

A review on bioremediation of pulp and paper mill effluent – An alternative to conventional remedial technologies

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Received: August 17, 2017; Revised received: November 24, 2017; Accepted: February 10, 2018

Abstract: At present, a large amount of water required for paper production and various chemicals has been identified in effluents, which is produced at different steps of paper making in paper mills. The pulp and paper industry is typically related to pollution difficulties related to high biological oxygen demand (BOD), chemical oxygen demand (COD), colour, suspended solids, lignin and chlorinated compounds. Several studies have been made on eliminate these difficulties of pulp and paper effluents, the problem still continues. Although the physical and chemical methods are on the track of treatment, they are not on par with biological treatment because of cost ineffectiveness and residual effects. The biological treatment is known to be effective in reducing the organic load and toxic effects of paper mill effluents. Some microorganisms including bacteria and fungi have been involved in degrading the chemicals present in pulp and paper mill effluent. This article is an overview of the attempts made by several researchers worldwide to use biotechnological methods for degradation of the toxic compounds present in pulp and paper mill effluents by using fungi, bacteria, algae and enzymes. The current study clearly shows that application of native dominant bacterial and fungal isolates may be used for the treatment of large pulp and paper mills effluents.

Keywords: Algae, Bacteria, Effluent, Enzymes, Fungi, Pulp and paper mill

INTRODUCTION

Development increases the financial value of a nation, but concurrently it leads to the degradation of the environment (Hossain and Rao, 2014). Financial profit of the pulp and paper industry has controlled it to be one of the most important industrial sections in the world. Still, now, pulp and paper mills are facing challenges with the management of the resulting pollutants (Kamali and Khodaparast, 2015). The pulp and paper industry has been considered to be a major consumer of natural resources and significant contributor of pollution to the environment. The pulp and paper industry is the sixth largest polluter globally, discharging a variety of gaseous, liquid and solid wastes into the environment (Monte *et al.*, 2009).

These pulp and paper industries generate 220-380 m³ of highly coloured and potentially toxic wastewater for every tonne of paper produced (Badar and Farooqui, 2011). That effluent needs appropriate treatment prior to release in the environment; otherwise, it represents a major environmental problem. The main problem is the persistent dark brown colour due to lignin and its derivatives (Prasongsuk *et al.*, 2009). The effluent cause huge damage to the receiving water if discharged untreated because it has high biological

oxygen demand (385 mg/l), chemical oxygen demand (792 mg/l), total dissolved solids (850 mg/l), lignin (436 mg/l), phenol (42 mg/l), sulphur and sulphur compounds (993 mg/l) (Raj *et al.*, 2014).

The increasing strict regulations established by many authorities and agencies are forcing the industry to treat effluents to the required permissible level before releasing in to the environment (D'Souza *et al.*, 2006). Various studies have been conducted regarding the environmental impacts and the control of the pollutants. But very few reports are present for the comparative assessment of various biological treatment processes (Hanafy *et al.*, 2007).

The term biodegradation has been used to describe the conversion of every type including those that yield products more complex than the starting material as well as those responsible for the complete oxidation of compounds to CO₂, H₂O, NO₃ and other inorganic compounds (Atlas and Bartha, 2003). Bioremediation is defined as the process in which organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities (Mueller *et al.*, 1996). The term mineralization is the ultimate degradation and recycling of organic molecule to its mineral

Table 1. Physicochemical characteristics of pulp and paper mill effluent (Raj *et al.*, 2014).

