Novel Biological approaches for the removal of Chlorophenolics [AOX] from Bleach plant effluent

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The bleach effluent from a pulp and paper industry has always been a matter of concern with respect to the environment and human health. Two most important attributes of the bleach effluent are the presence of organo-chlorides (AOX) and the dark color. Organo-chlorides are characterized by poor aqueous solubility, high lipophilicity, recalcitrance and high toxicity due to which they tend to bio-accumulate and thus bio-magnify in biological systems thereby posing a threat. Currently available treatment options include physical, chemical and electro-chemical treatments, that are either inefficient in treating such effluents to meet the ever increasing stringent discharge standards or economically not viable. Biological treatment is gaining importance due to low cost and high efficiency. This paper gives an overview of the pulp and paper manufacturing process in brief, the pollution and environmental impacts, various treatment options, with emphasis on biological treatment especially fungal techniques, for treating AOX containing bleach plant effluents and the regulatory discharge norms for the same.

Key Words: Bleach effluent, Pulp and Paper Industry, Organo-chlorides, AOX, Fungi

Introduction

The environment has become a strong concern to mankind globally. A diverse range of various anthropogenic activities have cumulatively led to the accumulation of pollutants in all the environmental compartments of the biosphere – air, water, soil, flora and fauna thus jeopardizing biodiversity and human health. Various industrial sectors meet the demands of the population by providing state of the art products and services and eventually to contribute to the national economy; they also jeopardize the environment by discharging pollutants beyond the assimilative capacities of the receptors. The Ministry of Environment and Forests has rated the pulp and paper industry as one of the twenty most polluting industries.

Wood is the conventional fibrous raw material for making paper pulp. With limited forest wood resources, other non-wood lignocellulosic fibrous resources like bamboo, bagasse and straws [rice, wheat] have been successfully used by the Indian paper industry. The chemical composition of these fibrous materials consists of cellulose, lignin and hemi-cellulose in varying proportions. Cellulose is the main fibrous component of the pulp and the other constituents like lignin and hemi-cellulose are separated and removed during the pulping and bleaching processes to produce different grades of paper.

Lignin is a three-dimensional network polymer based on phenylpropanoid monomers. It is inherently recalcitrant and biorefractory in nature and it is not readily degraded in nature. Delignification is achieved in commercial pulping processes through oxidative breakdown of the macromolecular structure of lignin. Lignin must be liberated to free the constituent cellulosic fibers by cleavage of the lignin – carbohydrate bonds, fragmentation and dissolution in the acidic / alkaline medium used for chemical pulping processes.

Degradation of recalcitrant lignin is an oxidative and nonspecific process [1, 2]. There are three commercial methods of pulping viz. the Kraft, the sulphite and semi-chemical pulping processes. The Kraft process is the dominating process because it readily accepts hardwood as well as softwood of any grade and species. Kraft pulping is performed in an aqueous medium composed of sodium hydroxide and sodium sulfide [white liquor] in a digester [170^{o}C, 10 atm] and up to 90 % of the lignin is removed from the chips.

The chemical pulping processes degrade nearly one – half of the digester furnish to give an average unbleached pulp yield of 50% and consisting mainly of cellulosic fibers with residual portions of lignin and hemi-celluloses. Residual lignin is removed by chlorination [C-Stage] and the chlorolignin derivatives are solubilized in alkali by caustic extraction [E – Stage] during bleaching. The dark brown unbleached pulp is subjected to multi-stage bleaching to produce pulp of the desired brightness. From environmental considerations, the major wastewater streams originate from the pulping [black liquor stream] and the bleaching stages. Dark colour, high alkalinity and high BOD, COD levels usually characterize the black liquor stream. The latter is amenable for treatment by anaerobic digestion and recovers potential energy as biogas in mini paper mills [capacity below 50 TPD]. The bleach plant effluents are considered to be highly toxic and polluting; contributing to 80 – 100 Kgs of colour imparting substances and 2 to 4 Kgs of organochlorine compounds per ton of paper in the bleach plant effluent [3, 4]. Chlorinated lignin derivatives are referred to as adsorbable organic halogens [AOX], which include chlorinated polyphenolics and dioxins that are highly toxic, recalcitrant and tend to bioaccumulate in biological systems due to their lipophilicity. Dioxins are well known for their high toxicity and carcinogenicity [5]. The pulp and paper industry generates about 80 - 150 m^3 of wastewater per ton of paper produced. Mills

have to install efficient effluent treatment systems to adequately treat the effluent before discharge into the water body for regulatory compliance.

Treatment methodologies currently employed include physical, chemical and biological treatment. Biological treatment is attractive in that it can deal with large volumes of wastewater and results in the degradation of organic materials to gases and biomass. Combinations of chemical and biological treatment methodologies would also lead to desired results. To gain an insight into various treatment methodologies it is essential to have an in-depth understanding of the sequences involved in the manufacturing process to identify potential sources of wastewater generation and its characteristics.

Pulp and paper manufacturing process:

Figure – 1 illustrates the major steps of wood preparation, pulping, bleaching and papermaking in a wood based integrated paper mill. Wood Preparation becomes simpler while using non-wood, like bamboo, bagasse or straws. The pulping step involves batch / continuous digestion process, in which the lignin is degraded and solubilized in the alkaline medium at high temperature and pressure. The residual pulp mass is subjected to multi-stage brown stock washing for the separation of unbleached pulp and the spent pulping solution as black liquor. The dark brown pulp is then brightened in the bleach plant by removing the lignin selectively without degrading the pulp cellulose fraction to retain the strength of the pulp. The bleached pulp is then sent to stock preparation and the paper machine for making paper. White water separated by drainage from the paper machine is recycled for mill use after recovering fine fibers.

Pulp Bleaching

Figure – 2 illustrates a generic schematic of the sequence of stages involved in pulp bleaching employing bleach chemicals such as Cl_2, ClO_2, OCl, NaOH and H_2O_2. The structure of the residual lignin in kraft pulps is significantly different from the structure of the lignin in wood the structure of dissolved kraft lignin [6].

