Bioremediation of paper and pulp mill effluents

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Pulp and paper mill effluents pollute water, air and soil, causing a major threat to the environment. Several methods have been attempted by various researchers throughout the world for the removal of colour from pulp and paper mill effluents. The biological colour removal process uses several classes of microorganisms—bacteria, algae and fungi—to degrade the polymeric lignin derived chromophoric material. White rot fungi such as Phanerochaete chrysosporium, Coriolus versicolor, Trametes versicolor etc., are efficient in decolourizing paper and pulp mill effluents. Gliocladium virens, a saprophytic soil fungus decoloured paper and pulp mill effluents by 42% due to the production of hemicellulase, lignin peroxidase, manganese peroxidase and laccase.

Keywords: Paper mill effluents, Pulp pollution, Microbes in bioremediation, Water pollution

Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less-harmful forms. Bioremediation could be employed for the treatment of various industrial effluents including sewage water, effluents from tannery, distillery, paper and pulp industry. The general approaches to bioremediation are to enhance natural biodegradation by native organisms (intrinsic bioremediation), to carry out environmental modification by applying nutrients or aeration (biostimulation) or through addition of microorganisms (bioaugmentation). Unlike conventional technologies, bioremediation can be carried out on-site. Bioremediation is limited to a number of toxic materials it can handle, but where applicable, it is cost-effective¹. This article is an overview of the attempts made by several research and development organizations around the world to use biotechnological methods for removal of the toxic compounds from the environment.

Major pollutants — Synthetic chemicals

The major chemical pollutants are synthetic chemicals like dichlorodiphenyl-trichloroethane (DDT) and polychloroprene chloride. The list also includes many chemicals such as trichloroethylene, polychlorinated biphenyls, nitroaromatic compounds like nitrobenzene, nitrotoluene and many others.

Polychlorobiphenyls (PCBs) are a class of synthetic, non-biodegradable chemicals, which were widely used all over the world before their harmful effects were realized. Polychlorobiphenyls were considered indestructible, super toxic pollutants just as heavy metals and dioxins. Although their manufacture in many countries has stopped for sometime, the PCBs continue to persist in the environment posing greater health risks.

Chlorinated organic compounds such as tetrachlorodibenzo-dioxin (TCDD) and tetrachlorodibenzo-furan (TCDF) formed during bleaching operations were reported to cause cancer in rats, but its effect on humans has been the center of much debate and is still being studied. Dibenzofuran (DBF) and dibenzodioxin (DBD) were found in deoamers and oils.

Apart from these synthetic chemicals there are various other non-degradable and degradable pollutants present in the environment such as lignin and its derivatives. They offer resistance to degradation due to the presence of carbon-to-carbon linkage of biphenyl type and other linkages in the molecule.

Microbes in bioremediation

The US army scientists for instance, stumbled on Altermonas sp., which can neutralize a whole range of biological warfare agents, nerve gas like sarin. This bacterium has an enzyme, organophosphorous anhydrase, which breaks down the nerve gas agents. Since Altermonas sp., is osmophile and alkali tolerant, scientists have suggested this organism for bioremediation applications.

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Similarly, *Ultramicrobacteria* (UMB), generally 0.3 μm in size, were isolated from deep oceans and they survive in a dormant state for more than 30 years. In this state, their adherence property is so modified that they move rapidly through porous media like layers of soil. Scientists are probing into the members of UMB population which can digest toxic wastes. They are toying with the idea of pushing such UMBs through waste dumps for cleaning up the toxic materials.

Recombinant DNA approaches have been used to construct desired bacteria to degrade the superoxic pollutant—PCBs. At the university of Minnesota, Professor Lawrence Wackett had designed such a recombinant PCB reducing bacteria. They have combined seven genes taken from different bacteria and which code for 2 types of oxygenase enzymes—the cytochrome P-450 coenzyme and toluene deoxygenase and transferred to *Pseudomonas*. The combination enabled the bacterium to dismantle pentachloroethane, a complex non-biodegradable organic chemical to CO₂, a feat never observed so far, in nature. The recent methods used in bioremediation are to put together different genes from different organisms into a single organism, so that they act in tandem to reduce the pollutant chemical to simple, harmless ones.