S.N.	Parameters	Calculated values
1	pH	8.2 ± 1.0
2	TDS	850 ± 30 mg/l
3	COD	792 ± 70 mg/l
4	BOD	385 ± 12 mg/l
5	Colour (CU)	2242 ± 56
6	Lignin	436 ± 18 mg/l
7	Total nitrogen	116 ± 32 mg/l
8	Sulphate	993 ± 6 mg/l
9	Phosphate	8.3 ± 0.3 mg/l
10	Nitrate	73.3 ± 6 mg/l
11	Total phenol	42 ± 2.5 mg/l
12	Heavy metals	
	Cu	0.09 ± 0.1 mg/l
	Fe	10.22 ± 9 mg/l
	Ni	5.03 ± 1 mg/l
	Zn	9.83 ± 1 mg/l
	Mn	0.04 ± 0 mg/l

constituents. The fundamental process of the bioremediation is the natural process of biodegradation, in which the concentration of pollutants can reduce and sometimes can completely oxidize the compound.

The biological colour removal process is particularly attractive since in addition to colour and COD it also reduces BOD and low molecular weight chlorolignins (Nagarthamma *et al.*, 1999). Microorganisms speedily degrade few chemicals and eliminate them from the environment, but some chemicals are degraded slowly, accumulate in the environment and occasionally exhibit toxicity (Alexander, 1981). Biodegradation of dangerous substances in the environment represents significant prospective methods when complex and ecologically unstable pollutants are converted into simpler substances (stable ones) by the action of microorganisms. The principle of biodegradation technologies is based on optimization of nutrient ratios to support the growth of selected microorganisms able to degrade the target contaminants and application of selected isolated microorganisms with relevant degradation abilities (Kucerova, 2006). Treatment of pulp and paper mill effluent (PPME) has not proved successful due to lack

of the desired microorganism, loss of genetic potentiality in adverse environmental conditions, the formation of recalcitrant compounds and poor process optimization for treatment at large scale. Although the physical and chemical methods are applied for treatment they are not better than biological treatment because of cost ineffectiveness and residual effects. The various enzymes implicated in the treatment of PPME are lignin peroxidase, manganese peroxidase and laccase (Malaviya and Rathore, 2007). Microorganisms showing good production of these enzymes have the strength to treat the effluent.

This article is a summary of the attempts made by several researchers worldwide to use biotechnological methods for degradation of the toxic compounds present in pulp and paper mill effluents by using fungi, bacteria, algae and enzymes.

Properties of pulp and paper mill effluent: The paper production process involves several steps and each step can be carried out by various methods. As a result, the final effluent is a mixture of waste water from each process. Table 1 summarizes the main pollutants, which are normally produced during pulp and paper making process (Raj *et al.*, 2014). In debarking process, the plant fibers are converted into smaller pieces called chips that remove the bark. In pulping process, the chips converted into pulp. After pulping, bleaching process is applied to the brown pulp to obtain the desired colour. In the washing process, the bleaching agents are removed from the pulp. Finally, the paper produced by mixing the washed pulp with appropriate fillers and sizing agents like resin and starch. The pollutants at various stages of the pulping and paper making process are presented in Table-2.

The pulp and paper industry produces effluents having large BODs and CODs. One of the specific problems that yet not been solved is the strong black brown color of the effluent, which is due to lignin and its derivatives, mainly from pulping, bleaching and chemical recovery stages. Due to brown color of the effluent water temperature increases and photosynthesis decreases, both effects may lead to decreased concentra-

Table 2. Pollutants from various sources of pulp and paper mill (US EPA, 1995).

Debarking process	The soils, dirt, and barks are removed from the wood and chips are separated from the barks and water is used to clean the wood. Thus the wastewater from this source contains suspended solids, BOD, dirt, grit, fibers etc.
Pulping process	The waste water generated from the digester house is called "black liquor". kraft spent cooking "black liquor" contains the cooking chemicals as well as lignin and other extractives from the wood. The wastewater contains resins, fatty acids, color, BOD, COD, AOX, VOCs (Terpenes, alcohols, phenols, methanol, acetone, chloroform etc)
Pulp washing	The wastewater from the pulp washing contains high pH, BOD, COD and suspended solids and dark brown in color.
Pulp bleaching	The waste water generated from the bleaching contains dissolved lignin, carbohydrate, color, COD, OX, inorganic chlorine compounds such as chlorate ClO_3^- , Organo chlorine compounds such as dioxins, furans, chlorophenols, VOCs such as acetone, methylene chloride, carbon disulphide, chloroform, chloromethane, trichloroethane etc.
Paper making	The wastewater generated from papermaking contains particulate waste, organic compounds, inorganic dyes, COD, acetone etc.

