



A Review on the Role of Microorganisms in Treatment of Paper and Pulp Industry Effluent

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ABSTRACT

The pulp and paper industries produce large amount of toxic and strongly coloured waste effluents, causing severe water pollution. This paper is the state of the art review of microorganisms for pulp and paper mill wastewater treatment and presentation of available treatment processes. A comparison of most usable treatment processes is presented. Fungal treatment is found to be efficient in colour removal. Combination of anaerobic and aerobic treatment processes are considered to be efficient in removal of soluble biodegradable organic pollutants.

INTRODUCTION

The manufacture of paper from pulp is water-intensive industry. It involves conversion of raw materials into pulp by digestion with various material and conversion of pulp into paper. Raw material used in this industry is mainly cellulose. The yield of pulp depends mainly on the raw material as given in Table 1.

Two trillion gallons of wastewater are discharged annually by the pulp and paper industry, much of which is highly coloured. The brown coloured effluents discharged by the pulp and paper industry result in poor water aesthetics as well as cause harm and disturbance to the surrounding aquatic environment. The receiving waters can potentially experience an increase in temperature and a decrease in photosynthesis as a direct result of the addition of these brown coloured effluents. In production of 1 ton of paper 150 m³ of effluent is generated, which is heavily loaded with organic compounds depending on nature of the raw material, process and chemicals used, finished product, and extent of water reused. The effluent generated at the pulping stage, called black liquor, contains various compounds like dissolved lignin and its degradation products, hemicelluloses, resin acid, fatty acids, tannins and phenols (Ali & Sreekrishnan 2001, Lara & Rodriguez-Malaver 2003, Malaviya & Rathore 2007). These organic compounds are also responsible for giving the effluent its characteristic dark brown colour and toxicity. Thus, it is obligatory to treat the effluent before disposal. Overall pollution effects of discharging raw wastewater from a paper mill are:

1. Oxygen depletion in the receiving body of water.
2. Presence of undesirable colour, odour and taste in the water.
3. Reduced photosynthesis.
4. Formation of blanket of suspended solids settling at the bottom of the receiving body of water.
5. The death of fish.
6. Toxicity added to the aquatic life due to formation of mercaptans, pentachlorophenol, sodium pentachlorophenate, etc.

These effluents are usually treated in aerated lagoons and activated sludge by biological processes, which reduce the chemical oxygen demand (COD) and the biochemical oxygen demand (BOD), but are not efficient in reducing colour, chlorophenols and high-molecular-weight chlorolignins (Cammarota & Avaliacao 1991). Many studies have demonstrated that the colour and the chlorinated organic compounds of kraft effluents can be reduced by white-rot fungi, which are considered promising for the treatment of these effluents (Cammarota & Avaliacao 1991). White rot fungi have the capacity to metabolize both lignin and its derivatives and therefore, have potentials in the lignin/phenolic wastewater treatment (Eaton & Chang 1982). These organisms have ability to perform decolourisation as well as to reduce absorbable organic halides and chemical oxygen demand (COD) (Livernoche et al. 1983). They produce extracellular oxidative enzymes, like lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. These enzymes are presented as the most functional in the biodegradation of lignin.

OBJECTIVES

- Development of an environmentally-friendly technology and cost-effective way for treatment of pulp and paper industrial effluent.
- Reduction of the environmental impact of wastewaters from the pulp and paper industry by using microorganisms (algae, bacteria, fungi) with special emphasis on wastewater biodecolourisation, biodegradation and bioremediation.
- Utilization of wastewaters from the pulp and paper industry to obtain valuable products such as enzymes (xylanases), single cell protein (SCP) and/or high-value fatty acids (gamma-linolenic acid).
- Reuse/recycling of treated effluent/feasibility studies.