Conventional bleaching is based on chlorine species like Cl_2 and OCl as bleaching agents. The advantage of using chlorine is low cost and effectiveness. Chlorination is the first stage of bleaching where the unbleached pulp is treated with elemental chlorine at low temperature and pH [0.5 – 1.5]. This first stage is known as the C – Stage. The chlorine reacts with the lignin by substitution of H – atoms with the Cl atoms, oxidation of lignin fragments to carboxylic acid groups, and to a small extent addition of Cl_2 across carbon – carbon double bonds. Only a small amount of lignin is removed in this stage. During chlorination, residual lignin is structurally modified, degraded, and chlorinated [3, 7]. Some of these chlorinated compounds (mostly low-molecular-weight material) are dissolved into the spent chlorination liquor. This stage is followed by an alkaline [NaOH] extraction stage [E – Stage] operating at 50 – 70°C

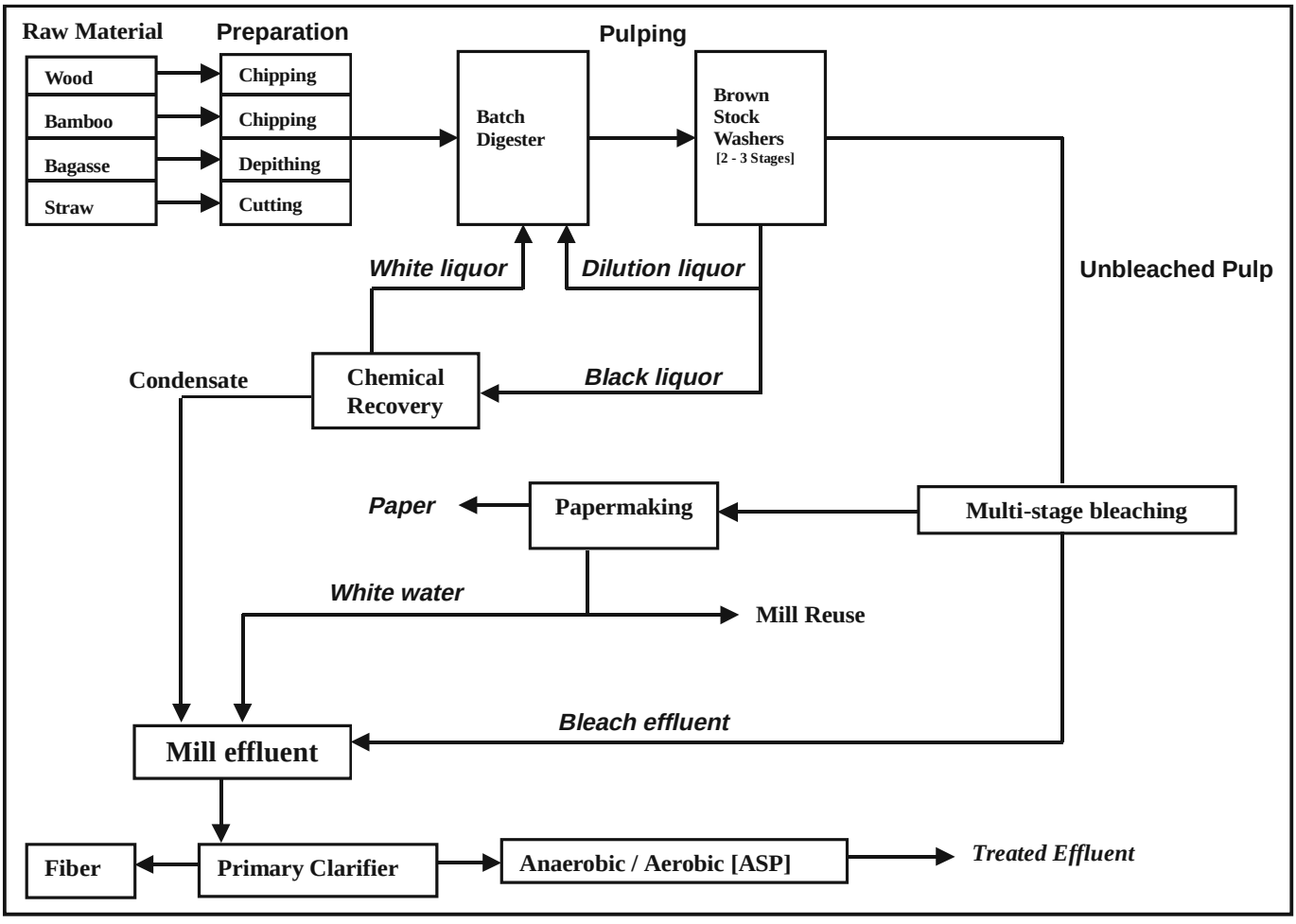


Figure 1: Sources of effluent generation during Pulp and Paper manufacturing

and pH [10 – 12]. In the extraction stage, chlorinated, oxidized lignins are solubilized thereby eliminating up to 90% of the organically bound chlorine in the pulp [8]. Subsequent bleaching with hypochlorite solution [NaClO, $Ca(OCl)_2$] in the H – Stage, is carried out at 35 - 45^{O}C for 1 – 2 hours at a pH of 10. Hypochlorite is more selective than elemental chlorine and extracts lignin as it gets depolymerized. The final bleaching is achieved by using relatively expensive oxidizing chemicals - chlorine dioxide [D – Stage] and hydrogen peroxide [P – Stage]. The D and P stages are very effective in oxidizing small portions of residual lignin and impart the desired brightness for the bleached pulp. The pollution loads from these latter stages are relatively small.

A typical bleach sequence in an Indian paper mill is represented by CEHP or CEHDP (C – Chlorination, E – Alkali Extraction, H – Hypochlorite, D – Chlorine dioxide, P – Hydrogen peroxide) and their variations [3].

