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REVIEW ARTICLE **OPEN ACCESS**

Recent trends of Environmental Biotechnology for bioremediation of pulp and paper mill effluents: A Review

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ABSTRACT
Pulp and paper mill effluent is the major source of pollution generating industry to be contingent on the model pulping processes and discarding huge amount of highly coloured effluent. The generated waste water from pulp and paper mills founds to liberate significantly polluting and highly dangerous substances in the environment, for now it become compulsory to treat these industrial waste waters before they exposed to the external environment. This literature of review counsel the circumstances related to treatability and availability of the multifarious treatment processes, characteristics of effluent, main sources of pollution and types of microorganisms involved in the treatment of pulp and paper mill effluents. The combination of aerobic and anaerobic treatment processes is mostly efficient in the removal of soluble biodegradable organic pollutants whereas certain fungal strain employed to treat lignin and to remove effluent colour. Although furthermore researches are needed to be looked by various scientists to discard Colour lignin, COD, BOD etc. from pulp and paper effluents.
***Keywords**: Pulp & Paper industry; Wastewater treatment; Microorganisms, Activated sludge; colour removal.*

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INTRODUCTION

The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment. Bioremediation is the most effective management tool to manage the polluted environment and recover contaminated soil. Such an attractive and successful cleaning technique of polluted environment has been used at a number of sites worldwide, with varying degrees of success. Microbes are very helpful to remediate the contaminated environment. Number of microbes including aerobes, anaerobes bacteria and fungi are involved in bioremediation process.

Pulp and paper industry has been ranked third in the world in terms of freshwater withdrawal and believed to be largest manufacturing user of water by the year 2000 [1]. It is one of India's traditional and core industrial sector. Although these industries are found to be generate a spacious amount of coloured and highly toxic effluents. Wastewaters discharged by the industries accounts for environmental pollution at very large level, particularly in the developing countries.

There are almost 500 hazardous chlorinated organic compounds have been recognised to be released from paper mill effluent [2]. Wide chemical diversity of these hazardous substances adversely affect the fishes and other aquatic living biota in the form of clastoges, carcinogens, and have endocrinic and mutagenic impact on the same [3, 4]. Some physical–chemical treatment systems like precipitation, adsorption, coagulation, and membrane filtration are traditionally applied for the removal of toxicity and colour from these effluents but their drawbacks does not allow them to be industrially applicable [5].

At present activated sludge process is most widely used biological treatment system which have potential to treat theses effluents by removing colour and toxicity [6]. There is a some bacterial strains have a ability to remove the colour, lignin and toxicity from the pulp and paper mill effluents but major problems is the high molecular weight of lignin, choloro-lignins and its derivates are not easily remove from the effluents. Recent researches have been relayed on biotechnological approaches with application of certain bacteria and fungi in in reference to their powerful enzyme system [7].

From the pulp and paper mill two trillion gallons of wastewater are discharged annually by the pulp and paper industry, much of which is highly coloured. The brown coloured effluents discharge of these

industries result in poor water aesthetics as well as cause harm and disturbance to the surrounding water bodies .The effluent released during the pulping stage, called black liquor which comprises a number of compounds like dissolved lignin and its degradation products, fatty acids, resin acid, hemicelluloses, tannins and phenols [3, 8, 9]. These organic compounds found to be responsible for the toxicity of effluent. Thus, it is mandatory to treat the effluent before disposal.
Many studies have demonstrated the implement of white-rot fungi to reduce the colour and chlorinated organic compounds of kraft effluents. White rot fungi have competence to metabolize lignin and its derivatives which make it a potential biochemical remedy for the lignin/phenolic wastewater treatment [10]. **B**iochemical properties of this fungal starin; production of certain extracellular oxidative enzymes like lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase makes them compatible to reduce absorbable organic halides and chemical oxygen demand [11]. So these enzymes are presented as the most functional in the biodegradation of lignin.