GENERAL CHARACTERISTICS OF PULP AND PAPER INDUSTRY EFFLUENT

High lignin content: The most important raw material for the production of pulp and paper is wood. Main element groups of wood are lignin, cellulose and hemicelluloses. Mainly lignins are degraded, heavily modified, chlorinated and finally dissolved in the effluent. Therefore, the effluent from such a process is dark brown due to content of chromophoric, polymeric lignin derivatives. The products of lignin transformation, primarily lignin peroxidase (Lip), are the main contributors of effluent colour in paper mill wastewaters (Perez et al. 1998). However, lignin degradation is dependent on the presence of a readily metabolizable co-substrate, such as glucose.

High absorbable organic halide (AOX) concentration: The content of chlorinated substances in effluents are usually ensured as total organically bound chlorine (TOCl) or high absorbable organic halide (Eriksson 1993). The molecular mass of chloroorganics has very serious effect when toxicity, biodegradation and decolourisation of effluent are examined (Martin et al. 1995).

Colour: Dyes and pigments are highly visible material. Thus, even minor release into the environment may cause the appearance of colour, for example, in open waters, which attracts the critical attention of public and local authorities.

Table 1: Yield of pulp from different sources.

Raw Material	Yield (%)
Rags	70-80%
Esparto grass/Straw	40-45% 40-50%
Wood(using sulphite process)	40-50%
Waste paper, waste fibers, bagging	70-90%
Bamboo	40%
Jute	50%
Bagasse	50%

In last few decades, the colour problems associated with pulp and paper mill wastewater have got a pretty important role in research (Dilek & Bese 2001). The colour causes an aesthetic problem and contributes to the BOD (Bajpai & Bajpai 1994). Pulp and paper wastewater treatment by the various steps involving biological technologies are tended towards removing BOD, colour is at best incompletely reduced (Perez et al. 1998). Moreover, the lignin derivative colour remains firm and unchanged in spite of chlorinated organic removal (Tarlan et al. 2002).

COD/BOD ratio: BOD represents only the organic matter which is capable of being degraded/oxidized by microbes whereas COD represents all the oxidizable matter, including organic matter in any particular effluent (Marmagne & Coste 1996). The low molecular weight fraction of the chlorolignins is the main contributor to the effluent BOD and acute toxicity. The high molecular weight chlorinated compounds contribute little to BOD and acute toxicity due to their inability to pass through cell membranes. They are the major contributor to effluent colour, COD and chronic toxicity (Eriksson et al. 1985).

Potential toxicity problems: The low molecular weight phenolics and their methylated counterparts, which are more lipophilic, cause toxicity and are bioaccumulable in fish (Kolar et al. 1985). However, Archibald et al. (1990) have reported that toxic levels of low-molecular weight chlorinated compounds do not accumulate during the natural degradation of chlorolignins. They found that the effluents indeed appear to stimulate the growth of algae and primary consumers, probably because of the nutrients they contain. About 75% of the dissolved organic material, 60% of the COD load, 40 to 50% of the organically bound chlorines, and 80% of the colour-imparting substances of bleach plant effluents are reportedly contributed by extraction stage effluents. The organic compounds in these effluents, mainly the chlorinated phenols, are toxic to aquatic organisms and resistant to microbial degradation.

Characteristics of raw wastewater: The wastewater is usually alkaline in nature and has high suspended solids, high total solids, high COD and relatively low BOD. The approximate analysis of wastewater from typical pulp and paper mill is given in Table 2.

Treatment of pulp and paper industrial effluent by bacteria and ligninolytic fungi: Over the past decade, many fungal strains have been studied for their abilities to degrade a wide variety of structurally diverse pollutants. White-rot fungi produce a large variety of extracellular enzymes like laccase, lignin peroxidase, phenol oxidase, Mn dependent peroxidase and Mn-independent peroxidase, which decompose the highly stable natural compounds such as lignin,

hemicellulose, cellulose, etc. (Moreira et al. 2003). For that purpose, white-rot fungi show as a valuable alternative because they are capable of oxidising compounds of complex structure such as lignin (Kirk & Farrell 1987). This feature is based on the extracellular secretion of highly oxidative oxidases and peroxidases; the most outstanding enzymes being lignin peroxidase (LiP) and manganese peroxidase (MnP) (Tien & Kirk 1988). The treatment of pulp and paper mill effluent has been studied using a number of fungi from quite some time. The enzyme system of the white rot fungi includes a group of nonspecific extracellular enzymes, which catalyse not only degradation of lignin and chlorolignins but also oxidation of several constant aromatic and halogenated compounds like lindane, DDT, PCP, benzopyrene, creosote, coal tars and heavy fuels, etc. (Eaton et al. 1982).

