial sludges may mix either with effluent of same pulp and paper industry or with water. This mixing will provide thick slurry of composite waste (sludge and effluent). Generally, pulp and paper sludge is obtained in wet condition; therefore, no presoaking is required like wheat straw. This slurry of waste treats with 1.25 ml/l of 40 % formalin solution (v/v) and 0.75 g/l (w/v) of antifungal substance bavistin for 18–24 h to remove unwanted organisms. Pulp fibres are collected by filtering the slurry by a clean cloth and spread on a clean surface, to remove excessive water for maintaining 80 % humidity. Further, these pulp fibres sterilise by steam for 1 h to overcome the disturbance of other microorganisms. After cooling, wet pulp inoculates with spawn and pack in porous polythene bags for incubation. Incubation temperature varies per the requirement of mushroom species. When *Pleurotus* mycelium fully colonises the substrate, polythene bags can be removed and substrate bundles can be hanged. These bundles can be sprayed with water twice a day to maintain humidity, and time can be recorded for pinhead formation, immature fruiting body and mature fruiting body formation.

After obtaining the mature fruiting bodies (basidiocarps), mushroom fruiting bodies can be harvested and analysed in the laboratory for its nutritional characteristics, toxicity and genotoxicity. In our recent investigation, it was found that mushroom fruiting bodies of *P. florida* do not possess basepair and frameshift mutagens (Kulshreshtha et al. 2011). However, these basidiocarps cannot be considered safe for consumption until found free of other toxicants and metallic content. Preliminary results obtained with mushroom cultivation on handmade paper and pulp industrial wastes indicate the extended application of this mushroom species not only for treatment but also for proteinaceous food.

4.7.2 Mill-made Paper and Pulp Industries

About 1800–1860, all work sequences previously performed by hand were mechanised for making paper. This included the rag preparation, the use of fillers, pulp beating, the paper machine with its various parts and the machines required for finishing the paper (the headbox, wire section, press section, dryer section and units for reeling, smoothing and packaging). The paper industry has came a long way, and evolution of new sheet-forming principles (with fluid boundaries between paper and nonwoven fabrics) and chemical pulp processes have been the main process improvements.

The heavy demand for the paper helps in steady expansion of paper industries. When India became independent in 1947, there were 17 paper mills with an installed capacity of less than 0.14 million TPA (tonnes per annum). The organised growth of this sector began in 1951, with many small and medium paper mills being set up due to the government policy. The annual increase in paper production since independence has been in the range of 0–13 % with a current dead capacity of 1.1 million tonnes. At present, there are an estimated 525 pulp and paper mills with a total installed capacity of around 6.25 million TPA. However, the situation on the global market, i.e. increased demand, above all in the Third World, trends in chemical pulp prices and problems of location, is again raising capital intensity and encouraging the formation of big company groups with international operations. On the basis of pulping procedures, these are of various types as mentioned in Table 4.5.

4.7.2.1 Wastes of Mill-made Paper Industries and Pollution

Mill-made paper industries are also producing two types of wastes: (1) effluent and (2) sludge. Generally, these industries are involved in making paper by delignification of wood. Besides this, these industries consume large amount of water in different steps of papermaking process which reappear in the form of effluent. The most significant sources of pollution among various process stages are wood preparation, pulping, pulp washing, screening, washing, bleaching and paper machine and coating operations. Among the processes, pulping generates a high-strength wastewater especially by chemical pulping containing wood debris and soluble wood materials. Pulp bleaching generates most toxic substances as it utilises chlorine for brightening the pulp. Moreover, depending upon the type of the pulping process, various toxic chemicals such as resin acids, unsaturated fatty acids, diterpene alcohols, juvaniones, chlorinated resin acids and others are generated during pulp and papermaking process (Pokhrel and Viraraghavan 2004). All these chemicals are present in the effluent generated by these industries which is discharge to the adjacent water bodies or soil after use, and therefore, these industries pollute not only water but also soil. These pulp and paper industries are one of the 17 most polluting industries listed by the Central Pollution Control Board

