Waste Water Bioremediation in the Pulp and Paper Industry

L Christov\(^1\,^2\)* and B van Driessel\(^1\)

\(^1\)Sappi Biotechnology Laboratory, Department of Microbiology, Biochemistry and Food Science, University of the Free State, P O Box 339, 9300 Bloemfontein, South Africa
\(^2\)Sappi Forest Products Technology Centre, Sappi Management Services, P O Box 3252, 1560 Springs, South Africa

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Effluents from the pulp and paper industry contain chromophoric compounds and can be partly mutagenic and inhibitory to aquatic biosystems. The presence of various pollutants produced during pulp and paper manufacturing necessitates the need for waste water pretreatment prior to discharge. Of all the methods investigated, bioremediation specifically holds promise in solving environmental problems in a cost-effective way. White-rot fungi have ability to process a variety of pollutants efficiently, however, development of suitable cultivation procedures has delayed industrial application. These and other issues affecting bioremediation of industrial wastewater, with special reference to application thereof in the pulp and paper industry, are reviewed in this paper.

Key words: bioremediation, waste water, pulp and paper industry, white-rot fungi, decolourization

Introduction

The release of the bleach plant waste waters with high adsorbable organic halogen (AOX) levels into the receiving waters has become one of the major environmental problems for the pulp and paper industry. Efforts are made to reduce the chloroorganic and chloride discharges by the substitution of chlorine-based bleaching chemicals with other more environment friendly agents. The waste waters containing chlorolignins can be highly coloured mainly due to the presence of solubilised lignin and its derivatives. Some of the chlorolignins of low molecular mass have mutagenic and toxic properties and are known to be highly resistant to biodegradation and to accumulate in aquatic organisms (Ander et al, 1977). In addition, a high concentration of chlorides in the waste waters contribute to their corrosiveness.

In response to the environmental concerns and stringent emission standards, modifications of the production process at the pulping and bleaching stages include the cooking time for additional lignin removal, introduction of oxygen delignification as a pretreatment step, and elemental chlorine-free (ECF) and totally chlorine-free (TCF) bleaching. Physical and chemical methods of removing chloroorganics, although quite effective in decolourisation of pulp and paper mill waste waters, are unattractive for industrial applications because of the high costs involved. However, biological methods of effluent treatment have the advantage of being cost-effective and in addition to colour removal, they can also reduce both the biological oxygen demand (BOD) and chemical oxygen demand (COD) of the waste water (Eaton et al, 1980).