A site poisoned by TCE near Savannah River in South Carolina, USA was cleared using the naturally occurring Methanotroph in 1992. With this method, TCE levels were reduced to 5 ppb (parts per billion) concentration in just half the time required by the conventional remediation methods, hence bioremediation procedures are attractive.

The release of genetically engineered organisms (GMOs) in the environment could be risky. Attempts were made to include indigenous mechanisms, which keep GMOs under control. They intend to include a “death gene” which will be switched on as soon as the microbes complete the task they are designed for.

The fungi are unique among microorganisms since they secrete a variety of extracellular enzymes. The

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<td>Bioventing on site; projected cost, $0.2 million</td>
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<td>Petroleum-hydrocarbon-contaminated soil - natural gas processing plant</td>
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*BTEx*, benzene, toluene, ethyl benzene, xylene; TCE, trichloroethene.
decomposition of lignocellulose is rated as the most important degradative event in the carbon cycle of earth. Enormous literature exists on the role of fungi in the carbon and nitrogen cycles of nature. The role of fungi in the degradation of complex carbon compounds such as starch, cellulose, pectin, lignin, lignocellulose, inulin, xylan, araban etc., is well known. *Trichoderma reesei* is known to possess the complete set of enzymes required to breakdown cellulose to glucose. Degradation of lignocellulose is the characteristic of several basidiomycetous fungi.

The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them in bioremediation. The white rot fungi are unique among eukaryotes for having evolved nonspecific methods for the degradation of lignin: curiously they do not use lignin as carbon source for their growth. Lignin degradation is therefore, essentially a secondary metabolic process, not required for the main growth process. Lamar et al. compared the ability of 3 lignin-degrading fungi, *Phanerochaete chrysosporium*, *P. sordida* and *Trametes hirsute* to degrade PCP (pentachlorophenyl) and creosote in soil. Inoculation of soil with 10% (wt/wt) *Phanerochaete sordida* resulted in the significant decrease of PCP and creosote. *P. sordida* was also most useful in the degradation PAHs (polycyclic aromatic hydrocarbons) from soil. Davis et al. showed that *P. sordida* was capable of degrading the three ring PAHs efficiently. However, four ring PAHs were less efficiently degraded.

### Table 2 — Genetic engineering solutions and benefits

<table>
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<tr>
<th>Limitation</th>
<th>Genetic engineering solution</th>
<th>Benefit</th>
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<td>Incomplete degradation</td>
<td>1. Uncoupling metabolism from degradation</td>
<td>1. Support activity with inexpensive, nontoxic substrates</td>
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<td>2. Deregulate genetic controls</td>
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<td>3. Achieve difficult clean up</td>
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<tr>
<td>Degradation</td>
<td>1. Select high-performance host organism</td>
<td>1. Use smaller, less expensive bioreactors</td>
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<td>2. Remove degradative bottlenecks</td>
<td>2. Decrease fermentation treatment cost</td>
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<tr>
<td>Recalcitrant target compound</td>
<td>1. Add substitution- specific functions (e.g., dehalogenation activity)</td>
<td>1. Increase range of treatable compounds</td>
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<td></td>
<td>2. Alter enzyme specificity</td>
<td>2. Increase substrate range of single organisms</td>
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<tr>
<td>Formation of toxic intermediates</td>
<td>1. Redirect metabolites</td>
<td>1. Extend treatment life</td>
</tr>
<tr>
<td></td>
<td>2. Add complementary activities/pathways</td>
<td>2. Extend range of treatable compound</td>
</tr>
<tr>
<td>Chemical mixtures (e.g., PCBs, mixed organic wastes)</td>
<td>1. Combine metabolic activities</td>
<td>1. Decrease fermentation costs (single organism)</td>
</tr>
<tr>
<td></td>
<td>2. Broaden substrate specificity</td>
<td>2. Select environmentally robust host</td>
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**Pulp and paper mill effluents — A major threat**