Table 4.5 Different pulping method-based paper and pulp industries and risks associated with them

Pulping process	Details	Risks
Sulphate pulping	Alkaline sulphate process independent of wood species yields high-fibre properties	Bleaching with chlorine-containing chemicals such as (elemental or gaseous) chlorine, hypochlorite or with chlorine dioxide Dissolved lignin extracted with alkali
Sulphite pulping	Several special paper qualities, e.g. tissue, wood-free printing and writing papers, grease proof papers, etc., are prepared Suitable raw material for this is spruce Pine, birch and other hardwood species are not good Sulphite cooking is possible using Ca, Mg, Na or NH4 as a base chemical in cooking the liquor Two methods: acid sulphite process or bisulphite cooking process	The acid sulphite pulping process waste liquor is normally burned in a recovery boiler in an oxidative atmosphere with about dry solid content of 55–57 %. Except for when using a sodium-based waste liquor, there is not a chemical smelt layer on the bottom of the boiler and no risk for smelt/water explosions like in the case of a black liquor recovery boiler
Recycled pulping (RCF) and deinked (DIP) pulps	Releasing of ink during pulping is the cause of colour and toxicity in the effluent	Exposure to some hazardous chemicals (e.g. peroxide) No risks stemming from a recovery boiler
Mechanical pulping (groundwood (GW), thermomechanical (TMP), chemithermomechanical (CTMP) and bleached chemithermomechanical (BCTMP) pulps	Adjacent sulphate pulp mill recovery boiler may be used in cross recovery for impregnated chemicals	Exposure to some hazardous chemicals, e.g. peroxide No recovery boiler risks

(CPCB). Most mills probably release suspended solids, such as fibre and bark, organic matter, which increase the biological oxygen demand, chemical oxygen demand and colour and suspended solids.

Most of the solid waste from the pulp and paper industry consists of "mill residues", which comprises bark, wood residues, refuse (pulp, paper and cardboard), ash from combustion facilities and sludge from treatment of process water and deinking. The major contributors of solid wastes in pulp and paper mill effluents are bamboos and wood dust, line sludge, coal ash and fly ash and other rejects. Discharging of sludge and effluent of mill-made paper industries lead to the pollution of soil and water.

4.7.2.2 Effluent Treatment

Effluent treatment of different types of mill-made paper industries is reported by various scientists till date. Two-step bioreactor is designed for the treatment of pulp and paper industrial effluent by Singh and Thakur (2006) in which at first effluent was treated in anaerobic condition and then treated by fungi *Paecilomyces* sp. in the second bioreactor. This technique leads to reduction in colour (95 %), AOX (67 %), lignin (69 %), COD (75 %) and phenol (93 %) by third day when 7-day anaerobically treated effluent was further treated by fungi. Fungi used for treatment of effluent of different types of pulp and paper industries and effect of fungal treatment on different parameters are given in Table 4.6.

4.7.2.3 Sludge Treatment

Generally, the sludge of this type of industries is used for landfilling purpose as well as composting purpose. However, there is scarcity of literature on the treatment of mill-made paper industrial sludge and its bioremediation with fungi.

The use of chemithermomechanical pulp for the cultivation of mushroom is reported (Sivrikaya et al. 2002). Chemithermomechanical pulp is used as a substrate by few mushroom cultivators, who used to cultivate mushroom in the mushroom farm. During the cultivation period, both phytohormones 2.4-D and PS A6 can be used. Mature fruiting bodies of *Pleurotus sajor-caju* can be collected and used for examination metals, respectively. Postharvesting mature fruiting bodies of basidiocarp can be cleaned, dried in a drying oven at 103 °C and homogenised by grinding. All the edible parts of the mushroom basidiocarp can be stored in closed containers at room temperature after pretreatment.