Bleach plant effluents

Bleach effluents are dark coloured and contain compounds that are toxic. They contain chlorinated and non-chlorinated derivatives of lignin and wood extractive compounds. During chlorination, the lignin macromolecule is structurally modified, degraded, and chlorinated. These are solubilized in alkali in the E – Stage. As a result, the effluent from the bleaching process is dark brown in colour due to the presence of chromophoric polymeric lignin derivatives. The amount of chlorinated organics produced during the pulp bleaching varies with wood species, kappa number of the pulp, bleaching sequence and the conditions employed [3, 8]. The effluent from the bleaching of softwood kraft pulp by the conventional sequence consists of color - 150 – 200 PCU, BOD_5, 8 – 17 mg /liter, COD - 50 – 70 mg / liter and TOCl [AOX] - 3 – 5 Kgs / ton of pulp bleached respectively. The pollution loads are generally lower from non-wood and hardwood kraft bleach plant as compared to softwood kraft bleach [3].

Most of the chlorine used in the bleach plant; [up to 90 %] forms NaCl and the balance 10 % will be bound to the organic material removed from the pulp. The organic bound chlorine is measured as Adsorbable Organic Halide [AOX] consisting of over 500 different chlorinated compounds.

Even though a considerable reduction in BOD, COD, and TSS of the bleach effluent may be achieved, using conventional treatment procedures no effect on color is observed because color-causing materials are not easily amenable to biological degradation. Most mills are reluctant to recycle bleach plant effluent to the chemical recovery system due to the corrosive nature of chloride ion and the substantial dilution of the black liquor.

A schematic classification of the chlorinated organics, present in the spent liquors from conventionally pulped and bleached softwood kraft pulp is depicted in Figure 3.

Environmental impacts

Chlorine chemicals are selective and efficient in oxidizing double bonds in organic matter, such as lignin. When chlorine is used for pulp bleaching, 6 to 10% of the chlorine is converted into organically bound form and measured as Adsorbable organic halide [AOX], consists of over 500 different chlorinated compounds, such as chlorinated lignin and small molecular size components, like chlorophenols chlorinated catechols, guaiacols, chlorate, resin acids, furans, dioxins, syringols, vanillins, etc [5].

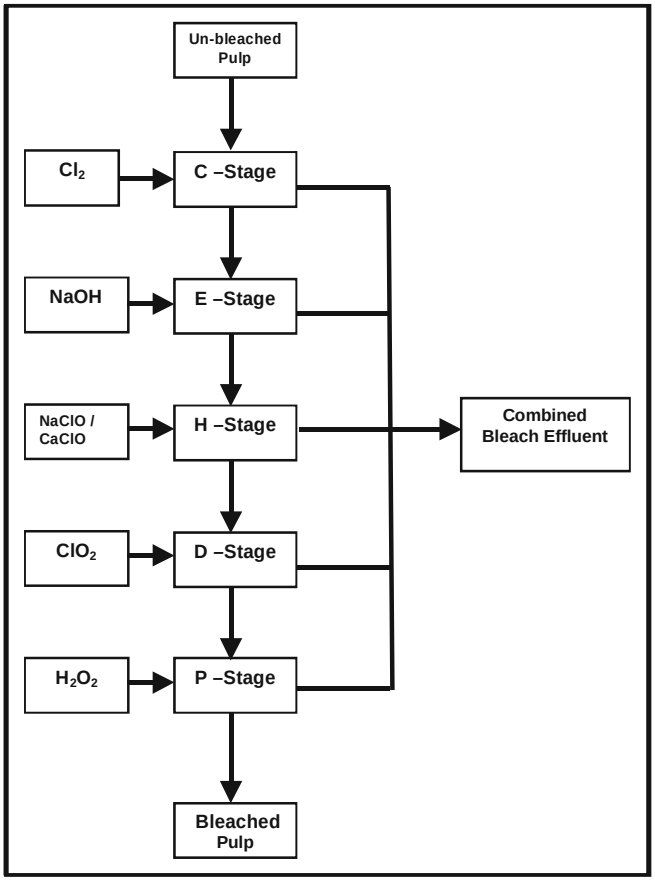


Figure 2 : Multi-stage Pulp bleaching sequence [1, 8]

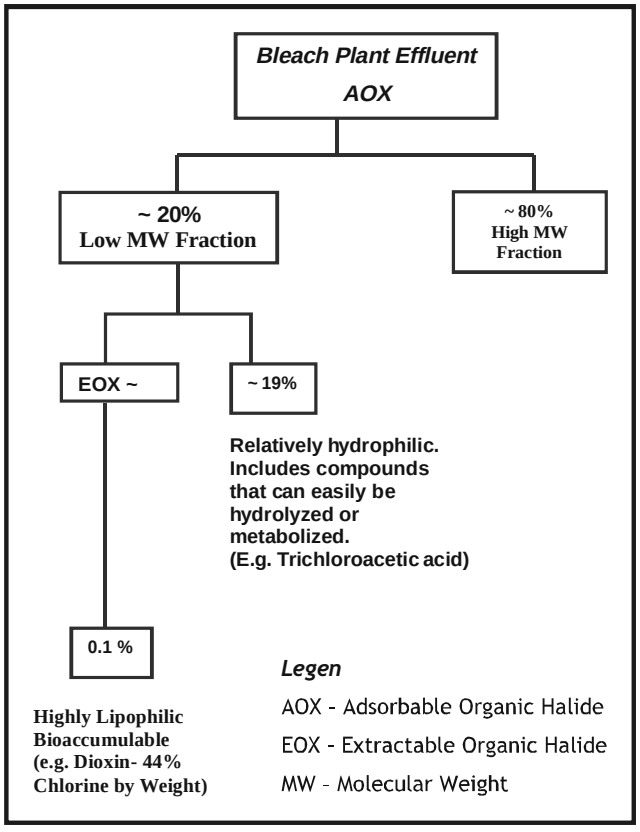


Figure 3: Classification of Major Organochlorine [AOX] compounds in bleach plant effluent [9]

Such compounds are believed to be highly toxic and harmful to the environment being bioaccumulative and carcinogenic. These compounds are known to be very toxic to aquatic organisms [3].