There are several investigations of potential ability of fungi for treatment of pulp and paper wastewater effluent. Recent studies using ligninolytic enzymes from filamentous fungi as *Aspergillus fumigatus* and *A. flavus* showed a removal from the paper effluent colour (Sahoo & Gupta 2005). In this case, *A. fumigatus* was able to decolorize 55.5% under stationary condition and 89.3% under shaking conditions, while *A. flavus* removed 53.5 and 84.0%, respectively, in the same conditions. The difference in the colour reduction was related to differences in aeration between stationary and shaking flasks conditions. Basidiomycetes were also applied in the paper effluent aiming decoloration, as *Pleurotus sajor-caju*, *P. platypus* and *P. citrinopileatus* (Ragunathan & Swaminathan 2004). After 6 days, *P. sajor-caju* decolorized 66.7% of the effluent. These studies show a decolorization between 55% and around 90%. However, in order to obtain such efficiencies, it is necessary 3 to 6 days of treatment, which cannot be applied industrially. *Aspergillus foetidus* was studied for its ability to remove colour, COD and lignin from bagasse-based pulp and paper mill wastewater (Sumathi & Phatak 1999). Nearly 90-95% of the total colour was removed, and the simultaneous reduction in colour and lignin level indicated a strong correlation between the decolorization and lignolytic processes. The degradation abilities of *Oxysporus* sp., *Phanerochaete chrysosporium* and *Schizophyllum commune* were evaluated for the removal of lignin from olive pomace (Haddadin et al. 2002). Among the three fungi, *Oxysporus* sp. was the most

effective for lignin degradation with efficiency of 70%, whereas *P. chrysosporium* achieved lignin removal of 60%. The effluent generated at the pulping stage (black liquor) contains various compounds like dissolved lignin and its degradation products, hemicelluloses, resin acid, fatty acids, tannins and phenols (Ali & Sreekrishnan 2001, Lara & Rodriguez-Malaver 2003, Malaviya & Rathore 2007).

Several researchers have examined biological treatment of black liquor by using various pure bacterial strains. The degradation efficiency of lignin in black liquor was 70-80%, and COD removal efficiencies of 70-80% were achieved with *Pseudomonas putida* (Sahoo & Gupta 2005) and *Acinetobacter alcoaceticus* (Jain et al. 1997). In a batch test on the treatment of black liquor from a Kraft pulp and paper mill by *Aeromonas formicans*, around 71% of COD and 78% of lignin were removed (Waraporn et al. 2006). Previous work has focused mainly on the decolorization of black liquor and the degradation of lignin by suspended- growth of white rot fungi (Garcia et al. 1987). Kerem et al. 1992). Among the biological methods tried so far, in this review we comprise some of current investigation (Table 3) using wood-degrading white rot fungi, which have the potential to successfully treat pulp and paper effluents. In case of pulp and paper industries, *Phanerochaete flavidoalba* (Perez et al. 1998), *Coriolus versicolor*, *Aspergillus niger* (Khanongnuch et al. 2004) and *Schizophyllum commune* (Kannan & Oblisami 1990) have shown to be able to remove the residual recalcitrant colour efficiently from paper and pulp mill effluents. Some white rot strains such as *Ceriopsis subvermispora* could decolourize Kraft-bleaching effluent at 90% and also resulted in reduction of COD up to 45%. However, higher percentages of COD, BOD and total solids reduction in pulp mill wastewater have been reported using a reduction-biological technique (Ghoreishi & Haghghi 2007).