SOURCES OF POLLUTION IN PULP AND PAPER INDUSTRY

Paper making process is the main source of pollution found in the pulp and paper industries. It generally comprises five basic steps which includes a variety of methods. Each step comprises different unit processes and methods which lead to the formation of a mixture of waste water and certain kinds of chemicals. These eventually resulted effluents found to be the major source of pollutants [4, 12].
Debarking: debarking of plant fibre refers to the removal of bark and processing of plant material into small pieces called chips. This process exploits the nature of plants raw material (hard wood, softwood, agro residues) and finally leads to the transfer of tannins, resin acids, etc (present in bark) which is further utilized in the processing of water. Softwood contains a much higher amount of resin acids than hardwood.
Pulping process: Second step includes the conversion of chips into pulp. Aim of pulping process is to the production of cellulose rich pulp by removing lignin and hemicelluloses from the raw material of plant. Required pulp material can be achieved by various methods i.e. mechanical, pulping, semi chemical, kraft, sulfite etc. Left over raw material can be further utilized for the same process [3].
Bleaching: Brown pulp obtained from the pulping process further processed with some bleaching agents to find desired coloured as dictated by product standards. There are kinds of bleaching agents are used including chlorine, chlorine dioxide, ozone, hydrogen peroxide, oxygen, etc. may be sometimes used singly or also in combinations. During bleaching process lignin, phenols and resin acids got transform into highly toxic xenobiotics by the process of chlorination.
Washing: Washing is done for the removal of bleaching agents from the pulp. This process generally involves alkali extraction stage which utilizes alkali caustic soda to extract bleaching agents and colour from the pulp [3].
In the last step paper and paper products are mixed with washed pulp with suitable filters like clay, calcium carbonate, titanium dioxide and sizing agents like resin and starch.

Table (1) List of pollutants released from pulp and paper making process

S.N.	Process stage	Pollution load	Waste water	Composition of Effluent	Reference
1	Raw material preparation	Low	Low	Suspended solids, including bark particles, fibre pigments, dirt, grit, COD and BOD	[3]
2	Pulping process	High	Low	Colour, bark particles, soluble wood materials, fatty acids, resin acids, AOX, VOCs, BOD, COD and dissolve inorganic materials.	[13]
3	Bleaching Process	High	High	Colour, dissolved lignin, COD, carbohydrate, inorganic chlorines, AOX, EOX, VOCs, halogenated hydrocarbons and chlorophenols,	[14]
4	Paper making process	Low	Depends on the extent of the recycling effluent	Particulate wastes, organic & inorganic compounds, COD & BOD	[15]

GENERAL CHARACTERISTICS OF PULP AND PAPER MILL EFFLUENT

The waste water of pulp and paper industry have high concentrations of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) that shows high toxicity and strong black-brown colour. In particular, organic chlorinated compounds like furans and dioxins furans may be formed by the chlorination of lignin in wood chips. There are some main characteristics of pulp and paper mill effluent such as-

Lignin content: In the pulp and paper industry wood is the most important raw material for the production of pulp and paper. The main wood components are lignin, cellulose and hemicelluloses. Mainly lignin are first degraded which follows modification at large scale, chlorination and finally dissolved in the effluent. The dark brown colour of effluent occurs due to high content of chromophoric, polymeric lignin and its derivatives. The products of lignin transformation, primarily lignin peroxidase (Lip), are the main source of effluent colour in paper mill wastewaters [16].
High concentration of absorbable organic halides (AOX): During bleaching process released high concentration of chlorinated compounds and its derivatives. The content of chlorinated components in effluents is usually ensured as total organically bound chlorine (TOCl) or high absorbable organic halide [17]. The molecular mass of chloroorganics has toxic and it is affect the biodegradation and decolourisation of effluent.
Colour Content: In pulp and paper mill effluent colour due to high content of lignin but some dyes and pigments are responsible for the colour and they are highly visible material. The colour problems associated with pulp and paper mill wastewater have got a pretty important role in research [18]. The colour causes an aesthetic problem and contributes to the BOD [19]. The treatment of Pulp and paper wastewater by the various steps involving biological technologies are tended towards removing BOD, colour is at best incompletely reduced [16]. The lignin derivative colour remains firm and unchanged in spite of chlorinated organic removal [20].
COD/BOD Ratio: COD refers to the sum of oxidizable and organic matter in any particular effluent by chemically oxidation whereas BOD represents only those organic matters which oxidized/degraded by the help of certain microbes [21]. The low molecular weight chlorolignins are accessory to effluent BOD and acute toxicity. Chlorinated compounds having high molecular weight does not have any major effect on BOD and acute toxicity because of the inability to cross cell membrane. Cholorolignins have low molecular weight which makes them main reason for acute toxicity and effluent BOD.
Potential toxicity problems in pulp and paper mill waste water: In pulp and paper mill waste water toxic levels of low molecular weight chlorinated compounds do not accumulate during the natural degradation of chlorolignins [22].These organic compounds in effluents specially chlorinated phenols founds to be very toxic for the aquatic micro organisms and their resistance to microbial degradation.