The pulp and paper industry is one of the largest and most polluting industries in the world. It is the third most polluting industry in North America. The world population used over 214 million tons of paper and board products in 1987 and all estimates showed that paper consumption will increase in the foreseeable future. There are about 500 kraft mills, and many thousands of other types of pulp and paper mills in the world. The Indian pulp and paper industry presently has an installed capacity of about 3.0 million tons per annum. Primary concerns include the use of chlorine-based bleaches and resultant toxic emissions to air, water and soil with global annual growth forecast at 2-5%. The industry and its negative impacts could double by 2025.

**Effects of pulp pollution**

**Water pollution**

Pulp mill effluents can seriously harm habitat near mills, reduce water levels necessary for fish and alter water temperature, a critical environmental factor for fish. Mill wastes continue to wreak havoc on surrounding ecosystem. Lignin and its derivatives impart an offensive colour to the water, which is not only aesthetically unacceptable, but also inhibit the natural process of photosynthesis in the stream due to absorbance of sunlight. This leads to a chain of adverse effects on the aquatic ecosystem as the growth of primary consumers as well as secondary and tertiary consumers is adversely affected.
Discharge of untreated or partially treated wastewaters from pulp and paper mills results in the colour persisting in the receiving body for a long distance. Recent studies have also indicated that lignin and its derivatives are toxic. A study by Roald\textsuperscript{12} showed that the growth rate of young rainbow trout was affected when exposed to a concentration of >160 mg/l ligno-sulphonate. Nazar and Rapson\textsuperscript{13} in an assay of the mutagenicity of kraft pulp bleaching plants found that the component of pulp is mainly responsible for the mutagenicity produced by chlorination of lignin.

Studies on the impact of pulp and paper mill effluents on the river Kallada in Kerala (India) also indicated the harmful effects of kraft pulp bleaching effluents on the fishes, copepods and other aquatic forms\textsuperscript{14}.

The major toxic compounds identified so far in chlorinated stage wastewaters in pulp mills are mixtures of chlorinated lignins. Das\textsuperscript{15} isolated tetrachloro-o-benzoquinone, a compound of low toxicity in wastewaters. Other toxic compounds present in caustic extraction stage wastewaters from a pulp mill are trichlororuguaicol, tetrachlororuguaicol, mono- and dichlorodihydroabietic acid, epoxystearic and dichloro stearic acid\textsuperscript{16}. It becomes necessary to remove colour due to lignin before they can be accepted into surface waters. Each Canadian mill produces an average of 40 oven-dry tonnes of sludge per day, which is de-watered and then either land filled or burned. Because of this disposal, sludge pollutes soil, air and water.

Air pollution

Air pollution from pulp mills is not well studied. Mills are monitored for a range of air emissions, such as particulate matter, CO\textsubscript{2}, SO\textsubscript{2}, H\textsubscript{2}S, volatile organic compounds, chlorine, chloroform and chlorine dioxide. Incomplete data from British Columbia’s Environment Ministry indicates that in 1997, mills in the Canadian province emitted 17000 tonnes of particulates and 2.7 million tonnes of CO\textsubscript{2}, plus other unreported emissions.

Air discharges from pulp mills contain hormone disrupting and carcinogenic chemicals such as chlorinated phenols, polycyclic aromatic hydrocarbons (PAHs) and VOCs. British Columbia’s coastal pulp mills are the largest provincial source of airborne dioxins and furans, which are among the most toxic substances known.

Biological decolourization

The problem of colour removal from pulp and paper mill wastewater has been a subject of study in the last few decades. The colour in these wastewaters is mainly due to lignin and lignin derivatives. Waste waters containing huge quantities of lignin from pulp and paper mill results mainly from processes such as pulping, bleaching and chemical recovery sections.

Several methods have been attempted for the removal of colour from pulp and paper mill effluents. Physical and chemical processes are quite expensive and removes high molecular weight chlorinated lignins, colour, toxicity, suspended solids and chemical oxygen demand but biological oxygen demand (BOD) and low molecular weight compounds are not removed efficiently. The biological colour removal process is particularly attractive since in addition to colour and chemical oxygen demand (COD) it also reduces BOD and low molecular weight chlorolignins.