Table 3. Microorganisms used for biodegradation of PPME.

Microorganisms	Authors
Fungi	
<i>Trametes versicolor</i>	Dashtbanet <i>et al.</i> (2010), Senthilkumaret <i>et al.</i> (2014), Demir <i>et al.</i> (2007) and Kamali and Khodaparast (2015)
<i>Phaenerochaete chrysosporium</i>	Tiku <i>et al.</i> (2010), Senthilkumaret <i>et al.</i> (2014) and Kamali and Khodaparast (2015)
<i>Tinctoporia borbonica</i>	Senthilkumaret <i>et al.</i> (2014), Demir <i>et al.</i> (2007), Verma and Madamwar (2002), Gomaa <i>et al.</i> (2008) and Kamali and Khodaparast (2015)
<i>Schizophyllum commune</i>	Senthilkumaret <i>et al.</i> (2014), Demir <i>et al.</i> (2007), Verma and Madamwar (2002) and Gomaa <i>et al.</i> (2008)
<i>Aspergillus niger</i>	Sarithaet <i>et al.</i> (2010), Senthilkumaret <i>et al.</i> (2014) and Kamali and Khodaparast (2015)
<i>Gloephyllum trabeum</i>	Patel and Madamwar (2002) and Gomaa <i>et al.</i> (2008)
<i>Trichoderma</i> spp.	Senthilkumaret <i>et al.</i> (2014) and Kamali and Khodaparast (2015)
<i>Paecilomyces variotii</i>	Senthilkumaret <i>et al.</i> (2014), Demir <i>et al.</i> (2007) and Kamali and Khodaparast (2015)
<i>Phlebia radiata</i>	Aftab <i>et al.</i> (2011), Senthilkumaret <i>et al.</i> (2014) and Demir <i>et al.</i> (2007)
<i>Bjerkandera</i> spp.	Aftab <i>et al.</i> (2011), Senthilkumaret <i>et al.</i> (2014) and Demir <i>et al.</i> (2007)
<i>Phanerochaete chrysosporium</i> and <i>T. hirsute</i>	Sarithaet <i>et al.</i> (2010), Senthilkumaret <i>et al.</i> (2014) and Demir <i>et al.</i> (2007)
Bacteria	
<i>Pseudomonas ovalis</i>	Tyagi <i>et al.</i> (2014), Raj <i>et al.</i> (2007) and Kamali and Khodaparast (2015)
<i>Pseudomonas aeruginosa</i>	Raj <i>et al.</i> (2007), Hao <i>et al.</i> (2000), Chandra <i>et al.</i> (2011), Chandra and Bharagava (2013), Keharia and Madamwar (2003), Tiku <i>et al.</i> (2010) and Kamali and Khodaparast (2015)
<i>Bacillus cereus</i>	Raj <i>et al.</i> (2007), Hao <i>et al.</i> (2000), Chandra <i>et al.</i> (2011), Chandra and Bharagava (2013), Tiku <i>et al.</i> (2010) and Kamali and Khodaparast (2015)
Algae	
<i>Microcystis</i> spp.	Iyovoet <i>et al.</i> (2010) and Sharma <i>et al.</i> (2014)
<i>Chlorella</i> , <i>Chlamydomonas</i>	Iyovoet <i>et al.</i> (2010), Sharma <i>et al.</i> (2014) and Kamali and Khodaparast (2015)
Enzymes	
Ligninase	Raj <i>et al.</i> (2014), Gao <i>et al.</i> (2010, 2013), Chakar and Ragauskas (2004) and Dashtbanet <i>et al.</i> (2010)
Cellulase	Raj <i>et al.</i> (2014), Gao <i>et al.</i> (2010, 2013), Chakar and Ragauskas (2004) and Dashtbanet <i>et al.</i> (2010)
Peroxidase	Raj <i>et al.</i> (2014), Gao <i>et al.</i> (2010, 2013), Chakar and Ragauskas (2004) and Dashtbanet <i>et al.</i> (2010)

tion of dissolved oxygen (Ragunathan and Swaminathan, 2004).