The dark color of pulp mill effluents leads to significant to water pollution problems, especially where the quality of the receiving stream has a low flow. The dark color not only is aesthetically unacceptable but also inhibits the natural process of photosynthesis in streams due to absorbance of sunlight. This leads to chains of adverse effects on the aquatic ecosystem, as the growth of primary consumers as well as secondary and tertiary consumers are adversely affected. Discharge of untreated or partially treated wastewaters from pulp and paper mills results in persistence of color in the receiving body over a long distance.

Chlorinated organics generated during pulp bleaching not only exert an oxygen demand ([BOD] and chemical oxygen demand [COD]) but also contribute to the color and toxicity (acute and chronic) of the effluent. Chlorinated organics in spent bleaching liquor are also responsible for the mutagenicity of the effluent. The low molecular- weight fraction of the chlorolignins will also contribute to the effluent BOD [indicating its partial biodegradability] and acute toxicity. The high molecular- weight chlorinated compounds being recalcitrant in nature contribute significantly to effluent COD, color and chronic toxicity [7].

Under natural conditions, these compounds are degraded slowly to various chlorinated phenolics, which may be methylated under aerobic conditions. The extraction-stage effluents can contribute to about 75% of the dissolved organic material, 60% of the COD load, 40 to 50% of the organically bound chlorines, and 80% of the color-imparting substances of bleach plant effluents.

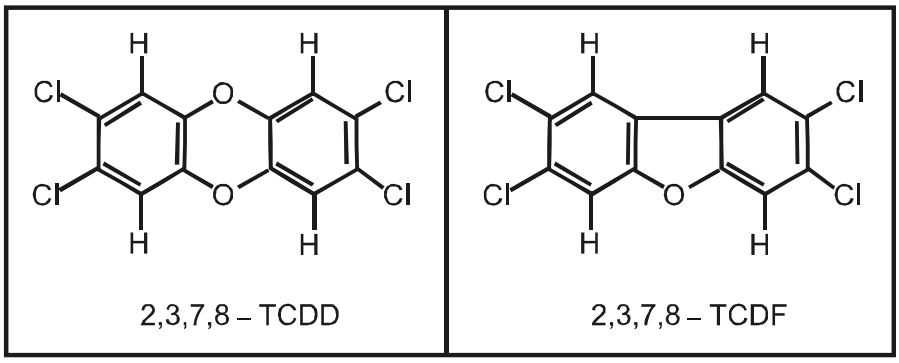
Any treatment method that can degrade, dechlorinate, and decolorize extraction-stage effluent can tackle most of the environmental problems associated with bleach plant effluents. Among the promising biological methods, techniques using wood-degrading white rot fungi have been reported to have the potential to successfully treat these effluents. The enzyme system of the white rot fungi includes a group of nonspecific extracellular enzymes, which catalyze not only degradation of lignin and chlorolignins but also oxidation of several persistent aromatic and halogenated organic compounds like lindane, DDT, PCP, benzopyrene, creosote, coal tars, and wastes such as olive-mill waste, etc [3, 10, 11, 12, 13, 14].

The major limitation associated with use of fungi for decolorization of bleach plant effluents has been the requirement for an easily metabolizable cosubstrate like glucose for the growth and development of ligninolytic activity. The use of co-substrate would lead to increased cost of treatment and the process also will require frequent replenishment of the fungal biomass [3, 7].

Environmental regulations

Recent regulatory requirements include limits on total organochlorine (TOCl) or adsorbable organic halides (AOX), in addition to the existing conventional parameters like BOD, COD, TDS, pH, SS, etc.

In an international perspective, the issue of AOX is not new. In some developed nations, limits have also been set on individual chlorinated organic compounds of bleach effluents. The Canadian Environmental Protection Act prohibits the discharge of final effluents that contains any measurable concentrations of 2,3,7,8 – Tetra-chlorodibenzodioxin [2,3,7,8 – TCDD] [>15 ppq] and 2,3,7,8 – Tetrachlorodibenzofuran [2,3,7,8 – TCDF] [>50 ppq] [3].



According to the Paris Commission [PARCOM], twelve European countries ratified the Paris Convention for Prevention of Marine Pollution for Land Based Sources and Rivers. The convention laid down standards and guidelines with regard to AOX discharge and set a discharge norm of 1 kg / ton of for bleached chemical pulp in 1995. The discharge limits were then lowered down gradually to 0.3 – 0.5 Kg / ton. All Kraft mills in Japan operate with an AOX discharge limit of less than 1.5 Kg / ton of pulp [5, 8]. Germany has banned the production of chlorine based pulping and also banned the consumption of pulps other than total chlorine free [TCF] pulps [8].

The pulp mills in Scandinavia and North America have stopped using chlorine bleaching so that they can meet government regulations on adsorbable organic halide (AOX) discharge. The USA proposed a cluster rule in 1992 to restrict the discharge limit at 2.5 – 5.0 ppb for all polychlorinated phenolics [5, 8].

However, in the Indian context, the awareness and concern regarding AOX and its control has grown only recently. Most of the pulp and paper industries in the developed nations have switched over to new fiber line [involving modern technologies in pulping, bleaching, washing, etc.], as a result of which they have reduced the AOX discharge below the toxicity level and are no longer a major issue. However, due to techno-economical reasons the industries in India continue using elemental chlorine for bleaching in most cases [9]. With growing public awareness, preference for eco-friendly products in the national and international market and stricter environmental legislations, the pulp and paper industries have been forced to develop / adopt suitable strategies to reduce the AOX levels. Further, the "Charter on Corporate Responsibility for Environmental Protection [CREP]" introduced recently by the Central Pollution Control Board [CPCB] has forced industries to reduce the AOX levels within a specified time frame [15]. Achieving the AOX discharge standards is a challenge as the level of AOX discharged in the Indian paper industry varies between 1.0 – 2.5 Kgs / ton of paper produced in large mills and 4.0 – 6.0 Kgs / ton of paper produced in small agro based mills. As indicated in the charter [Table 1], the large pulp and paper mills have to achieve the AOX discharge level of 1.5 kg / ton of paper produced within two years and 1.0 kg / ton of paper produced within the next five years. Small mills have to either install chemical recovery systems or shift to waste paper recycling [15].