MICROORGANISMS INVOLVED IN PULP AND PAPER MILL EFFLUENT TREATMENT

Biological (bioremediation) treatment methods for pulp and paper mill effluent involve the use of certain microorganisms like bacteria, fungi, algae and also some enzymes. These methods can be performed as single step treatment or can be applied in combination with other physical and/or chemical methods [23]. Most of the biological processes have not effectively performed for the removal of colour and recalcitrant compounds from paper and pulp wastewater. Bioremediation is the process of utilization of certain microorganisms like bacteria, fungi, algae in reference to catalyze the degradation or transformation of many toxic chemicals into their less harmful forms which makes it to employed for the treatment of many industrial effluents specially paper and pulp mill effluent [24, 25, 26].
Remediation by the Bacteria: There is a variety of bacterial species evaluated for their decolourization abilities, some of them have also been used commercially. Some studies demonstrated numerous types of bacteria some of which can decompose monomeric lignin substructure models whereas few bacterial strains founds to have ability to attack lignin derivatives or resultants obtained from multiple pulping processes [27, 28, 29].
According to Raj et al. 2007 and Tiku et al. 2010 [32, 40] bacterial strain *Bacillus cereus* and *Pseudomonas aeruginosa* are capable to decolorize bleach kraft effluent. The bacterial culture *Pseudomonas aeruginosa* accounts to alleviate the level of kraft mill effluent colour by 26-54%. The process of bioremediation by *P. aeruginosa* founds to be more effective under aerobic conditions [30]. Bacterial strain *Bacillus stearothermophilus* T6 produce a thermostable alkali tolerant enzyme xylanase, which is successfully used on a large scale to bleach pulp optimally at 65°C temperature and pH 9. *Streptomycetes badius* and *S. viridosporous* are also used as sole carbon source against commercial kraft lignin.
Acienetobacter calcoaceticus and *Pseudomonas putida* both bacterial processed with sontinuous reactor strain degrade black liquor from a kraft pulp and paper mill. They removed 70 - 80% of COD and lignin and found to have 80% colour removing efficiency within 8 days [26, 31]. *Pseudomonas*, *Ancylobacter* and *Methylubaclerium* strains are able to remove organochlorine from paper mill effluents and bleached kraft pulp. These strains were tested for growth on chlorinated acetic acids and alcohols for absorbable organic halogen (AOX) reduction in batch cultures of sterile bleached Kraft mill effluents from different sources.
Pure bacterial culture strains such as *Bacillus spp., E. coli, Zooglea, Commamonas, Flavobacterium spp., Pseudomonas, Chaetomium cochliolidae, Acaligenes eutrophus, Anthrobacter, Sphinomonas, Mortierella isabella, Corticum sasaki and Fomes annosus* found to degrade resin acid effectively [12, 32]. A variety of

mesophilic bacterial strains have been isolated and characterized for their ability to degrade resin acid. Two bacterial species *Pseudomonas*, IpA1 and IpA2 are used as a carbon source and electron donor 41 [33]. Both of these bacterial species are generally found to grow on isopimaric acid. Some Actinomycetes, have ability to exploit sulfite bleach effluents from a paper mills.