The generation of waste water and characteristics of PPME depends totally on manufacturing process adopted and the extent of reuse of water employed in the plant. The effluent of kraft pulping is highly polluted and characterized by parameters such as colour, absorbable organic halides (AOX) and related organic compounds. The alkaline extraction stage of bleach plant effluent is the major source of colour due to lignin and its derivatives (Bajpai *et al.*, 1993). Lignin wastewater is discharged from the pulping, bleaching and chemical recovery sections. Lignin is a heterogeneous, three dimensional polymer, composed of oxyphenylpropanoid units. The high chlorine content of bleached plant reacts with lignin and its derivatives converted into highly toxic and recalcitrant compounds and the responsible for high BOD and COD. Trichlorophenol, trichloroguaiacol, tetrachloroguaiacol, dichlorophenol, dichloroguaiacol and pentachlorophenol are major contaminants formed in PPME (Leuenberger *et al.*, 1985).

Effects of pulp and paper mill effluent: The most noticeable effects on receiving water were reduced

oxygen levels, eutrophication and deposition of sludges and associated microbial growth. Many chlorinated organic compounds have been detected in PPME, in receiving water and in biota exposed to pulp and paper mill discharges (Dey *et al.*, 2013). These chlorinated compounds are highly toxic included phenolics, fatty acids and resin acids as well as dioxins and furans (Ali and Sreekrishnan, 2001). Pulp and paper industry is one of the most polluting industries contributing 100 million kg of toxic pollutants every year in the environment (Dey *et al.*, 2013). More than 260 chemicals have been identified in PPME which are produced at different stages of papermaking (Hawkins *et al.*, 2002). The toxic nature is due to several naturally occurring and xenobiotic compounds which are formed and released during various stages of processing (Sharma *et al.*, 2007). The PPME absorb more light and heat and retain less oxygen due to a large amount of tannins, thereby negatively affecting the aquatic flora and fauna. The condensed tannins from spruce bark are toxic to methanogens present in PPME and also to aquatic organisms like fish by changing their behavioral response, development and growth, impact on the immune system, impact on enzymes and reproductive

(Dey *et al.*, 2013; Mishra *et al.*, 2011).

Various studies have reported damaging effects of PPME on animals living in water bodies receiving the effluent. The effects are in the form of respiratory stress, oxidative stress, liver damage and geno-toxicity (Vass *et al.*, 1996). Health impacts such as diarrhea, vomiting, headaches, nausea and eye irritation in children and workers were reported due to the PPME discharge to the environment (Mandal and Bandana, 1996). The effluent has a high chemical diversity of organic chemicals present in it. Many of them are carcinogenic, mutagenic, clastogenic and endocrine disrupters. A study on *Bacillus Subtilis* reported the mutagenic effects by the effluent of kraft paper mill (Kinae *et al.*, 1981).

Another study reports the toxic and mutagenic effects of PPME contaminating lake Baikal (Lindstrom-Seppa *et al.*, 1998). Exposure to the PPME adversely affects diversity and abundance of phytoplankton, zooplankton and zoobenthos, disrupting benthic algal and invertebrate communities (Karrasch *et al.*, 2006). Therefore it is mandatory to treat the effluent before disposal.

Biodegradation of pollutants in PPME: Since last many years, the pollutants removal from PPME is the major problem and a subject of study. The colour is the main problem in PPME which is mainly due to lignin and lignin derivatives. A Large amount of lignin produced from various processes such as pulping, bleaching and recovery sections.