Biological decolourisation methods use several classes of microorganisms—bacteria, algae and fungi to degrade the polymeric lignin derived chromophoric material. Among these, wood degrading white rot fungi have been shown to efficiently and completely degrade and metabolize lignin, resulting in rapid decolourization of the effluents\textsuperscript{17-20}.

Decolourization with bacteria

Bacterial cultures of Pseudomonas aeruginosa are capable of reducing Kraft mill effluent colour by 26-54\% or more under aerobic conditions\textsuperscript{20}. Bourbonnais and Paice\textsuperscript{21} tested Bacillus cereus and 2 strains of P. aeruginosa for decolourization of bleach Kraft effluent. Colour was primarily removed by adsorption. Kawakami\textsuperscript{22} found that P. ovalis degraded alkali lignin more readily than Kraft lignin sulphonate. A mixed population of bacteria and protozoa derived from lake bottom sediment near the effluent Kraft paper mill was shown to degrade lignin sulphonate source. Although numerous bacteria can decompose monomeric lignin substructure models, only a few strains are able to attack lignin derivatives obtained from different pulping processes.

The extracellular xylanase from Bacillus stearothermophilus T-6 is a thermostable alkali tolerant enzyme that bleaches pulp optimally at pH-9 and 65°C and was successfully used in a large-scale bio-bleaching. Streptomyces badius and S. viridosporous were able to use a commercial Kraft lignin as sole carbon source. The acid precipitable polymeric lignin derived from this degradation was characterized by Fourier transformed infra-red spectroscopy, amino acid analysis, elemental analysis.
for C, H, N and high performance size exclusion chromatography. Two bacterial strains Pseudomonas putida and Acinetobacter calcoaceticus were studied for degradation of black liquor from a Kraft pulp and paper mill in a continuous reactor. They were able to remove 70-80% of COD and lignin while the colour removal efficiency was around 85% in 8 days. The degradation of dissolved and colloidal substances from thermo-mechanical pulp (TMP) by bacteria isolated from a paper mill was studied. Burkholderia cepacia strains hydrolysed triglycerides to free fatty acids and the liberated unsaturated fatty acids were then degraded to some extent. Saturated fatty acids were not degraded. However, the branched antioleaptenoic fatty acid was degraded almost like the unsaturated fatty acids. About 30% of the steryl esters were degraded during 11 days, increasing the concentration of free sterols. Approximately 25% of the dehydroabietic and 45% of the abietic and isopimaric resin acids were degraded during 11 days. The degree of unsaturation seemed to be of greater importance for the degradation of fatty acids.

The effect of pH, nutrient and aeration on the removal of colour and reduction of BOD, COD and heavy metals, nitrogen and phosphorous was studied. Active microbial consortia effectively degraded recalcitrant compounds. The isolated bacteria - Pseudomonas putida (S1), Citrobacter sp. (S4) and Enterobacter sp. (S5), not only decolorized effluent upto 97% but reduced BOD, COD, phenolics and sulphide upto 96.63, 96.80, 96.92 and 96.67% respectively within 24 hrs of growth and the heavy metals were removed upto 82-99.80%. The TSS and TDS were sharply reduced due to degradation.

A comparison of organochlorine removal from bleached kraft pulp and paper mill effluents by dehalogenating Pseudomonas, Ancylobacter and Methylobacterium strains were assessed. These bacteria were tested for growth on chlorinated acetic acids and alcohols for adsorbable organic halogen (AOX) reduction in batch cultures of sterile bleached kraft-mill effluents (BKME) from three sources. A. aquaties (A7) exhibited the broadest substrate range, but could only effect significant AOX reduction in softwood effluents. Methylobacterium CP13 exhibited a limited substrate range, but was capable of removing significant amounts of AOX from both hardwood and softwood effluents. By contrast, Pseudomonas sp.P1 exhibited a limited substrate range and poor to negligible reduction in AOX levels from both effluent types. Mixed inocula of all three species combined and inocula of sludge from mill treatment systems removed as much AOX from softwood effluents as did pure populations of Methylobacterium CP13. When BKME was hydrolysed prior to AOX analysis, the subsequent estimates of recalcitrant or non-hydrolysable, AOX levels were far less variable than their counterpart total AOX measures. It is suggested that this is a relevant and useful measure of AOX for biodegradation studies.