Santos et al. (2002) used the ligninolytic fungus *Pleurotus ostreatoroseus* to treat the first alkali extraction stage (E1) effluent of a Kraft bleach plant. The best results were obtained with 20% fungus and shaking of the effluent. During the last three days of the experiment, the average percent removal of colour, total phenols and lignin/chlorolignin was 84.4±6.1, 82.1±5.7 and 72.4±8.9, respectively. The results indicate the potential of *Pleurotus ostreatoroseus* for use in the treatment of E1 effluent (Santos et al. 2002). In previous works some other authors used diluted E1 effluent without an extra carbon source and the fungus *Lentinus edodes*, and obtained 73% colour removal after five days of treatment (Esposito et al. 1991). Livernoche obtained 60% decolorization using fungus *Coriolus versicolor* in liquid culture, and 80% with the fungus immobilized on calcium. They used E1 effluent enriched with glu-

Table 2: Characteristic of raw wastewater.

Raw Material	Yield (%)
pH value	8.0-9.0
Total solids, mg/L	1500-2500
Suspended solids, mg/L	600-1500
COD, mg/L	300-2500
BOD ₅ , mg/L	150-1000

cose (Livernoche et al. 1983). These experiments are comparable to those obtained by Wang who used the fungus *Ganoderma lacidum*. In experiments with the E1 effluent diluted and enriched with nutrients and 5 g/L of glucose, the authors obtained 88% colour reduction after four days of treatment (Wang et al. 1992).

Singhal & Thakur (2009) have isolated 8 fungal isolates and 4 bacterial strains from pulp and paper effluent. Among fungi, *Paecilomyces* sp. exhibited significant reduction in phenol (40%), lignin (66%), COD (75%), colour (81%) in 100 L bioreactor supplemented with 1% carbon and 0.2% nitrogen source at 6 h retention time. Treated effluent of fungus subsequently treated by bacterial strain *Microbrevis luteum* showed reduction in phenol 77%, colour 84%, COD 83% and lignin 72% in one day (Singhal & Thakur 2009). They conclude that fungus is more efficient than bacteria in colour removal and less in chlorinated phenol.

Prabu & Udayasoorian (2005) have reported that white rot fungus *Trametes versicolor* was capable of decolorization and degradation of phenol from paper mill effluent. There was 76% effluent depolarization along with 78% COD reduction. The effluent chlorinated phenol degradation was 85% by *Trametes versicolor* when added with 1% glucose as co-substrate (Prabu & Udayasoorian 2005).

Juan Wu et al. (2005) have investigated the lignin degrading capacity of attached-growth white rot fungi. Five white rot fungi, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Lentinus edodes*, *Trametes versicolor* and S22, grown on a porous plastic media, were individually used to treat black liquor from a pulp and paper mill (Juan Wu et al. 2005). Over 71% of lignin and 48% of COD were removed from the wastewater. Among that three white rot fungi, *P. chrysosporium*, *P. ostreatus* and S22 showed high capacity for lignin degradation at pH 9.0-11.0. The addition of 1 g

Table 3: Comparison of fungi used to treat wastewater effluent of pulp and paper industry, optimal culture conditions and the effect of fungal pretreatment.