Table 1: Charter on Corporate Responsibility for Environmental Protection - Guidelines for Pulp and Paper Industry [15, 16]

A. Large Pulp and Paper	
Directive	**Implementation Schedule**
Discharge of AOX kg/ton of paper	AOX 1.5 kg/ton of paper within 2 years AOX 1.0 kg / ton of paper in 5 years
Installation of lime kiln	Within 4 years
Wastewater discharge cum / ton of paper	Less than 140 cum/ton of paper within 2 years Less than 120 cum / ton in 4years for units installed before 1992 Less than 100 m3 / ton of paper per units installed after 1992.
Odour control by burning the reduced sulfur emissions in the boiler/lime-kiln	Installation of odor control system within 4 years.
Utilization of treated effluent for irrigation	Utilization of treated effluent for irrigation wherever possible
Colour removal from the effluent	Indian Paper Manufacturers Association to take up project with Central Pulp & Paper Research Institute
B. Small Pulp and Paper	
Compliance of standard of BOD, COD & AOX	Recovery of chemicals by installation of Chemical recovery plant or utilization of black liquor with no discharge from pulp mill within 3 years OR Shift to waste paper
Upgradation of ETPs so as to meet discharge standards	ETPs to be upgraded within 1 year so as to meet discharge standards.
Waste water discharge/ ton of paper	Less than 150 cum/ton of paper within 3 years
Utilization of treated effluent for irrigation	Utilization of treated effluent for irrigation wherever possible
Colour removal from the effluent	Indian Agro and Recycled Paper Manufacturers Association to take up project with CPPRI.
Note: Non-complying units not meeting notified standards under Environment (Protection) Act, 1986 would submit action plan with PERT Chart along with bank guarantee to SPCBs by June 30, 2003.	

Review of treatment Methodologies.

The various treatment options studied can be classified as physical treatments, chemical treatments and biological treatments, each being capable of treating bleach effluents with varying degrees of efficiency.

Physical treatment

Nancy et al. [17] have used fly-ash as an adsorbing medium for removing chlorinated organics and colour efficiently form bleach effluents. Shawwa *et al.* [18] reported the use of delayed petroleum coke to bring about significant removal of AOX from bleach plant wastewaters at an initial concentration greater than 15000 mg/lit and reported that the treated bleach effluent was more amenable to biological treatment. *Torrades et al.* [19] described a photo-catalytic treatment, which removed the entire colour and most of the TOC, AOX and COD from D – Stage effluent. *Moiseev et al.* [20] have also reported the use of photo-catalytic oxidation as a pretreatment step, which enhanced the biodegradability of wastewaters containing similar recalcitrant or inhibitory compounds. Also such a treatment can be an alternative for an energy intensive total pollutant mineralization process. *Yao et al.* [21] have used of ultrafiltration membranes for AOX reduction and reported 99% reduction in the AOX levels. Reverse osmosis with pressures in the range of 3.5 – 5.5 MPa have been used for AOX removal [5]. *Seiss et al.* [22] have used nanofiltration in combination with electro-dialysis at the pilot scale. However all these membrane based techniques require a high degree of pretreatment and are capital intensive. Membrane fouling is another major problem with all these techniques.

Chemical treatment

Among the chemical methods, Milstein et al. [23] have reported 75%, 59% and 80% removal of AOX, COD and colour, respectively, with the use of polyethylene and modified starches as chemical additives. Milosevich and Hill [24] could achieve 60 – 70 % AOX removal in 1 hour at 50^{O}C by neutralization of bleach plant effluent with lime mud, followed by the addition of alkaline sulfide process liquor. Stephensen and Duff [25] have demonstrated the reduction in toxicity of bleached kraft mill effluent with the use of chloride and sulfate salts of iron and aluminum. Ganjidoust et al. [26] have used chitosan as a coagulant and reported 90% and 70% reduction in colour and TOC respectively. Hostachy et al. [27] have reported complete detoxification and removal of residual COD of bleached kraft mill effluent at low ozone doses. A major limitation of chemical treatment methods is the generation of large volumes of sludge, posing disposal problems.

In general, the physical and chemical treatment technologies for removal of AOX are not economical for large scale field operations. Consequently, it is necessary to develop economical, sustainable and eco-friendly alternative approaches for the removal of AOX compounds.

Biological treatment

Among the biological options, aerobic processes are the most widely used techniques to remove BOD and the organics can be completely oxidized to CO_2 and water. The rate of aerobic degradation is proportional to the dissolved oxygen concentration. Among the aerobic bacteria, Pseudomonas sps, Ancylobacter aquaticus and Methylobacterium sps have been reported to degrade AOX [5]. A major drawback in using the aerobic treatment system is the requirement for supplementation of oxygen [aeration], which invariably demands large inputs of energy to operate the aerators, making the process overall expensive.