Resin acids, a group of diterpenoid carboxylic acids present mainly in softwood species are present in many pulp mill effluents and are toxic to fish in receptant waters. They are considered to be readily biodegradable. However, their removal across biological treatment systems has been shown to vary. Recent studies indicated that natural resin acids and transformation products might accumulate in sediments and pose acute and chronic toxicity to fish. Several resin acid biotransformation compounds have also been shown to bioaccumulate and to be more resistant to biodegradation than the original material. Although wood inhabiting fungi have been shown to decrease the level of resin present in wood, there is no conclusive evidence that fungi can completely degrade these compounds. In contrast, a number of bacterial isolates have recently been described which are able to utilize dehydroabiestic or isopimaric acids as their sole carbon source.

An alkalophilic strain of Bacillus SAM3, producing high levels of cellulase-free xylanase active and stable at alkaline pH, was isolated from a soda lake. The enzyme was tested as a means of bleaching sugarcane bagasse pulp from a paper mill where the bagasse was subjected to hot alkali cooking and washing with water to neutrality. Enzymatic treatment for 2 hr at 60°C and pH 8 with an enzyme dose of 1.2 IU/g pulp led to a decrease of 4 units in the kappa number. Similar treatment of the pulp at pH 7 and pH 9 indicated that the SAM-3 xylanase was effective at lowering the kappa number of the pulp over a wide pH range.

The optimal conditions for the decolorization of a paper mill effluent by several strains of Streptomycetes, were investigated. Strains able to decolorize this effluent were identified from 50 test strains isolated from lignocellulosic substrates. The decolorized effluent was also partially characterized. Fractionation of the decolorized effluent by gel permeation chromatography suggested that the fractions of high,
Decolourization with algae

It has been reported that some algae can decolourize diluted bleach Kraft mill effluents. It was found that pure and mixed algal cultures removed up to 70% of colour within 2 months of incubation. All cultures exhibited a similar colour reduction pattern consisting of a phase with declining rate. Colour removal was most effective during the first 15 to 20 days of incubation, then gradually declined. Complete removal of colour did not occur. Colour removal by algae is caused by metabolic transformation of coloured molecules to non-coloured molecules with limited assimilation or degradation of molecular entities. Adsorption is not a major colour removal mechanism.

Decolourization with fungi

The wood-degrading white-rot fungi are capable of degrading lignin efficiently. Schizophyllum commune, Tinteporia borbonica, Phanerochaete chrysosporium and Trametes versicolor have been found to degrade lignin and metabolize it along with carbohydrates. Aspergillus niger and Trichoderma sp., one of the fungi imperfecti, are also capable of degrading lignin and decolourizing effluent of hardwood pulp bleaching.

Belzare and Prasad reported that the effluent from bagasse based pulp and paper mills could be decolourized with the white rot fungus S. commune. However, this fungus could not degrade lignin unless a more easily metabolizable carbon source was made available simultaneously. The addition of carbon and nitrogen not only improved the decolourizing efficiency of the fungus but also resulted in a reduction of BOD and COD of the effluent. Sucrose was found to be the best co-substrate for the breakdown of lignin. A 2 day incubation period was sufficient for lignin degradation by S. commune. The efficiency of treatment of effluent with this fungus was highest at pH 4-5 and was further improved by intermittent aeration. Under optimum conditions, S. commune removed the colour of effluent by 90% and also reduced BOD and COD by 70 and 72% respectively in 2 days incubation.