Organisms	Waste water analysis	Optimization experimental conditions	Reactor medium handling, Co-substrate	Parameters	References
Fungi: <i>Trichaptum</i> sp., <i>Datronia</i> sp. and <i>Trametes</i> sp.	pH 5.2, Temperature 24.1 °C, DO 4.75 mg/L, TSS 75 mg/L, TDS 4065 mg/L, COD 1154 mg/L Color (Pt Co) 1317	30°C with shaking. speed of 135 rpm for 7 days	Shake-flask; Effluent from fungal mycelium + pulp and paper + co substrate (glucose 10 g, K ₂ HPO ₄ 1g, MgSO ₄ .7H ₂ O 0.5 g, KCl 0.5 g, FeSO ₄ .7 H ₂ O 0.01 g and NH ₄ NO ₃ 1.75 g per 1 L effluent)	Decolorization: <i>Trichaptum</i> sp. 54.4% <i>Datronia</i> sp. 54.9% <i>Trametes</i> sp. 53.7% Much higher COD value	Waraporn Apiwatanapiwat et al. (2006)
Fungi: <i>Trametes versicolor</i>		Centrifuged at 7000 rpm for 10 min. CU values measured for 7 days after inoculation at 465 nm by spectrophotometer	Inoculated flask; homogenized fungal mycelia mats + effluent + cosubstrate; carbon source (glucose, fructose, starch) Nitrogen source (ammonium sulphate, sodium nitrate, diammonium phosphate)	Decolorization: 76% (glucose + ammonium sulphate) COD reduction: 78% (glucose + ammonium sulphate) Chlorinated phenol degradation: 85% (when add 1% glucose)	Prabu & Udayasoorian (2005)
Bacteria: <i>Bacillus pumilus</i> and <i>Paenibacillus</i> sp.	The colour of the untreated effluent at pH 7.0, 9.0 and 11.0 respectively were, 887, 781 and 597 at Pt-Co scale. COD in the untreated effluent was 2158, 2222 and 2298 mg/mL, when the pH was corrected to 7.0, 9.0 and 11.0 respectively	Shaking at 200 rpm at 45°C during 24 h or 48 h, and centrifuged at 10.000 rpm under refrigeration	Shake-flask; effluent + bacteria inoculation (8% v/v, equivalent to 108/mL) Gel permeation chromatography	Decolorization: 42.30% for <i>Paenibacillus</i> sp. and 41.87% for <i>B. pumilus</i> COD reduction of 22% at pH 9.0 for both bacteria (after 24, 48h) GPC: peak areas were reduced nearly 60% and 70% with <i>B. pumilus</i> , and <i>Paenibacillus</i> sp. respectively	Patricia Lopes de Oliveira I et al. (2009)

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Fungi: <i>Datronia</i> sp. KAPI0039	Temperature 25.8 °C pH 7.7 Brown Color, Pt-Co unit 1,400 Turbidity, FAU < 10 BOD 560, mg/L COD 1120, mg/L Dissolved solids, 2,100 mg/L TSS 35, mg/L	Shaken at room temperature for a period of studied time.	Shake flask; Fungal mycelium + effluents with essential nutrients and co- substrate (glucose 10 g, K ₂ HPO ₄ 1 g, MgSO ₄ ·7H ₂ O 0.5 g, KCl 0.5 g, FeSO ₄ ·7H ₂ O 0.01 g and NH ₄ NO ₃ 1.75 g per L of effluent)	Decolorization: 89.8 to 90.0% COD reduction: 34.5 to 40.4%, Highest enzyme activity for MnP (6.3 unit/L) and for Laccase (5.8 unit/L) No LiP activity detected	Torpong Kreetachat et al. (2007)
Fungi: <i>Cryptococcus</i> sp.		Fungi + 10% MSM effluent incubated at 30°C, pH 7 in a rotary shaker, rpm 125 for 10 days Taguchi approach: temperature (30–35 °C), shaking condition (125 rpm), dextrose (1.0% w/v), tryptone (0.1% w/v), inoculum size (7.5% w/v); pH 5 and duration 24 h	Shake flask; 10% MSM effluent i.e. MSM (in g/L): Na ₂ HPO ₄ ·2H ₂ O, 7.8, KH ₂ PO ₄ , 6.8; MgSO ₄ , 0.2; Fe(CH ₃ COO) ₃ NH ₄ , 0.01; Ca(NO ₃) ₂ ·4H ₂ O, 0.05) having 10% effluent, Co-substrate (sucrose, dextrose, sodium acetate and sodium citrate) and nitrogen sources (yeast extract, tryptone, peptone and sodium nitrate)	Decolonization 50–53% at optimum conditions Lignin reduction: 35- 40%, pH from 5 to 6 had most significant effect, Temperature from 30°C to 35°C had no effect, toxicity by alkaline single cell (comet) gel electrophoresis (SCGE) assay using <i>Saccharomyces</i> <i>cerevisiae</i> MTCC 36 as model organism, indicated 45% reduction.	Singhal & Thakur (2009)
Fungi: <i>Daedaleopsis</i> sp., <i>Schizophyllum</i> <i>commune</i> PT, and <i>S.</i> <i>commune</i> SL, <i>Phanerochaete</i> <i>chrysosporium</i>		Fungi + effluent incubated at 35°C in an orbital shaker (130 rpm), wastewater from the pulping process (wastewater No. 1) with (pH 8.07, COD 4347 mg/L) and wastewater from the pulping process combined with that from the paper recycling process (wastewater No. 2) with (pH 6.94, COD 4000 mg/L)	Shake flask, Cell pellet of fungi + wastewater (No. 1 or No. 2) + 1% (w/v) glucose and 0.12% (w/v) NH ₄ Cl	Decolorization: <i>Daedaleopsis</i> sp. No. 1 (52%) <i>P. chrysosporium</i> No. 2 (86%), COD: all fungi by 59-71% (No. 1) and 66-83% (No. 2). All tree fungi show Laccase activities, only <i>Phanerochaete</i> <i>chrysosporium</i> show MnP activity	Shehanat Prasongsuka et al. (2009)