Bioremediation in the Pulp and Paper Industry

The pulp and paper industry discharges waste water, which usually contains halogenated organic materials that can pose environmental problems (Wang et al, 1992; Kallas & Munter, 1994). Chlorinated organic compounds, produced during the chlorination bleaching phase, are made soluble in dilute alkali and extracted from the pulp in the subsequent extraction phase (Prasad & Joyce, 1991). Chloroorganics are often toxic to aquatic life, many are genotoxic and have the potential to migrate widely throughout the ecosystem, ultimately accumulating in the fatty tissues of organisms (Suntio et al, 1988). During bleaching, chromophoric and highly oxidised, polymeric lignin derivatives are formed that give rise to a dark colourisation in the waste water (Livernoche et al, 1983; Bergbauer et al, 1991). The colour poses an aesthetic problem and contributes to BOD (Bajpai & Bajpai, 1994). Conventional biological treatments cannot effectively remove the colour attributed to the recalcitrance of chlorolignins and the difficulty in
mineralising these compounds (Boman & Frostell, 1988; Prasad & Joyce, 1991; Mehna et al., 1995). White-rot fungi have the ability to degrade lignin and its chlorinated derivatives effectively (Eriksson, 1991; Baijpai & Baijpai, 1994). However, lignin cannot serve as the sole carbon source for these fungi because its degradation is apparently a very energy-intensive process (Leisola et al., 1983; Boman & Frostell, 1988). The specific cultivation conditions, necessary for white-rot fungi to degrade lignin (Boman & Frostell, 1988), have been devised such as the patented Mycor process, continuous flow systems (Prasad & Joyce, 1991), immobilization of white-rot fungi (Livernoche et al., 1983; Kirkpatrick et al., 1990), etc. Other organisms such as soil inhibiting fungi imperfecti (Rodriques et al., 1996), algae (Lee et al., 1978) and streptomyces strains (Hernandez et al., 1994) have also been studied for their ability to decolourise chromophoric substances. White-rot fungi produce a variety of ligninolytic enzymes, including peroxidases and laccases (Lamar, 1992; Manzanares et al., 1995). These enzymes convert and biotransform phenolic or polyphenolic substances, and can be used to treat waste waters (Paice & Jurasek, 1984; Roy-Arcand & Archibald, 1991; Al-Kassim et al., 1994; Limura et al., 1996). The use of membrane reactors for the immobilization of biomass and/or enzymes leads to increased stability and allows continuous use of enzymes (Van der Walt et al., 1997). Adsorption of coloured compounds on biomass is reported to occur during cultivation (Livernoche et al., 1983; Royer et al., 1985; Feijoo et al., 1995). Oxidised phenolic compounds have a high affinity for chitosan (Muzzarelli, 1994), which exhibits polycationic characteristics at acidic pH-levels that could facilitate sorption of anionic substances. Chitosan-coated hollow-fibre membrane process have been used for the bioremediation of a phenolic containing waste water (Edwards et al., 1997). Certain physico-chemical methods for the treatment/pre-treatment of waste water (Kallas & Munter, 1994) include adsorption using ion exchange resins or activated carbon, ultra-filtration, chemical precipitation (Boman & Frostell, 1988), oxidation (ozonation, hydrogen peroxide, etc.) using chemical methods (Feijoo et al., 1995), and photo-catalysis, involving irradiation of titanium oxide (Sierka & Bryant, 1994). However, the decolourisation of paper mill effluents has not been completely solved because the technical difficulties encountered when cultivating white-rot fungi and the problems of setting up an effective and economically viable process for bioremediation of paper and pulp waste waters (Manzanares et al., 1995).

Chemical Composition of Waste waters

The content of chlorinated substances in waste water is usually measured as total organically bound chlorine (TOCl) or AOX (Eriksson, 1993). Chloroorganic material from pulp mill waste water is generally described in terms of their molecular mass. The molecular weight of these materials has serious implications in terms of biodegradability, toxicity and colourisation (Martin et al., 1995; Jokela & Salkinoja-Salonen, 1992).

High Molecular Mass (HMM) Compounds

More than one-half of the colour load from bleach plant waste water originates from HMM lignin-derived material in the alkaline extraction stage (Paice & Jurasek, 1984). Waste water from the extraction stage accounts for 80% of the colour, 30% of the BOD and 60% of the COD of the mill pollution load (Prasad & Joyce, 1991). The HMM substances are generally believed to be stable and therefore biologically inert (Jokela & Salkinoja-Salonen, 1992). However, HMM material in spent liquors from the chlorination and alkali extraction stages of bleaching softwood kraft pulp were chemically unstable under conditions prevailing in receiving water systems, because these materials slowly released chlorinated catechols and guaiacols (Eriksson et al., 1985). Martin et al. (1995) concluded conflicting results on the aromatic nature of HMM compounds, whereas both association by adsorption and chemical bonds might account for the presence of some chloro-aromatics in HMM material. With HMM fractions (>30,000 Da), sequential depolymerisation does not necessarily lead to a complete degradation of aromatic compounds (Bergbauer et al., 1991). Once degradation of lignin derivatives by Trametes versicolor had reached a certain level, neither additional cosubstrates, nor inoculation of the culture filtrate with fresh mycelium, could induce any further degradation. Thus, it seems that the end result of using this fungus could be the accumulation of highly recalcitrant compounds in the ecosystem.