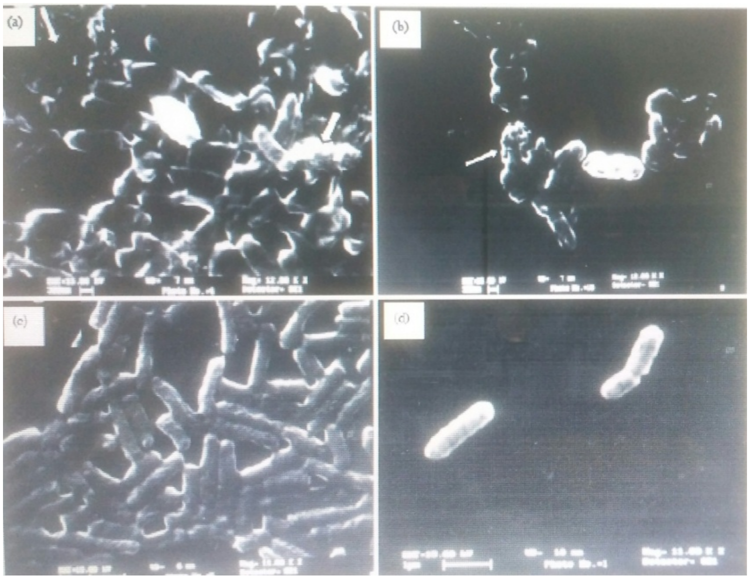

Figure (1). SEM *of Pseudomonas aeruginosa* (DSMZ 03504), (a, b) Bacterial cells showing clumping and aggregation of cells with effluent components while exposing to the wastewater for decolourisation, some amount of exopolysaccharide like material was also produced during process, (c) Clusters of unexposed cells and (d) Individual, unexposed cells of *P. aeruginosa.* Source: [34].

Remediation By fungi: Accounts of fungi found to be involved in the degradation of lignin and its derivatives positively. The white-rot fungi which are known for the wood degradation are found capable to degrade lignin. Certain fungal strains like as *Tinctoria borbonica, Trametes versicolor* and *Phanerochaete chrysosporium* have also been found to degrade lignin and its derrivatives and metabolize it along with monosaccharides*. Aspergillus niger* and *Trichoderma spp*., also have capacity to degrade lignin and decolorize effluent of hardwood pulp bleaching [34, 15].

Tinctoria borbonica decolourizes the kraft waste liquor into light yellow colour, there is about 90.99% reduction in colour achieved after 4 days of incubation [26] and it can also decolorize the hardwood effluent. *Tinctoria borbonica* was most effective utilized carbohydrate; and it stimulates substantial colour reduction without promoting the pollution load like COD. It require optimal condition (pH 4.0) to remove up to 85% of the total colour and decreased COD up to 25%, after cultivation for 3 days [15, 32]. *Gliocladium virens*, ubiquitous soil saprophyte, is capable to grow in the effluents and can decolourize them up to 42%. This fungal strain is also found to decrease the level of lignin (52%), cellulose (75%) and BOD (65%) present in effluents [15].

White rot fungus *Phanerochaete chrysosporium* known as the most common fungal strain to degrade lignin and decolourization of effluent and have special ability to degrade the copious aromatic polymer lignin [35, 36, 37]. *Schizophyllum commune*, another white-rot fungus found to be capable to decolourize the effluent produced by bagasse based pulp and paper mills. But, these fungi cannot degrade lignin unless metabolizable carbon source [34]. *Schizophyllum commune* shows highest effluent treatment efficiency at4.5 pH which can be enhanced by intermittent aeration. This fungal strain can also remove 90% colour of effluent. *Schizophyllum commune* is capable to reduce BOD and COD by 70% and 72% respectively within 2 days of incubation under optimum conditions [15, 38].