Other approaches are based on the use of anaerobic bacteria for AOX degradation in bleach plant effluents. Many pure cultures of anaerobic bacteria, which can degrade chlorinated organics, have been isolated in recent years. Anaerobic bacteria capable of degrading chlorinated organics have been classified as alkyl dehalogenators and aryl dehalogenators [28]. Some anaerobic bacteria are capable of utilizing the energy generated in the process of dechlorination of organochlorines. This phenomenon is often referred to as dehalorespiration. Anaerobic treatment is technically simple, relatively inexpensive technology and requires very little power inputs. Both, the dechlorination and degradation efficiencies are good. A major drawback of using the anaerobic treatment is that the conventional anaerobic digester is highly unstable with respect to AOX loading rates. Some of the other compounds in the bleach effluent have been reported to be toxic to anaerobic microorganisms [8]. Moreover, higher AOX loading is highly inhibitory to the methanogens in the digester and as a result, all full-scale anaerobic systems are currently used as a primary treatment process [5]. Actinomycetes isolated from different soil samples have been tested for their abilities to utilize the chlorolignins in bleach effluents from a sulfite paper mill. Winter et al. have demonstrated the ability of actinomycetes to degrade and partly dehalogenate chlorinated compounds in bleach effluents [29].

Combinations of treatment methodologies involving chemical and biological treatment such as hypochlorite oxidation and anaerobic treatment, ozone and fungal treatment have also been reported [30, 31, 32].

Ek et al. [33] have investigated a combination of physical treatment using ultrafiltration with biological treatment. They have reported a good reduction in the AOX, COD, Chlorate and Chlorophenol levels employing a sequential system involving an ultrafiltration followed by the anaerobic and aerobic treatment respectively.

Fungal/enzyme treatments have a potential advantage in that the enzymes that are produced are extracellular. Also most of the fungal enzymes are relatively nonspecific in nature and can accomplish degradation of most of the detrimental organics [10, 11]. This means that a wide variety of substrates may serve as a substrate for degradation by the enzymes and these can form the basis for fungal bioremediation techniques as a relatively inexpensive and environmental friendly treatment option. A majority of the fungi reported to degrade and dechlorinate bleach plant effluents belong to the family of white-rot basidiomycetes. The most commonly reported among them include Phanerochaete chrysosporium and Trametes versicolor [3, 34, 35, 36, 37]. Phanerochaete chrysosporium is widely accepted as a model organism for the degradation of lignin and lignin derivatives including chlorophenols. It has also been reported to degrade a wide range of xenobiotics and structurally diverse organo-pollutants. Bumpus et al. have demonstrated the degradation of a few organic xenobiotics using Phanerochaete chrysosporium [37]. This ability arises due to the fact that this fungus produces lignin peroxidases [9] and manganese peroxidases [10] together, which effectively act on lignin and lignin derivatives. Pellinen at al. [34] have reported a 50 % reduction in the concentration of chlorolignins using Phanerochaete chrysosporium. Chan et al. [35] have reported degradation of 30 mg/lit of 2,4 – Dichlorophenol to trace level concentrations using Phanerochaete chrysosporium.

Ünal and Kolankaya [38] have investigated a number of fungi and reported the dechlorination activity of Trametes versicolor to be the highest. Among the other white-rot fungi reported till date include Ceriporiopsis subvermispora, Phlebia radiata [4, 39, 40]. The ligninolytic system of the white-rot fungi Trametes villosa and Panus crinitus have been reported to efficiently degrade all fractions of different molecular mass contained in E1-Stage bleaching effluent [41]. Rhizopus oryzae, a zygomycete, is reported to decolorize, dechlorinate, and detoxify bleach plant effluent at lower cosubstrate concentrations than the basidiomycetes previously investigated [2]. Trichoderma sps, one of the fungi imperfecti, is reported to be capable of degrading lignin and decolorizing [almost 85 %] the hardwood extraction-stage bleach effluent [42]. Raghukumar et al. [43] have reported two marine fungi viz. Halosarpheia ratnagiriensis and Sordaria fimicola capable of degrading and dechlorinating bleach plant effluents. These organisms and the enzymes that are produced by them are widely considered to have the potential for industrial applications, such as biodegradation of environmental pollutants, bio- conversion of lignin, biobleaching and biopulping of wood chips, desulfurization of petroleum and coal, and delignification of agricultural plant residues [1].

Three main enzymes are responsible for the degradation of AOX and specific chlorophenols. They include the lignin peroxidases, manganese peroxidase and laccase or phenol peroxidase. Lignin peroxidases secreted by Phanerochaete chrysosporium has been most extensively studied. However a few other white-rot fungi including Plebhia radiata also secrete this enzyme. This lignin degrading system consists of a family of peroxidases, which are able to catalyze the initial oxidative depolymerization of lignin. Though most studies concerned with lignin peroxidases are associated with Phanerochaete chrysosporium, the oxidative extra-cellular enzyme system is probably not identical in all white-rot fungi. For instance, Niku-Paavola et al. [40] have reported Plebhia radiata to degrade lignin better than Phanerochaete chrysosporium, under certain experimental conditions. Kannan and Oblisami [44] have reported decolourization of pulp and paper mill effluent employing Aspergillus niger.

Among the ligninolytic enzymes produced by fungi, only lignin peroxidase can directly oxidize non-phenolic lignin models, whereas manganese peroxidase and laccase are supposed to mainly degrade phenolic units [45]. Nagarathnamma et al. [4] have reported the secretion of laccase and manganese peroxidase by the fungus Ceriporiopsis subvermispora and have also reported its effect on the treatment of chlorophenols. The fungus did not produce lignin peroxidase. The studies by several researchers have shown that manganese peroxidase plays an important role in the decolourization of bleach effluent. Laccase has also been implicated in the degradation and decolourization of bleach effluents. Archibald et al. [36] have reported that laccase plays a primary role in the decolourization of bleach effluents by Trametes versicolor. Archibald et al. [46] have also demonstrated that Trametes versicolor laccase, in the presence of phenolic substrates was able to generate Mn(III) chelates similar to those produced by manganese peroxidase which was reported by Lackner et al. [47] to be responsible for the oxidation of bleach effluents. Mn(III) is a nonspecific oxidant, which in turn oxidizes a variety of organic compounds [11]. Laccase catalyzes a single-electron abstraction from phenolic hydroxyl groups, a substrate common in lignin, to produce a phenoxy radical which will, via nonenzymatic steps, result in quinone formation or in polymerization or depolymerization of the substrate thereby bringing about degradation [48]. Ünal and Kolankaya [38] reported that the addition of a laccase inducer, xylidine, into the culture medium of Trametes versicolor led to an increase in dechlorination activity.