Mushroom basidiocarps can be tested for the presence of toxic metals. Metal analysis of basidiocarp can be done by mixing 2 g of basidiocarp with 20 ml concentrated HNO_3, and this mixture is allowed to stand overnight. The mixture is heated carefully on a hot plate until the production of red NO_2 fumes ceased, and then samples are analysed for metallic content. These metals can be most conveniently determined by either the flame emission (FE) or atomic absorption (AA) methods.

Metal analysis of postharvesting mature fruiting bodies revealed the presence of trace element contents in mushroom *Pleurotus sajor-caju* cultivated on chemithermomechanical pulp with phytohormones PS A6 and 2.4-D. Fruiting bodies were found to possess many metals above the acceptance level which makes it unsuitable for consumption (Sivrikaya et al. 2002). Hence, fruiting bodies were found to possess many metals above the acceptance level which makes it unsuitable for consumption.

Table 4.6 Mycoremediation of paper and pulp industries effluent and parameters measured

Type of paper and pulp industry	Effluent type	Fungi used	Parameters reduced	References
Bagasse-based paper and pulp mill	Dissolved bagasse lignin, raw black and alkali stage liquors	*Aspergillus foetidus*	90–95 % of initial colour, COD removal	Sumathi and Phatak (1999)
	Dissolved bagasse lignin, raw black and alkali stage liquors	*Schizophyllum commune*	90 % colour reduction, 70 % BOD and 72 % COD reduction	Belsare and Prasad (1988)
Bagasse pith-based pulp and paper mill	Black liquor	*Ceriporiopsis subvermispora*	90 % colour, 45 % COD, 62 % lignin, 32 % AOX, 36 % EOX (extractable organic halide)	Nagarathnamma and Bajpai (1999)
Paper and pulp mill	Coloured effluent	*Gliocladium virens*, *Phanerochaete chrysosporium*, *Coriolus versicolor*, *Trametes versicolor*	42 % initial colour removal	Murugesan (2003)
	Black liquor contain colour, highly toxic chlorinated lignin-degradation products	*Fomes lividus* and *Trametes versicolor*	COD reduced to 1,984 mg/l (59.3 %), 68 % decolourisation with *T. versicolor*, 103 % inorganic chloride with *T. versicolor*	Selvam et al. (2002)
	Black liquor	Marine-derived fungus NIOCC #312	Better than NIOCC #2a strain of marine fungus in decolourisation of bleach plant effluent due to production of peroxidases	Raghukumar et al. (2008)
		Marine-derived fungus NIOCC #2a	Decolourisation of bleach plant effluent due to production of laccases	Raghukumar et al. (2008)
		Aspergillus niger	81 % decolourisation	Kannan et al. (1990)
		Trametes versicolor	93 % decolourisation	Bajpai et al. (1993) and Martin and Manzanares (1994)
		Trichoderma spp.	85 % decolourisation	Prasad and Joyce (1991)

(continued)

Table 4.6 (continued)