Various methods have been used for the removal of colour from PPME. Physical and chemical methods are relatively expensive and eliminate some chlorinated lignins, colour, toxicity, suspended solids and COD but BOD and some other chemicals can not be removed efficiently. The biological method is a comparatively better option to reduce colour and COD; it also reduces BOD and other chemicals.

Bioremediation is a technology that utilizes biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. So, bioremediation is employed for the treatment of various industrial effluents like PPME (Wu *et al.*, 2005; Yang *et al.*, 2008).

Biological treatment: Biological treatment methods involve the utilization of microorganisms like fungi, bacteria, algae and enzymes, as a single step treatment or in combination with other physical and/or chemical methods (Singhal and Thakur, 2009). Biological methods for wastewater treatment are comparatively cost effective, eco-friendly and appropriate for reduction BOD as well as COD from the effluents.

Treatment with fungi: The fungi are naturally present in PPME as well as sludge (Yang *et al.*, 2011) and they produce extracellular enzymes (Kamali and Khodaparast, 2015). *Schizophyllum commune*, *Tinctoria borbonica*, *Phanerochaete chrysosporium* and *Trametes versicolor* have been found to be helpful for

degradation and metabolism of lignin along with carbohydrates. *Aspergillus niger* and *Trichoderma* sp. are also capable of degrading lignin and decolorizing effluent of hardwood pulp bleaching (Dashtban *et al.*, 2010). The nutrients can improve the decolourizing efficiency of the fungus and also reduce BOD and COD of the effluent. Sucrose was found to be the best co-substrate for the degradation of lignin (Tiku *et al.*, 2010; Kamali and Khodaparast, 2015). The use of *Tinctoria borbonica* has removed 90-99% color in 4 days of incubation (Abd El-Rahim and Zaki, 2005). *Schizophyllum commune* can be removed the 90% color of effluent and also reduced BOD and COD by 70% and 72% respectively under optimum conditions in 2 days incubation (Saritha *et al.*, 2010).

Gliocladiumvirens has been employed for the treatment of PPME, and it was observed that the fungus grew efficiently in the presence of effluent and decolourized upto 42% and also decreased lignin (52%), cellulose (75%) and BOD (65%) in effluents (Kamali and Khodaparast, 2015).

Phanerochaete chrysosporium can degrade the lignin and remove the color of PPME, therefore which has been studied in much detail by Senthilkumar *et al.* (2014), Demir *et al.* (2007) and Gomaa *et al.* (2008). *Coriolus versicolor* secretes an extracellular enzyme laccase, which helps in lignin degradation (Aftab *et al.*, 2011). *Coriolus versicolor* in liquid culture reduced 60% color of the effluent in 6 days incubation in the presence of sucrose. Fungus *Coriolus versicolor* immobilized in calcium alginate beads have been used in airlift bioreactor for the treatment of PPME (Verma and Madamwar, 2002).

The *Cyrus stercoreus* can also degrade lignin as efficiently as like as other white rot fungi did (Achoka, 2002; Saritha *et al.*, 2010). The *Trametes versicolor* strain B7 oxidized the chromophores present in PPME in the presence of the carbohydrates, and highest decolourization was reported in the presence of glucose at optimum condition, pH 4.5-5.5 and temperature 30°C (Diez *et al.*, 1999).

Decolourization of PPME was reported maximum decolourization of 34% by *Trametes versicolor* on third day incubation in effluent supplemented with 1.0% (w/v) glucose as co-substrate and 0.2% (w/v) urea as nitrogen source (Singhal and Thakur, 2009).

Sordariafimicola, *Halosatphea* sp. and *Basidiomyces* sp. can produce the lignin modifying enzymes; laccase, manganese peroxidase (MNP) and ligninperoxidase (LP) (Kamali and Khodaparast, 2015). Sumathi and Phatak (1999) investigated the capability of *Aspergillus foetidus* to colour removal, COD reduction and lignin metabolism. The cellulose degradation by *Pleurotus sajor-caju* was rapid at the initial stages of growth. The activity of endoglucanase, exoglucanase and beta-glucosidase were maximum at 8, 12 and 26 days of incubation, respectively (Atkins and

Clark, 2004; Ramos *et al.*, 2009).