A white, rot fungus, Tinteporia borbonica, has been reported to decolourize the Kraft waste liquor to a light yellow colour. About 99% colour reduction was achieved after 4 days of cultivation. Measurement of the culture filtrate by ultraviolet-spectroscopy showed that the chlorine-oxy lignin content also decreased with time and measurement of the culture filtrate plus mycelial extract after 14 days cultivation showed the total removal of chlorine-oxy lignin content.

Addition of carbon and nitrogen source was found to improve decolourization of pulp and paper mill waste water by A. niger, leaving 19% of the original colour and reduced about 43% BOD and 41% COD after 2 days of incubation.

Prasad and Joyce used Trichoderma sp. to decolourize the hardwood effluent. Glucose was found to be the most effective carbohydrate utilized by the fungus as it stimulated substantial colour reduction without any increase in COD. Addition of nitrogen did not stimulate the decolourization process indicating that it is not a rate-limiting factor. The optimum pH for decolourization and growth was 4.0. Under optimal conditions, total colour and COD decreased by almost 85 and 25% respectively, after cultivation for 3 days.

Gliocladium viride, a saprophytic soil fungus has been employed for the bioremediation of paper and
pulp mill effluents. It grew efficiently in the effluents and decolourized them by 42% and also decreased the level of lignin (32%), cellulose (75%) and BOD (65%) in the effluents.

*Phaeoacreatus chrysosphorium* has the ability to degrade lignin and lignin derivatives efficiently.[9,20,38,42] Lab scale studies with *P. chrysosphorium* to paper mill effluent supplemented with glucose and asparagines or urea, resulted in a significant reduction in colour in 5-6 days.[37]

The optimum conditions favouring fungal growth are quite different from those favouring decolourization. The pH range for optimum growth was 4.3-4.8 and decolourization is greatly retarded below pH 4 or above 5 because of poor growth.[19] However, if the fungus was grown at an optimum pH, decolourization occurs, even at a pH as low as 3. In the pH range of 5-7, the situation is less clear since fungal decolourization results in the formation of acids, which lowers the pH rapidly.[40] Thus it appears that decolourization is less sensitive to pH decrease than fungal growth. The optimum temperature for the growth of fungus was 40°C, whereas decolourization is not limited to the same narrow range of temperature but takes place with a little decrease in rate at temperature as low as 25°C.[19] The fungal decolourization requires oxygen and a co-substrate but the addition of nitrogen source is not necessary.

Another white rot fungus, *Coriolus versicolor*, has shown good performance. It produces an extracellular laccase, which plays a role in lignin biodegradation. It was found that production of extracellular enzyme, such as laccase, did not follow any specific profile. No correlation could be developed between laccase production and the rate of decolourization. Consequently, laccase production could not be considered as a safe indicator of lignolytic activity in these experiments.[15]

It requires a growth substrate such as cellulose or glucose for the decomposition of lignin.[44] The culture conditions favouring lignin degradation are similar to those favouring fungal decolourization. Livernoche *et al.*[44] showed that *C. versicolor* in liquid culture removed over 60% of the colour of the combined bleach kraft effluent within 6 days in the presence of sucrose. Decolourization of effluents was more efficient when the concentration of sucrose and inoculum was high.

Biological reactors of the airlift type using calcium alginate beads with immobilized fungus *C. versicolor* have been used to study the continuous decolourization of the kraft mill effluents.[5] Royer *et al.*[46] described the use of the pellets of *C. versicolor* to decolourize ultrafiltered kraft liquor in non-sterile condition with a negligible loss of activity.

Lignin degradation by basidiomycetes fungi has been studied by Abbott and Wicklow.[47] The gasteromycete *Cyathus stercoreus*, which is associated with litter decomposition, degrades lignin as efficiently as any other white rot fungi.

Bajpai *et al.*[38] used pellets of *Trametes versicolor* strain B7 for decolourization of E1 effluent. The mycelial pellets oxidized the chromophores of the effluent in presence of either of the carbohydrates, sucrose, glucose, starch, ethanol, carboxy methyl cellulose, microcrystalline cellulose, pulp and malt extract. The highest decolourization was obtained in the case of glucose. Optimum pH and temperature was 4.5-5.5 and 30°C respectively.