Type of paper and pulp industry	Effluent type	Fungi used	Parameters reduced	References
		Pleurotus sajor-caju, *P. platypus*, *P. citrinopileatus*	66.7 % decolourisation, 61.3 % COD reduction, inorganic chloride reduction	Raghunathan and Swaminathan (2004)
		Merulius aureus syn. *Phlebia* sp., *Fusarium sambucinum* Fuckel MTCC 3788	78.6 % colour, 79.0 % lignin and 89.4 % COD reduction	Malviya and Rathore (2007)
Bleached kraft pulp mill	Processing Eucalyptus globulus	*Trametes versicolor*	74–81 % COD	Freitas et al. (2009)
		Phanerochaete chrysosporium	Reduction in BOD and COD	Freitas et al. (2009)
		Rhizopus oryzae	Biodegradation of organic compounds	Freitas et al. (2009)
Bleach plant effluent	Black liquor contain colour, highly toxic chlorinated lignin-degradation products	Marine fungi *Sordaria fimicola* (NIOCC #298)	65–75 % decolourisation due to producing MnP	Raghukumar et al. (1996)
		Marine fungi *Halosarpheia ratnagiriensis* (NIOCC #321)	65–75 % decolourisation due to producing MnP	Raghukumar et al. (1996)
Eucalyptus paper pulp	Pitch-causing lipophilic compounds present	Laccase from the basidiomycete *Pycnoporus cinnabarinus*	Free and conjugated sitosterol removed	Gutiérrez et al. (2006)
Spruce pulp	Pitch-causing lipophilic compounds present	Laccase from the basidiomycete *Pycnoporus cinnabarinus*	Resin acids, sterol esters and triglycerides removed	Gutiérrez et al. (2006)
Flax pulp	Pitch-causing lipophilic compounds present	Laccase from the basidiomycete *Pycnoporus cinnabarinus*	Sterols and fatty alcohols removed	Gutiérrez et al. (2006)
Pine kraft black liquor	Black liquor	*Polyporus versicolor*	70 % decolourisation	Marton et al. (1969)
Kraft pulp and paper mill	Black liquor	*Aspergillus* spp.	Decolourisation	Dutta et al. (1985)
		Phanerochaete chrysosporium	Decolourisation	Thomas et al. (1981)
		Tinctoporia sp.	Decolourisation	Fukuzumi (1980)
Kraft pulp mill using chlorine bleaching	Black liquor	*Rhizopus oryzae*	Decolourisation and detoxification	Nagarathnamma and Bajpai (1999)

4.7.3 Cardboard Industries

Cardboard is a thick layer of paper made by shortened cellulosic fibres produced due to repeated recycling. Cardboard is a heavy wood-based type of paper notable for its stiffness and durability. It is used for a wide variety of purposes generally for packaging purposes. The growing demand of paper led to a greater mechanisation processes and materials. This produces a thick sheet of paper of much poorer quality. Due to shortage of rags, other papermaking materials are using nowadays which are available in plentiful amount and economic. In this context, various types of printed, nonprinted, coloured, laminated, non-laminated, paper and paperboards are collected for removing the waste from environment and recycle it into cardboard. These industries are based on the recycling of wastepaper and cardboards and laminated board. Recycling of newspaper and wastepaper and boards saves trees.

It is first invented in China in the fifteenth century. The first commercial cardboard box was produced in England in 1817, and first machine for producing large quantities of corrugated cardboard was built in 1874 by G. Smyth, and in the same year, Oliver Long improved upon Jones' design by inventing corrugated cardboard with liner sheets on both sides.

4.7.3.1 Wastes of Cardboard Industries and Pollution

These industries recycle the wastepaper, however, pollute the environment by discharging two types of wastes: (1) effluent and (2) sludge. Mostly, cardboard is prepared from unused paper and pulp fibres without using any type of chemicals for delignification of pulp fibres. However, these are considered as pollution-causing industries due to having low pH, high BOD and COD. Effluent of cardboard industries is highly coloured and generally possesses brown colour due to release of natural colour of lignin. Another possibility of brown-coloured effluent is the release of inks, dyes and chemicals in the pulping liquor during the pulp-making process. These wastepaper-based mills consume a large amount of raw water which is discharged as effluent. These industrial effluents are characterised by a high organic load in the form of shortened pulp fibres. The brown colour of the effluent is due to the release of ink, dyes and chemicals in the water during pulping used for making papers. The discharge of untreated effluent from these industries into water bodies causes poor water quality, and the brown colour of untreated effluent is detectable over long distances. Discharging of untreated effluent can cause eutrophication due to having organic nature. Due to economical and environmental reasons, consumption of water has decreased overtime, and today, there exist several zero-discharge recycled paper and cardboard mills. However, closing a water system leads to several different problems, such as increased demand for retention aids, decreased product quality and reduced felt life and corrosion.

Cardboard industries also cause pollution due to repeated recycling which results in shortening of pulp fibres due to repeated recycling. In the last stage of making

paper, cardboard and pulp fibres become too short to be recycled which accumulate in adjacent area of industries. In this case, accumulation of nonrecycled left over pulp residues is the main cause of soil pollution, and biodegradable organic pulp residue waste will produce enormous odour in the area.