The optimum conditions for fungal growth are quite different from degradation. The pH range for optimum growth was 4.3 to 4.8 and degradation is retarded below pH 4.0 and above pH 5.0 due to lowering growth. Therefore, suggesting that the pH does not play a critical role in the treatment of PPME (Saritha *et al.*, 2010). The optimum temperature for the growth of fungus was 40°C where as the treatment is not much affected to the same narrow range of the temperature but slightly decrease in the rate at a temperature as low as 25°C (Tiku *et al.*, 2010). The fungal degradation needs oxygen and a co substrate but not requires the addition of nitrogen source (Verma and Madamwar, 2002).

Treatment with bacteria: Various bacterial species have been evaluated for their decolorization abilities. *Bacillus subtilis* and *Micrococcus luteus* were found competent of reducing BOD up to 87.2%, COD up to 94.7% and lignin content up to 97% after 9 days under shaking conditions and brought down pH of PPME to neutral (Tyagi *et al.*, 2014).

Pseudomonas aeruginosa is capable of reducing PPME color by 26-54% or more under aerobic conditions (Ramsay and Nguyen, 2002). Tiku *et al.* (2010) and Raj *et al.* (2007) were tested *Bacillus cereus* and two strains of *Pseudomonas aeruginosa* for the decolorization of PPME.

Streptomyces badius and *S. viridosporous* were able to use a commercial kraft lignin as sole carbon source which was characterized by fourier transformed infrared spectroscopy, amino acid analysis, elemental analysis for C, H, N and high performance size exclusion chromatography (Abd El-Rahim and Zaki, 2005; Chandra *et al.*, 2011).

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 80% in 8 days (Murugesan, 2003).

Burkholderia cepacia strains hydrolysed tri-glycerides to free fatty acids. Nearby 30% of the stearyl esters, 25% of the dehydro abietic and 45% of the abietic and iso-pimaric resin acids were degraded during 11 days (Aftab *et al.*, 2011).

Apart from the investigation, mixed consortia of aerobic and anaerobic microbes for resin acid degradation, researchers have employed pure cultures of several bacteria which include *Bacillus* sp., *E. coli*, *Flavobacterium* sp., *Pseudomonas*, *Acaligenes eutrophus*, *Anthrobacter*, *Sphinomona*, *Zooglea*, *Commamonas*, *Mortierella isabella*, *Chaetomium cochliolidae*, *Corticium sasaki* and *Fomesannosus* (Tiku *et al.*, 2010; Raj *et al.*, 2014). Wilson *et al.* (1996) isolated two species of *Pseudomonas*, IpA-1 and IpA-2 which were capable of growing on isopimaric acid as the sole carbon source and an electron donor. These isolates were also

found to grow on pimaric acid and dehydroabietic acid.

Bacillus SAM3 producing high levels of cellulose free xylanase active and stable at alkaline pH (Chandra and Singh, 2012). *Actinomycetes*, isolated from different soil samples were tested for their ability to utilize sulphite in PPME. The AOX values of the higher molecular weight fractions were also reduced. Extracellular peroxidase and cell wall-bound catalase activities were produced during growth of the microorganisms on bleach effluents (Chandra *et al.*, 2012).

Pseudomonas putida, *Citrobacter* sp. and *Enterobacter* sp. can decolorized effluent up to 97% and also can reduce BOD, COD, phenolics and sulphide upto 96.63%, 96.80%, 96.92% and 96.67% respectively within 24 h of growth and the heavy metals were removed upto 82-99.80% (Keharia and Madamwar, 2003).