Decolourization of papermill effluent was studied *in vitro*, using glucose as a cosubstrate and urea as a nitrogen source. Sterilization of substrate and adjusting the pH to lower values were unnecessary for the sedimentation of the chromophoric compounds. A maximum decolourization (34%) by *T. versicolor* was
observed on third day in the effluent supplemented with 1.0% (w/v) glucose cosubstrate and 0.2% (w/v) urea. Further, colour reduction (24%) was observed in the effluent supplemented with glucose. It is concluded for effluent decolourization by *T. versicolor*, the effluent should be supplemented with glucose as a cosubstrate and urea as the nitrogen source. Decolourization of the effluent occurred rapidly due to the growth of *Gliocladium viride* except in the effluents amended with peptone. Effluents supplemented with carbon (glucose, sucrose) sources showed quicker and efficient colour reduction, when compared to that of unamended controls.

The ability of 3 marine fungi (*Sordaria fimicola*, *Halosphaeria ratnagiriensis* and an unknown *basidiomycetes*) to produce the lignin modifying enzymes; laccase, manganese peroxidase (MNP) and lignin peroxidase (LiP) and to mineralize 14 C ring labelled synthetic lignin was demonstrated. The ability of these marine fungi to decolorize paper mill bleach kraft effluent was also demonstrated for the first time.

Experiments were conducted to study the ability of *Aspergillus foetidus* to remove colour, decrease COD (chemical oxygen demand) and metabolize lignin from dissolved bagasse-based pulp and raw black and alkali-stage liquors in nutrient medium. Approximately 90-95% of the total colour was removed from growth media containing lignin at 0.05 or 0.1% or diluted black liquor or alkali liquor. Decolorization and lignolytic and COD removal processes occurred principally during the exponential growth phase of the fungus, with concomitant utilization of the primary growth-supporting substrate. Strong correlations existed between the timing of decolorization and lignolytic processes.

The degradation of cellulose by *Pleurotus sajorcaju* was rapid at the initial stages of growth. The activities of endoglucanase, exoglucanase and beta-glucosidase were maximum at 8, 12 and 26 d of growth, respectively. The activities of lignin-degrading enzymes were maximum at the later stages of growth. Such a delignification process is considered to have potential applications in the conversion of paper-mill sludge into food, animal feed and fibre products.

**Decolourisation with enzymes**

Some enzymes also seem to have the potential to remove colour from pulp and paper mill effluents. Ligninase, cellulase, peroxidase etc., are the most important enzymes, especially peroxidase, which is used for colour removal in bleaching effluents. It is also possible to mix enzymes together with special microbes, which normally do not have high enzyme activity. White rot fungi uses glucose as substrate and produce peroxidase, an extracellular enzymes. It seems that this enzyme oxidizes the chromophores and removes the colour from bleaching wastewater. The colour removal from effluents at neutral pH by low levels of H$_2$O$_2$ was enhanced by the addition of peroxidase.

**Conclusion**

The comparison of decolorization by different organisms show that white rot fungi particularly *P. chrysosporium* and *C. versicolor* are suitable for efficient degradation of the recalcitrant chromophoric material in bleaching plant effluents. However, the requirements for high oxygen tension and a growth substrate constrain the practical implementation of fungal decolorization. Further research is needed to develop fast biodegradation processes, which are likely to provide an economically feasible colour removal process.

**Future outlook**

It may be possible to clone genes for the efficient degradative enzyme into bacteria, which could be further transferred to suitable fungi. The high surface-to-cell ratio of filamentous fungi makes them better degraders under certain niches like contaminated soils. Fungi have been shown to even solubilize partially coal, a highly polymeric substance more complex than lignin. There is no doubt that fungi can be harnessed more in environmental bioremediation work in future.

**References**


16 Leach J M & Thakore A N, Isolation and identification of constituents toxic to juvenile rainbow trout on caustic extraction effluent, J Fish Res Bd Can., 32, 1249.