4.7.3.2 Effluent Treatment

Closing of drainage system of cardboard industries gives rise to problem of pungent odour, blockage, etc. Hence, it is necessary to find out the solutions to overcome these problems. One method is to reduce the compounds in the wastewater, on which the fungi feed on. As fungi used all the pollutants as source of energy and carbon, these are removed from the effluents.

An anaerobic/aerobic biological process for treating wastewater from a recycled paper mill was evaluated in both mesophilic and thermophilic conditions in laboratory-scale experiments. The pilot plant trials were carried out at Munksjo Lagamill AB, Sweden. Effluent can also be treated in an in-mill biological treatment of wastewater by using fungi, and it has been shown to be an attractive alternative for mycoremediation of wastewater from recycled paper/board production. Presently, there is no mycoremediation technology for the wastewater of paperboard or cardboard mill due to which Indian cardboard mills discharge brown-coloured water, treatment of which is meagerly reported. This untouched problem opens a good opportunity of research in the field of mycoremediation of waste.

4.7.3.3 Sludge Treatment

Sludge of these industries contains nonrecyclable pulp residues and is reported to be used for the cultivation of mushroom. Sludge is processed and inoculated as mentioned earlier for handmade paper industries. The results of this study show that when waste is used alone, mushroom cultivation required very long time as compared to straw. However, the same sludge is processed and mixed with wheat straw in equal amount; mushroom fruiting bodies can be obtained in short time with increased biological efficiencies (Kulshreshtha et al. 2010b).

4.8 Degradation of Paper, Pulp and Cardboard Industrial Pollutants

New treatment technologies must be designed to degrade those chemicals that pose the greatest threat to human health that are toxic and/or mutagenic, have a tendency to bioaccumulate, and are difficult to degrade. Using these criteria, the degradation of all mutagenic compounds, resin acids, chlorinated phenols and chlorinated aliphatic hydrocarbons should be the focus of new treatment processes.

4.8.1 Lignosulphonics

Generally, pulp, paper and cardboard industrial effluents are brown in colour which is due to the presence of lignin and its degradation products formed by the action of chlorine on lignin. This is a serious cause of concern from aesthetic and biological point of view. In the kraft process of making paper, lignin is converted to thio- and alkali lignin due to ligno-sulphonate in the sulphate process.

4.8.1.1 Mycoremediation of Lignosulphonics

These compounds give brown colour to the effluents. Many fungi can be used for the treatment of black liquor and mentioned in Table 4.6.

4.8.2 Heavy Metals and Inorganic Compounds

The main sources of Al, Cu, Cr, Ti, Fe, Mg and Zn in pulp and paper mill effluents are the chemicals used in pulping, additives used in papermaking.

4.8.2.1 Mycoremediation of Heavy Metals

Many fungi have been used for the bioremediation of heavy metals for long times. These include several fungi (*Aspergillus* spp. and *Rhizopus* spp.) and mushroom species (*Agaricus* spp., *Pleurotus* spp.). However, there is scarcity of report on the mycoremediation of heavy metals from paper and pulp industrial effluents. Metals absorbing fungi provide us a good opportunity of research to use them for mycoremediation of heavy metal content of paper and cardboard industrial effluent and sludge.

4.8.3 Dioxin and Dibenzofurans

Dioxins are the family of halogenated dibenzo-*p*-dioxin congeners. Dioxins are toxic, and environmentally persistent compounds arise as trace pollutants in the manufacture of pulp and paper bleaching which is the most considerable pollutant for bioremediation. Dioxins and furans are persistent and tend to accumulate in sediments and in human and animal tissues.

4.8.3.1 Mycoremediation of Dioxins

The metabolism of 2,7-dichlorodibenzo-*p*-dioxin by *Phanerochaete sordida YK-624* has been reported (Takada et al. 1996). Moreover, the oxidation of dibenzo-*p*-dioxin by fungal enzyme lignin peroxidase is also reported by Joshi and Gold (1993).