It was observed that as the pH of PPME was dropped to as low as 2.0 because of the settling properties of the effluent are increased tremendously. This could be due to the precipitation of negatively charged lignin components and the bacterial cells due to increased protonation of the medium. The color intensity increased, as the pH was made alkaline up to 10. Thus, suggesting that pH plays an acrucial role in the color of PPME. Additional carbon source plays an important role in degradation process. A number of studies revealed that the color removal ability increased by about 20-25% by the addition of nutrients like glucose and sucrose. There is a negative impact in practical applicability due to excessive growth of biomass, leading to high turbidity in the samples. So, the use of additional nutrient sources of carbon was summarily rejected by the workers, considering the practical applicability of the technology. In decolourization studies, generally, a range of 50-200 rpm was selected. It was observed that percentage of removal of color was highest at about 150 rpm, after which it became stable. Different effluent biomass ratios (1:1, 1:0.5, 1:0.25 and 1:2) were tried, and finally, the results suggested that *P. aeruginosa* (DSMZ 03504) and consortium produced the best results when used in the 1:1 ratio (Tiku *et al.*, 2010). Adsorption is playing a major role to contribute the removal of color. *P. aeruginosa* was taken out from the wastewater, after the decolorization experiment and analyzed by scanning electron microscopy. Scanning electron microscopy studies showed that the bacterial cells adhering to the colored material present in the wastewater exhibited some extra cellular secretion visible on the cell surfaces which was absent in the control cells. The surfaces of the cells growing in the wastewater were not as smooth as seen in the control cells. This would mean that adsorption could be a major contributor to color removal. However, the extent of adsorption was not clear and further study is required (Tiku *et al.*, 2010).

Treatment with algae: It has been reported that some algae like *Microcystis* sp. can decolourize diluted bleach kraft mill effluents (Iyovo *et al.*, 2010; Sharma *et al.*, 2014). According to Chandra and Singh (2012), pure and mixed algal cultures removed up to 70% of colour within two months of incubation. Mostly cultures showed a similar colour reduction pattern consisting of a phase with declining rate. The decolorization was maximum remedial during the first 15-20 days of incubation and then gradually declined. The decolorization by algae is caused by metabolic transformation of coloured molecules to colourless molecules. Mixed culture of *Chlorella*, *Chlamydomonas* and *Microcystis* were used for removal AOX and colour, and it was reported 70% AOX reduction while 80% color reduction in 30 days. Many authors concluded through analysis of alkaline extraction of algal biomass that the main color removal mechanism was metabolism rather than adsorption (Sharma *et al.*, 2014; Chandra and Singh, 2012).

Treatment with biological enzymes: Few enzymes like ligninase, cellulase and peroxidase are showing potential to remove organic matter from PPME and also improve the quality of wastewater. Out of them 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 for decolourization process. Some fungus produces peroxidase, 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 hydrogen peroxide (H₂O₂) was enhanced by the addition of peroxidase (Raj *et al.*, 2014; Gao *et al.*, 2013).

Conclusion

The comparison of treatment by different methods showed that native microbes isolated from the site of pulp and paper mill have the ability to use lignin, as a carbon source and reduce the COD, BOD and other contaminants. With the increased demand for paper, the treatment of effluents emerges as a most pressing problem in environmental protection. Presently, bioremediation is taken to be an attractive option for reducing the pollution load from contaminated water because of its high efficiency and economical impact than the chemical remediation. The current study clearly showed that application of native dominant bacterial and fungal isolates could be used for the treatment of large pulp and paper mills effluents. The comparison of decolourisation by different organisms showed that some fungus (*Aspergillus niger*, *Phanerochaete chrysosporium* and *Trametes versicolor*) and some bacteria (*Pseudomonas ovalis*, *Pseudomonas aeruginosa* and *Bacillus cereus*) are suitable for efflu-

ent degradation of recalcitrant chromophoric material in paper mill effluent. The fungal and bacterial isolates having appropriate enzyme activity with optimized proper physical conditions are playing a significant role in the bioremediation process. Further research is needed to develop fast biodegradation processes which are likely to provide an economically feasible process.

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