of glucose and 0.2 g of ammonium tartrate was beneficial for the degradation of lignin by the white-rot fungi studied. Waraporn et al. (2006) have reported three fungus isolates, which were able to grow well on agar containing synthetic lignin indicating that they could utilize synthetic lignin as nutrient for their growth. These fungal strains include *Trichaptum* (KAPI25), *Datronia* (KAPI39) and *Trametes* (KAPI50), which were isolated among 64 fungal strains. They were the most efficient strains able to decolorize the pulping wastewater by 54.4, 54.9 and 53.7%, respectively (Waraporn et al. 2006).

Torpong et al. (2007) have reported the use of white rot fungi *Datronia* sp. (KAPI0039) for decolorization and or-

ganic removal of the pulp and paper effluents and found 89.8 to 90.0% and 34.5 to 40.4% colour and COD removal within 4-7 days respectively. This experiment confirmed that *Datronia* sp. (KAPI0039) could remove colour or able to degrade lignin-derived compounds in the effluents. For the period of time, the ligninolytic enzymes MnP and laccase were produced during the decolorization process whereas LiP was not detected.

Singhal & Thakur (2009) isolated three fungal strains from sediments of pulp and paper mill in which PF7 reduced colour (27%) and lignin content (24%) of the effluent on 5th day. PF7 was identified as *Cryptococcus* sp. The process of decolourisation, optimized in shake flask experiments, by

Taguchi approach indicated optimum conditions: temperature (30-35 °C); shaking condition (125 rpm); dextrose (1.0% w/v); tryptone (0.1% w/v); inoculum size (7.5% w/v); pH (5) and duration (24 h). Overall evaluation criterion (OEC) value before optimization was 32.3. There was 38% improvement in the process with final OEC value, 44.6±2.02 at optimum conditions. The colour content of the effluent reduced by 50-53% and lignin content 35-40% after treatment at optimum conditions. Variation in pH from 5 to 6 has most significant effect on decolourisation (72%) while variation in temperature from 30°C to 35°C has no effect on the process. Treated effluent was further evaluated for toxicity by alkaline single cell (comet) gel electrophoresis (SCGE) assay using *Saccharomyces cerevisiae* MTCC 36 as model organism, indicated 45% reduction. The results showed significant reduction in colour, lignin and toxicity of the effluent and this process can be scaled up to industrial level. However, very few studies have optimized the process. Studies related to the interaction of various factors among them are lacking. For *Cryptococcus* sp., there was no significant effect of temperature variation (30-35°C) on effluent treatment. Hence, temperature was dropped from the analysis. The most suitable shaking condition was 125 rpm. One possible reason may be very rapid movement leads to the shear and tear of fungal mycelium (Mandels & Stenberg 1976, Broche-Due et al. 1994).