4.8.4 Organochlorine Compounds (AOX)

Absorbable organic halogens (AOX), total organochlorine (TOCl) and tertachlorinated dibenzodioxins (TCDD) are the most hazardous compounds of pulp and paper mill effluents, which besides being toxic, mutagenic, persistent and bioaccumulating are also known to contribute much to the pollution load in the effluents. These compounds are produced mainly by the reactions of residual lignin present in the wood fibre and chlorine used for bleaching which is a matter of great concern in the pulp and paper industry.

4.8.4.1 Mycoremediation of AOX

Mycoremediation of AOX from paper and pulp industrial effluent and degradation of chlorinated compounds such as mono-, di-, tri-, penta- and chlorophenols has been done by fungal isolates that were obtained from different effluent streams of pulp and paper industry and soil irrigated with this wastewater.

4.8.5 Resin Acids

The wastewaters of the pulp mills are found to contain measurable concentrations of resin acids and resin acid biodegradation products. Resin acids are the group of deterpenoid carboxylic acids which are toxic to fishes in recipient waters. Commonly found resin acids can be classified into two types: abietanes (abietic, dehydroabietic, neoabietic, palustric and levopimaric acids) and pimaranes (pimaric, isopimaric and sandaracopimaric acids). The most abundant resin acids are two abietanes, dehydroabietic acid (DHA) and abietic acid (ABA). The toxicity of chlorinated compound increases with increasing number of chlorine atoms on organic compounds. Recent studies indicate that natural resin acids and transformation products may accumulate in sediments and pose acute and chronic toxicity to fish. Several resins and their biotransformation compounds have also been shown to bioaccumulate and to be resistant to biodegradation than the original material.

4.8.5.1 Mycoremediation of Resin Acids

Resins are considered to be readily biodegradable. However, their remediation has been shown to vary. Until recently, the microbiology of resin acid degradation has received only scant attention. There is no conclusive evidence that fungi can completely degrade these compounds. In contrast, a number of bacterial isolates and activated sludge (Kostamo and Kukkonen 2003) have recently been described which are able to utilise dehydroabietic and isopimaric acids as their sole carbon source.

4.8.6 Lignin Cellulose Degradation

Lignin is a highly complex, stable and irregular polymer which is structurally similar to the resins. The lignin-degrading system has broad substrate specificity, includes a large range of oxidoreductases and hydroxylases such as laccases and high redox potential LiP, MnP, VP and others (see Sect. 4.3). Cellulose is a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1 → 4)-linked D-glucose units. Cellulose is also an organic compound with the formula $(C6H10O5)_n$, possessing a complex structure, but it is easily degradable by cellulolytic fungi. When it is present in complex form with lignin, then its degradation occurs only after the removal of lignin.

4.8.6.1 Mycoremediation of Lignin and Cellulose

Sporotrichum pulverulentum was found to degrade lignin in the presence of phenol oxidase enzyme. The lignin degradation abilities of wild type, a phenol oxidase-less mutant and a phenol oxidase-positive revertant were compared by Ander and Eriksson (1976) to determine if phenol oxidase activity is necessary for lignin degradation by white-rot fungi. They found that the phenol oxidase-less mutant was unable to degrade kraft lignin. The phenol oxidase-positive revertant, however, regained the ability of the wild type to degrade kraft lignin.

Besides white-rot fungi, some non-white rots are also able to degrade lignin. For example, *Aspergillus foetidus*, an isolated fungus, reduced lignin content along with decolourisation, indicating strong correlation between the decolourisation and lignolytic processes. *Paecilomyces* sp. exhibited significant reduction in lignin (66 %) in sequential bioreactor supplemented with 1 % carbon and 0.2 % nitrogen source in 6 h of retention time (Singh and Thakur 2004).