Remediation by the algae: Process of bioremediation especially decolourization of diluted bleach kraft mill effluents can be achieved by certain algae like as *Microcystis spp*. [39]. Some studies demonstrated 70% removal of colour within 2 months of incubation period. Sharma et al., 2014 and Chandra and Singh, 2012 point out the use of a mixed culture of algae which can remove 70% AOX and create 80% reduction in colour of effluent within 30 days under continuous lighting conditions. Such algal mixture includes *Chlorella*, *Chlamydomonas, Microcystis*, etc. By analysis of alkaline extraction of algal biomass and material balance findings, several authors suggested that the mechanism of colour removal was due to metabolism rather than adsorption.

Table(2) Microorganisms used for decolonization of pulp and paper mill effluent.

Types of microorganisms	References
Alage	
Microcystis spp.	[39]
Chlorella, Chlamydomonas.	[15, 39]
Bacteria	
Pseudomonas ovalis	[40, 15]
Pseudomonas aeuginosa	[40, 27, 28]
Bacillus cereus	[40, 27, 28, 29]
Fungi	
Aspergillus niger	[15, 38]
Trametes versicolor	[34, 35]
Phaennerochaete chrysosporium	[32, 35, 15]
Tinctoporia borbonika,	[35, 15]
Schizophyllum commune	[35, 15]
Gleophyllum trabeum	[37, 41]
Trichoderma spp.	[15, 35]
Phlebia radiate	[35, 42]
Bjerkandera spp.	[35, 42]

TREATMENT PROCESS OF PULP AND PAPER MILL EFFLUENT

For the treatment of pulp and paper mills effluent activated sludge methods is most successful and these operate at high oxygen levels. Treatment process generally includes three steps where tertialy step is not common for all process. First step involve the primary clarification, main treatment process for pulp and paper mills which is followed by secondary treatment generally of a biological nature. Tertiary treatments applied for the removal of colour, although these treatments are rare at present, but may become more common in the future if legislation becomes more stringent.

Primary clarification: In pulp and paper industries primary clarification of waste water is achieved by either sedimentation or flotation. On an average, primary clarification units achieved >80% high removal of suspended solids. Dissolved air flotation is an accepted process for the high removal of suspended solids and it is applied in many waste water treatment process streams. 30% of the raw material is recycled waste paper and waste water generated from its processing is further clarified by dissolved air flotation prior and then succeeding to secondary biological treatments [43].

Secondary treatment or Biological treatment systems

Aerobic treatment: Amongst the numerous biological treatment systems, activated sludge process is most commonly used in pulp and paper industries. The performances activated sludge plants being used for the treatment of pulp and paper mill wastewaters show that a very high removal efficiency of COD and BOD. Aerobic treatment of pulp & paper waste water includes two critical operational aspects of an activated sludge plant one is maintaining a proper control of the dissolved oxygen (DO) concentration in the aeration tank whereas second one involved the maintenance of a suitable settling sludge. The exact mechanisms of formation of sludge flocs are unknown, a general assumption suggest that floc-forming bacteria attached on the matrix which is produced by filamentous bacteria. Settlement problems occur with the growing filamentous species as they start too grown out from the floc [44]. Different theories are given in support to the presence and growth of filaments and floc-forming bacteria [45].

Kinetic theory; According to this theory floc-forming bacteria and filamentous bacteria have unequal maximum growth rates depends on the substrate concentration.

Accumulation & regeneration; Under transient (unbalanced) conditions floc-former bacteria shows great ability for intracellular storage of energy compounds than filaments bacteria. Here storage products are typically glycogen and poly-hydroxybutyrate.

Sludge age; Sludge age have considerable impact on the distribution of microbial species in the activated sludge which depends on the decay rate and specific growth of microbes and it results into enabling the different filamentous bacteria to become dominant.

Certain filamentous bacteria are more at low concentrations of dissolved oxygen (DO). This is especially notable at the inlet zone of aerators and some filaments bacteria have a competitive edge in a low nutrient environment and it has been suggested that total inorganic nitrogen should be >1 mg/l and soluble orthophosphate phosphorous >0.2 mg/l.

Temperature; Temperature has a great impact over the growth rates of the bacteria thus it plays a significant role during the treatment process. Growth of non- filamentous bacterial species will also favoured by nutrient gradients in the aeration tank.