Additionally, fungal biomasses are susceptible to engineering improvements and regeneration of their capabilities. Attempts have been made to use a Rotating Biological Contactor [RBC] for treating synthetic lignin derivatives / AOX-model compounds like chlorophenols, chloroguaiacols, etc [35]. The RBC however employs a mutant strain of Phanerochaete chrysosporium capable of adhering to the RBC disc [10]. The MyCoR [Mycelial Color Removal] process is developed as a fixed film reactor inoculated with a suitable fungus and charged with nutrients. This process is reported to convert 70 % of the organic chlorides to inorganic chlorides in 48 hours and simultaneously reduce the BOD and COD by 50%. A variant of such a process, termed the MyCoPoR process developed by Messner et al. [49] uses porous material inoculated with the spores of Phanerochaete chrysosporium, is reported to bring about 50 – 80 % decolourization and about 70 % reduction in the AOX levels. With regard to organic pollutants fungi can remove them from wastewaters, leading to a decrease in their toxicities. However, the detoxification rates seem to be dependent on media and culture conditions. The post-treatment by anaerobic bioprocesses of effluents pretreated with fungi can lead to higher biogas production than the original effluents [12]. A major drawback of most

fungal treatments are the need for supplementation of an easily utilizable co-substrate like glucose, which is essential for fungal growth, but often increases the cost of the treatment. A challenge for future research may involve the assessment of various co-substrates, which would be equally efficient in promoting fungal growth and thus treating the bleach effluent at a reasonable cost. A xylan-rich waste product of an Australian paper mill has been reported to give results equivalent to glucose [49]. Also fungal treatment demands frequent replenishment of the fungal biomass. Hence approaches towards using fungal enzymes instead of whole cells could probably be the future of effluent treatment.

Studies by the Author

Recent laboratory scale studies by the author [50] focused on the assessment of different fungi for degrading model AOX compounds especially chlorophenols. The chlorophenols selected included mono-chlorophenols, di-chlorophenols, and a tri-chlorophenol. The objective of the work was to compare the degradation efficiency of the isolated fungi with that of Phanerochaete chrysosporium, which has been widely accepted as a model organism for degrading lignin and lignin derivatives. A standard culture of Phanerochaete chrysosporium was procured from the National Collection of Industrial Microorganisms [NCIM No.1197, ATCC No.34541, CMI No.284010]. The fungi were isolated from the effluent of a pulp and paper industry and from the soils in the vicinity of a pulp and paper mill. The optimum growth conditions for the fungi were determined and maintained. The minimum inhibitory concentration of Phanerochaete chrysosporium was found to be 30 ppm of 2,4 – di-chlorophenol. Considering a concentration lesser than the threshold tolerance, 25 ppm was chosen for the study.

A list of the various fungi isolated for this study is given below:

Source of isolation.	
Penicillium aurantiogriseum Dierckx	*Aspergillus fumigatus Group*
Aspergillus wentii Group	*Alternaria alternate (Fr.) Keissler*
Penicillium aurantiogriseum Dierckx	*Penicillium species close to Penicillium helicum (Raper and Fennell)*
Aspergillus niger Group	

All the isolated fungi including the standard culture of Phanerochaete chrysosporium, was subjected to 25 ppm of 2,4 – dichlorophenol for a period of 6 days incubation and the residual concentration in the medium determined by HPLC techniques are shown in the Figure 4.

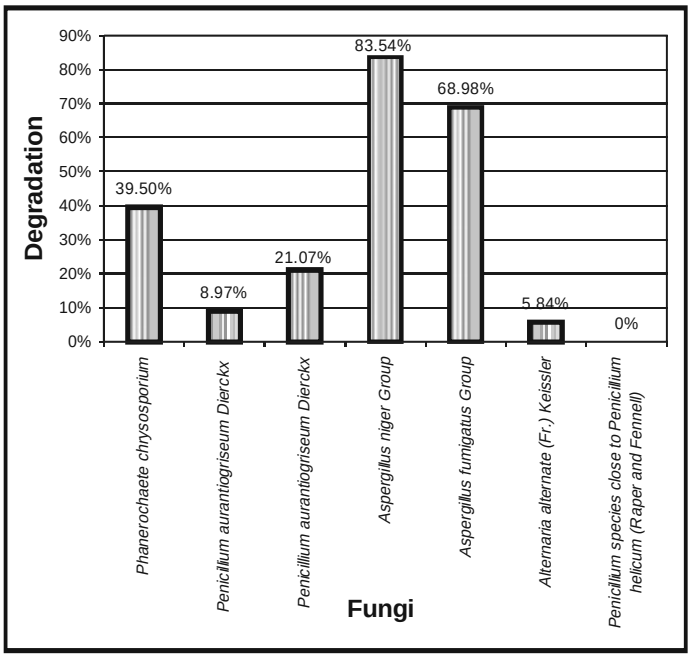


Figure 4: Degradation of 2,4 - Dichlorophenol by different fungal cultures

Aspergillus niger exhibited nearly twice the degradation activity as that of Phanerochaete chrysosporium. Since Aspergillus niger exhibited maximum degradation activity of 83.5%, it was selected for further subsequent experiments. It was then subjected to 25 ppm of mono-chlorophenols, di-chlorophenols and a trichlorophenol [Figure – 5].

The experimental observations after 6 days of incubation are shown in Figure 6. The chlorophenols were degraded to varying extents, with 2,4 – Dichlorophenol exhibiting maximum of 93.83 % and minimum degradation of 49.50 % in the case of 3 – chlorophenol. Among the monochlorophenols tested, 2-chlorophenol was degraded to a maximum extent of 81.57 % followed by 4 – chlorophenol which was degraded to an extent of 77.97 % and lastly 3 – chlorophenol. Among the dichlorophenols tested, 2,4 - chlorophenol was degraded to a maximum extent of 93.83 % followed by 3,4 – chlorophenol which was degraded to an extent of 90.24 % and lastly 2,3 – dichlorophenol exhibiting degradation to an extent of 59.15 %.