4.8.7 Phenolic and Chlorophenolic Compounds

Chlorinated phenolic compounds are the most abundant recalcitrant wastes produced by the paper and pulp industry which accumulated in the effluents after secondary treatments. These compounds are produced upon the partial degradation of lignin during bleaching process. These compounds pose a big concern to human and environmental health due to their high toxicity to a wide range of organisms.

4.8.7.1 Mycoremediation of Phenolic and Chlorophenolic Compounds

White-rot fungi are a group of organisms very suitable for the removal of chlorinated phenolic compounds from the environment. Chlorophenols act as substrates for laccase and Mn-peroxidase that requires hydrogen peroxide and Mn (II) for reaction, which can react with all the chlorophenols including the most recalcitrant pentachlorophenol (PCP). The free-cell cultures of white-rot fungus *Panus tigrinus*, when adapting to high concentrations (up to 2,000 mg/l) of 2,4, 6-trichlorophenol (2,4,6-TCP), transformation of a mixture of 2,4-DCP, 2,4,6-TCP and PCP achieved (Leontievsky et al. 2000). The lifetime and reactivity of the biomass can be achieved by immobilisation of free cells on to a support matrix, which produces higher cell densities, higher and time-extended enzyme activities, greater enzyme stability and subsequently a reduction in treatment cost.

Exposure of *Trametes versicolor* to increasing amounts of pentachlorophenol (PCP) from 200 to 2,000 ppm leads to acclimatisation of the fungus to these toxic pollutants. Free-cell cultures of acclimatised *Trametes versicolor* were compared with cultures immobilised on nylon mesh for transformation of PCP and 2,4-dichlorophenol (2,4-DCP). A total addition of 2,000 ppm of 2,4-DCP and 3,400 ppm PCP were removed from the immobilised cultures with 85 % of 2,4-DCP and 70 % of PCP transformed by enzymes (laccase and Mn-peroxidase), 5 % 2,4-DCP and 28 % PCP adsorbed by the biomass and 10 % 2,4-DCP and 2 % PCP retained in the medium at the termination of the fermentation. In contrast free-cell cultures in the same medium with the same addition regime of PCP and 2,4-DCP transformed 20 % 2,4-DCP and 12 % PCP by enzyme action, adsorbed 58 % 2,4-DCP and 80 % PCP by the biomass and retained 22 % 2,4-DCP and 8 % PCP in the medium. This shows that immobilised acclimatised fungus can remove chlorophenols more efficiently than free acclimatised fungal culture (Selvam et al. 2002).

Lamar et al. (1993) compared the abilities of *P. chrysosporium*, *P. sordida* and *Trametes hirsuta* to degrade PCP and found that *P. chrysosporium* is best in degrading PCP. Extracellular peroxidases play an important role in the degradation of chlorophenols by *P. chrysosporium*. Extracellular laccases and peroxidases carry out the first productive step in the oxidation of chlorophenols, forming *para*-quinones and consequently releasing a chlorine atom. Further degradative steps involving several enzymes and highly reactive, nonspecific redox mediators

produced by the fungus render it capable of efficiently degrading several toxic compounds. *Paecilomyces* sp. exhibited significant reduction in phenol (40 %) in sequential bioreactor supplemented with 1 % carbon and 0.2 % nitrogen source in 6 h of retention time (Singh and Thakur 2004).

Recently, the degradation of 2-chlorophenol (2-CP), 2,4-dichlorophenol (2,4-DCP), 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) was found to be bioremediated by white-rot fungus *Trametes pubescens* (González et al. 2010). These fungi may be used possibly for the bioremediation of paper and pulp industrial wastes in the near future.

4.8.8 Colour

Paper and pulp industries discharge highly coloured effluents. The brown colour of the effluent can be noticed many kilometres away from discharging point. The reason of this colour is possibly the lignin and its derivatives.