Sehanat et al. (2009) collected eight isolates, which could be cultured to test for thermotolerance and to screen for the presence of ligninolytic enzymes using *Phanerochaete chrysosporium* as a reference. Of the eight isolates, only three species *Daedaleopsis* sp., *Schizophyllum commune* PT, and *S. commune* SL were able to grow above 40°C. All the three species exhibited 2, 20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)-oxidizing activity on ABTS agar plates. Only *P. chrysosporium* could oxidize manganese on MnCl₂ agar plates. Both *P. chrysosporium* and *Daedaleopsis* sp. were able to decolourize wastewater on wastewater agar plates. All fungal cell suspensions tested decolourized wastewater No. 1 (pH 8.07, COD 4347 mg/L) from the pulping process, and wastewater No. 2 (pH 6.94, COD 4000 mg/L) from the pulping process combined with that from the paper recycling process. *Daedaleopsis* sp. and *P. chrysosporium* exhibited the highest ability to decolourize wastewater No. 1 (52%) and No. 2 (86%), respectively. Laccase activities were detected in the decolourized effluents and all fungi tested reduced the COD by 59-71% (No. 1) and 66-83% (No. 2). The use of bacterial enzymes for effluent colour removal in the paper industry was reported previously (Joyce et al. 1984, Chairattananokorn et al. 2006, Nimchua et al. 2008). Bacterial aerobic and anaerobic treatment systems can reduce the BOD and are able to remove the dark

colour in the effluents as verified previously (Chairattananokorn et al. 2006, Nimchua et al. 2008). In a current study, Patricia Lopes de Oliveira et al. (2009) applied *Bacillus pumilus* and *Paenibacillus* sp. on the paper mill effluent to investigate the colour removal. Inocula were individually applied in effluent at pH 7.0, 9.0 and 11.0. The real colour and COD removal after 48h at pH 9.0 were 41.87% and 22.08% for *B. pumilus* treatment and 42.30% and 22.89% for *Paenibacillus* sp., respectively. Gel permeation chromatography was used to verify the molar masses of compounds in the non-treated and treated effluents showing a decrease in compounds responsible for the paper mill effluent colour.

CONCLUSION

In general, the high volumes and complexity of effluent produced by the pulp and paper industry present formidable problems for the treatment of these wastewaters. Biological treatment methods that utilize ligninolytic microorganisms, especially white-rot fungi, which are able to degrade lignin, cellulose, and hemicellulose concurrently at approximately equal rates. These fungi secrete three extra cellular enzymes, which are critical for wood degradation, but the requirement for a co-substrate and the need for aeration would seriously hamper their utilization from an economic perspective.

Cultivation conditions have an important impact on the efficiency of white-rot fungi during biological treatment of bleach plant effluent. Greater biodegradation efficiencies are required in order to develop a practical biotreatment process. Any way, the possibility of wastewater bioremediation prior to wastewater discharge has been demonstrated. The use of continuous biosystems such as trickling filters, rotating biological contactors and activated sludge systems have been shown to reduce the environmental impact of these wastewaters. The most efficient treatment system was proved to be the rotating biological contactor where colour, bacterial growth inhibition levels, adsorbable organic halogen and chemical oxygen demand were decreased to a significant extent. Definite differences in the decolouring mechanisms between the white-rot fungi (adsorption + biodegradation) and the mucoralean fungi (adsorption) have been observed.

Because of the complexity of treating paper mill effluent, combinations of physical, chemical and biological treatment strategies might lead to a synergistic beneficial outcome that would facilitate the development of economic and efficient treatment procedures.

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