Low Molecular Mass Compounds

Certain compounds (chlorinated phenols, quaiacols, catechols, chlorosyringols and chloroaliphatics) in
pulp and paper mill effluents (mole mass, < 1000 g/mol) can be acutely toxic to aqueous organisms (Limura et al, 1996; Roy-Arcand & Archibald, 1991). Such compounds are divided into acidic, phenolic and neutral categories (Suntio et al, 1988). Chlorinated phenols in bleach waste waters are typical chlorinated lignin degradation products that contain methoxyl groups. The predominant compounds of the neutral fraction are chloroform, chlorodimethylsulfoines and chloroacetones, while minor concentrations of additional chlorinated hydrocarbons, ethers, ketones, aldehydes, lactones and thiophenes have also been detected (Smith et al, 1994). Chlorinated acidic compounds in bleaching waste water are largely aliphatic acids, mainly chloroacetic acid. Although a variety of low molecular substances do occur in the waste water streams, most organic chlorine present is associated with HMM material (Smith et al, 1994).

Cultivation Strategies for Decolourisation of Waste Waters

None of the traditional biological methods (aerated lagoons, activated sludge systems and anaerobic cultivation) can degrade HMM chromophoric compounds effectively (Boman & Frostell, 1988; Archibald et al, 1990). Aerated lagoons can reduce TOCl by about 25% at relatively high costs. Activated sludge systems can reduce chlorinated compounds by 40%, a substantial part of which is adsorbed onto the surface of the biomass, which could lead to disposal problems of excess sludge. Ultrafiltration of the E-stage waste water seems promising, but this will also result in a disposal problem (Boman & Frostell, 1988). Anaerobic treatment have gained popularity in the pulp and paper industry because of low energy consumption and the low production levels of well stabilised excess sludge. However, bleach plant waste waters are more diluted than desired for a low retention time and the varying waste water composition can lead to toxic shock conditions, detrimental for the efficient functioning of the anaerobic systems. Boman & Frostell, (1988), Anaerobic treatment have gained popularity in the pulp and paper industry because of low energy consumption and the low production levels of well stabilised excess sludge. However, bleach plant waste waters are more diluted than desired for a low retention time and the varying waste water composition can lead to toxic shock conditions, detrimental for the efficient functioning of the anaerobic systems. Boman & Frostell, (1988), by combining ultrafiltration of the E-stage waste water and incorporating permeate with the chlorinated waste water, followed by anaerobic filter treatment, achieved an overall reduction of 62% in chloroorganics. However, they observed that white-rot fungi can degrade both high and low molecular weight compounds. One major drawback is the need for the addition of an easily degradable, inexpensive carbon source and the complicated physiological demands of some of these fungi when degrading lignin. The requirements for high oxygen tension and a growth substrate can impede the practical implementation of fungal decolourisation (Eaton et al, 1982). Various parameters and cultivation strategies need to be considered in an effort to develop a successful treatment process.

The Mycor Process

In the Mycor-process, called a FPL/NCSU Mycor method, the fungus, *P. chrysosporium* is immobilized on the surface of rotating disks that can be enclosed for additional oxygen supply (Eaton et al, 1982). Before decolourisation commences, a growth stage is necessary so that nutrient nitrogen is depleted and the fungus becomes ligninolytic. The Mycor process reduced colour in an alkaline stage spent liquor by 80% and furthermore converted 70% of the organically bound chlorine into chloride at a hydraulic retention time of 2 days (Boman & Frostell, 1988). Although steam treatment initially might be beneficial, further aseptic procedures would not be required with fungi like *P. chrysosporium*. Waste waters (temp, 28-40°C; pH, 4.5), in laboratory scale bench reactors, could be treated in over 60 days. Some limitations of the Mycor process seem to be the poor surface to volume ratio since mycelia is in constant contact with substrate to an extent of only about 40%. Moreover, the mycelia is present in a thick layer that could result in deficient oxygen and nutrient supply and a lower productivity in general.