4.8.8.1 Mycoremediation of Colour

Terrestrial white-rot basidiomycetous fungi and their lignin-degrading enzymes laccase, manganese peroxidase and lignin peroxidases are useful in the treatment of brown-coloured effluent of paper and pulp industries. Fungi involved in decolourisation of paper and pulp are given in Table 4.6. Sometimes decolourised effluent was found to regain colour after prolonged incubation which is possibly due to high molecular weight colour components. To avoid decolourisation instability, fungal bioreactor can be coupled with ultrafilteration.

4.8.9 Lipophilic Extractives

Primary clarifier effluent (PE) and a secondary clarifier effluent (SE) from a treatment plant of a Finnish elementally chlorine-free (ECF)-bleached kraft pulp and paper mill was found to possess lipophilic organic compounds (Koistinen et al. 1998) originating from kraft pulping and papermaking and identified by straight gas chromatography/mass spectrometry (GC/MS) analyses. Lipophilic extractives also exert a negative impact in pulp and paper manufacturing causing the pitch problems and poor product quality (Back and Allen 2000).

4.8.9.1 Mycoremediation Lipophilic Extractives

Phlebia radiata, *Funalia trogii*, *Bjerkandera adusta* and *Poria subvermispora* strains are the most promising organisms for the degradation of both free and

esterified sterols present in the effluent of eucalypt wood-based paper and pulp industries. *Pycnoporus cinnabarinus* and its laccase enzyme are used to treat different model lipids—alkanes, fatty alcohols, fatty acids, resin acids, free sterols, sterol esters and triglycerides—in the presence of 1-hydroxybenzotriazole as mediator. Unsaturated lipids attack via the corresponding hydroperoxides. The enzymatic reaction on sterol esters largely depended on the nature of the fatty acyl moiety, i.e. oxidation of saturated fatty acid esters initiated at the sterol moiety, whereas oxidation of unsaturated fatty acid esters initiated at double bonds of the fatty acid. In contrast, saturated lipids decreased when the laccase-mediated reactions carried out in the presence of unsaturated lipids suggesting participation of lipid peroxidation radicals. These results suggested the remediation of lipid mixtures containing paper and pulp industrial wastes using laccase-mediator system of different fungi.

4.8.10 Dissolved and Colloidal Substances

These substances are present in thermomechanical pulp and newsprint mill process waters. These are derived from the woody component and can be degraded by microbes in natural condition.

4.8.10.1 Mycoremediation of DCS

White-rot fungi *Trametes versicolor* possessed the highest growth on white water and highest activity against the DCS components present in thermomechanical white water. It shows significant decrease in the total dissolved and colloidal substances (DCS), carbohydrates and extractives in 2 days and at 30 °C.

4.9 Conclusion

Insufficient and inappropriate disposal of sludge and effluent wastes of paper and cardboard industries leads to various environmental and health problems. Therefore, management of the increasing quantities of solid and effluent of paper and cardboard industries is a global environmental issue. There is a lack of knowledge, organisation and planning in waste management due to insufficient information about regulations and due to financial restrictions in both large-scale and small-scale paper and cardboard industries. The issue of waste mycoremediation is necessary due to the increasing quantities of waste and an inadequate management system. Mycoremediation technology is a great boon which will definitely help us in improving the existing situation and to minimise environmental and health problems associated with paper and cardboard industrial wastes and

pollutants. Therefore, in this chapter, we have discussed about the fungi, their enzymes and the role of both in mycoremediation process and factors affecting the process. Besides this, we have also discussed about the types of wastes and pollutants generated by handmade paper, mill-made paper and cardboard industries and strategies adopted for their mycoremediation. It was found that fungi have great capability to degrade not only lignin or cellulose but also a variety of pollutants. Therefore, these can be used successfully for remediating the paper and cardboard industrial waste in free or immobilised form. However, there is a great lacuna in mycoremediation technology of paper pulp and cardboard industrial waste which will provide us good opportunity of research. In brief, it is suggested that every industry should adopt mycoremediation technology and established treatment plant at industrial level before discharging the waste into environment for contributing in sustainable development without affecting the environment and production.

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