Future scope

Future line of work may include the biodegradability assessment of other AOX model compounds and other chlorinated organics as well. Assessment of various pre-treatment options such as Fenton's oxidation, ozonization, etc needs to be considered. Most importantly, it is essential to assess the effect of different co-substrates other than glucose and the effect on AOX degradation efficiency. Treatability assessment employing the bleach effluent would help gain an insight into the possibility of potentially using Aspergillus niger for reducing AOX levels. Studies on bio-avaliability and enzymatics are essential.

Summary

Bleach plant effluents have two important polluting attributes viz: the presence of AOX and the dark color. AOX being highly toxic and carcinogenic needs to be remediated, by treating the effluent. With a diversity of treatment options available, biological treatment seems to be the most promising due to several merits over the physical and chemical treatment methodologies.

Aerobic and anaerobic bacterial treatment, treatment with actinomycetes, and fungal treatment have been used with varying degrees of efficiency in treating AOX. Studies by the author indicate treatment with Aspergillus niger, can be potentially effective in remediating specific chlorophenols reportedly in the bleach effluent.

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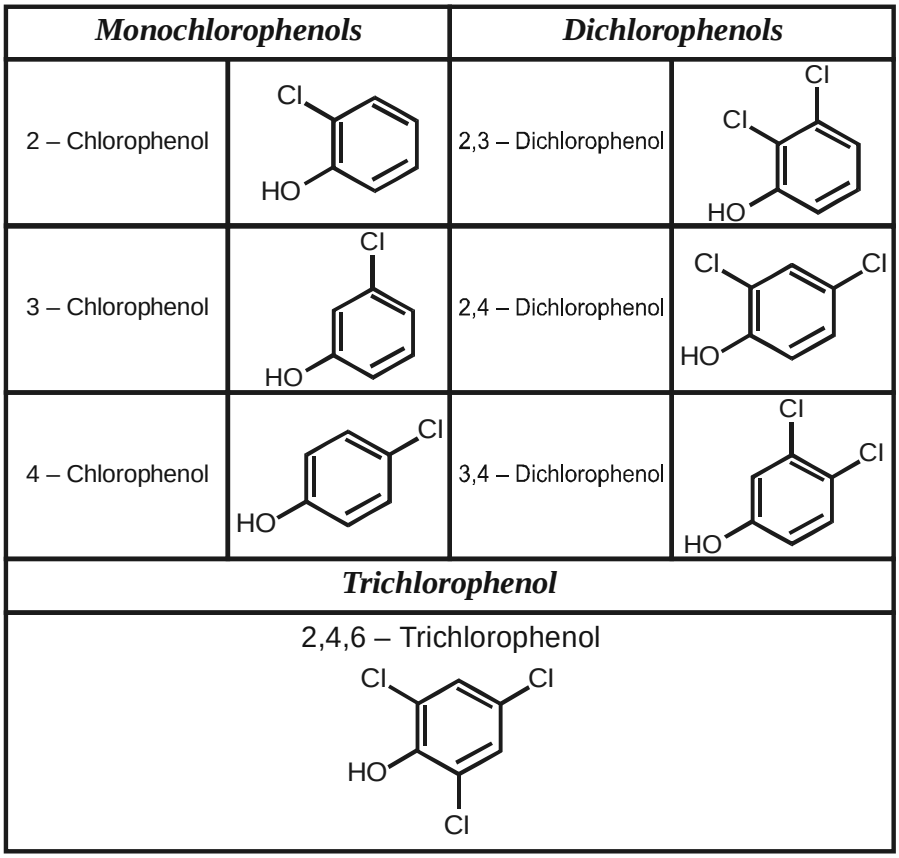

Figure 5: Structure of chlorophenols model compounds

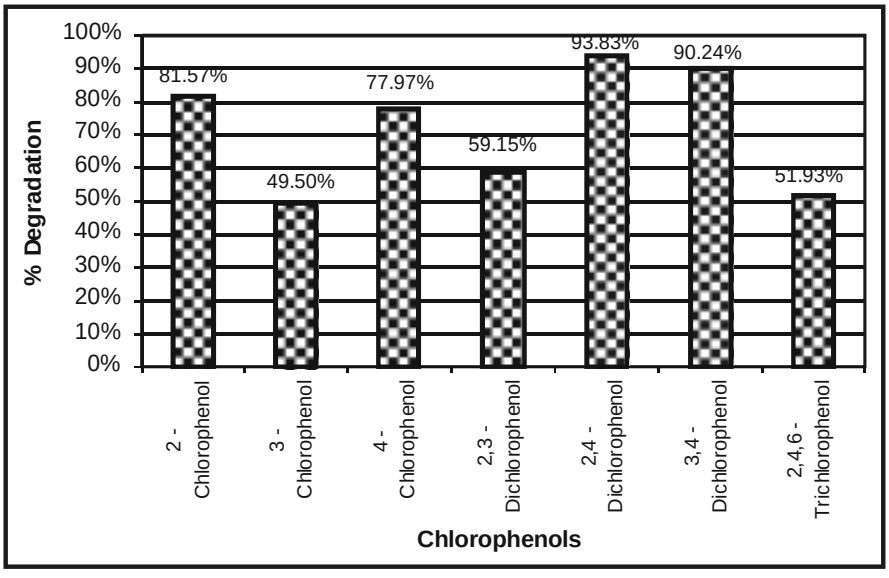

Figure 6: Degradation of Chlorophenols by Aspergillus niger

a-Keto-g- hiomethylbutyric acid and lignin model dimers. Appl Environ Microbiol, 1999; 65:916 – 922.

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