Anaerobic treatment: Anaerobic treatment or anaerobic digestion is a successively deputed process for the secondary treatment of industrial waste waters. Anaerobic treatments are not widely used as compared to activated sludge method [46]. Anaerobic method of biological treatments has significant advantages over aerobic treatment such as lower chemical consumption, lower sludge production, smaller land requirements and it can be performed at mesophilic temperatures, 35°C to 37°C.

The COD removal data of paper mill wastewaters shows a relatively constant removal efficiency of about 80%. Anaerobic treatment is not found to be a convenient treatment option because it has potential for hydrogen sulphide conformation due to presence of high sulphur content in pulp and paper mill effluent [47]. According to Buisman et al., 1991 [48] sulphide can be removed by colourless sulphur bacteria which have ability to convert the sulphide ions into elemental sulphur.

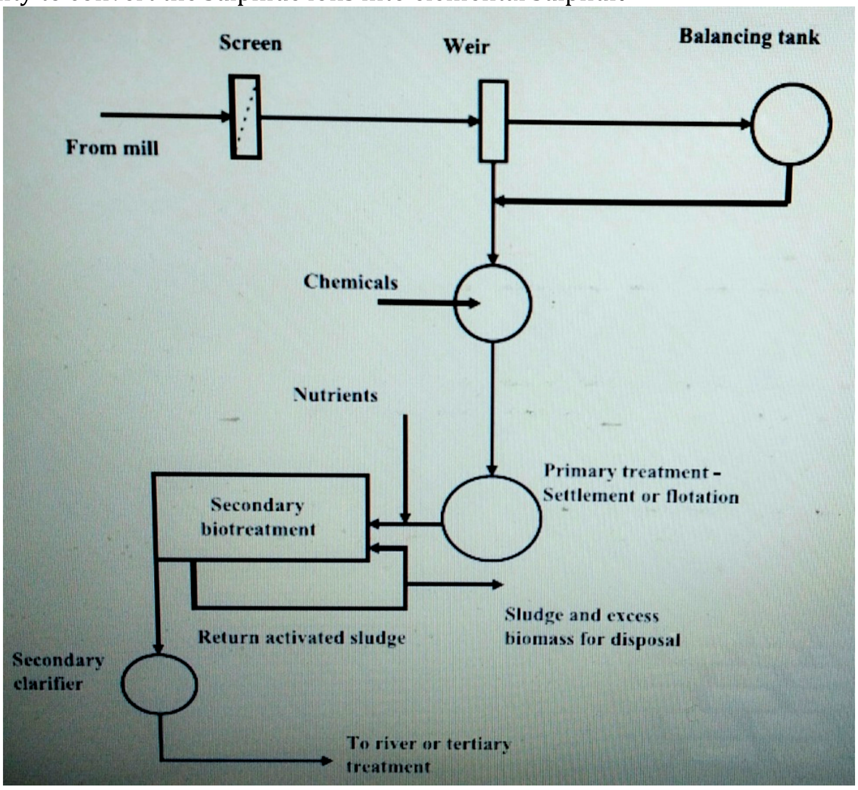

Figure (2) Generalised schematic diagram of the plant for the treatment of paper mill effluent. Source: [49]

CONCLUSION

The pulp and paper industries play an extraordinary role as industrial polluter in terms of organic discharges and wastewater volumes. The most efficient treatment system was proved to be activated sludge process where colour, bacterial growth inhibition levels, absorbable organic halogen and chemical oxygen demand were decreased to a significant extent and it has been shown to alleviate the environmental impact of these waste waters. Some moicroorganisms such as *Pseudolllonas putida*, *Citrobaterer spp*. and *Enterobacter spp.* founds to be suitable for efficient degradation of pulp and paper mill effluents. Certain White rot fungi (WRF) supposed to be best for dexterous degradation of the recalcitrant chromophoric material in bleach plant effluents. Furthermore researches are much needed for the renovation and proliferation of fast biodegradation processes which are likely to confer an economically feasibility also.

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