Continuous-flow Systems

Three continuous-flow laboratory decolourisation strategies were examined (Prasad & Joyce, 1991) as follows:

**Alternative I.** It consisted of a set-up similar to a conventional oxidation basin with a surface area of 510 cm² for fungal cultivation. Fungal mats of *Trichoderma* sp, were transferred to the reactor and decolourisation was carried out without aeration.

**Alternative II.** It utilized a vessel in which the height was increased by decreasing the surface area and four baffles were introduced to divide the vessel into compartments. *Trichoderma* packed in synthetic wire bags were suspended in the middle of each zone and continuous aeration supplied.

**Alternative III.** It was similar to the Mycor process in that fungal mats were clasped between circular
wires, supported by outer central rings and securely fixed in a metallic flame. The discs were rotated continuously.

These systems could reduce colour by at least 50% for the first 6 days. However, Alternative III gave the best results with a total delolourisation of extraction-stage effluent of more than 78% as well as a COD reduction of 25%. Colour removal targets in excess of 57% were sustained for 18 days with Alternative III.

**Fungal Pellets**

_Coriolus (Trametes) versicolor_ as mycelial pellets was used to decolourise lignin-containing kraft E-stage waste water (Royer et al, 1985). Decolourisation was optimum (temp, 25-30°C; pH, 4-5) and practically non-existent at 40°C. It corresponded to the temperature of the liquor waste water at the E-stage. Magnesium ions improved the consumption of chromophores, which might indicate that an enzymatic mechanism took place since magnesium ions serve as activators of many enzymes. At an initial colour level of 7000 colour units (CU), the mean colour removal proceeded at a rate of 300 CU/g mycelium/hr. The authors suggested the use of airlift reactors employing the pelleted form of the fungus with the possibility of minimising operational costs. This strategy would facilitate recycling and the use of large amounts of fungal biomass. Mehna et al (1995) were able to achieve a colour reduction of 92% with a COD elimination of 69% after 7 days using _T. versicolor_.

**Flask Cultivations**

_Trichoderma_ sp. was initially cultivated in shake flasks and washed. Under optimal conditions (pH, 4.0; carbon source, glucose), this fungus decreased the colour of kraft bleach plant waste water by 85% (68.6% without using additional carbon source) and reduced the COD by 25% after 3 days of incubation. Other carbon sources such as pulp and pith, which are abundant and inexpensive, increased the decolourisation after 6 days, since they were not metabolised immediately. Therefore, treatments should be carried out strictly under defined conditions (Prasad & Joyce, 1991).

**Fermentor Cultivations**

_Trametes (Coriolus) versicolor_ was cultivated in a laboratory fermentor with 0.8% glucose and 12 mM ammonium sulphate at a controlled pH level of 5.0 under optimal aeration. This resulted in 88% reduction in the colour units within 3 days, whereas 80% reduction of waste water was observed in flask cultures after 6 days (Bergbauer et al, 1991).

**Immobilised Culture Treatment Strategies**

The application of immobilised filamentous fungi in a continuous culture would alleviate the inherent problems of biomass adherence, steady state instability and non-Newtonian behaviour (Anselmo & Novais, 1992). In liquid cultures, _C. versicolor_ removed over 60% colour of a combined bleach kraft waste water within 6 days in presence of sucrose. This contrasted drastically with the results obtained when the same waste water was treated with fungus immobilised in beads of calcium alginate gel, where 80% decolourisation was attained after 3 days in the presence of sucrose. Recycled beads could remove colour efficiently in air repeatedly but not under anaerobic conditions (Liveroche et al, 1983). A fluidised bioreactor, containing polyurethane immobilised _T. versicolor_, operated at a residence time of 9 hr and reduced colour of a bleach plant waste water by 65%. A simultaneous reduction in AOX-levels of 57% was recorded. Biological activity and mechanical stability were maintained for 45 days. Up to 73% of colour and 55% of AOX were removed from bleach waste waters by mucoralean and white-rot fungi immobilised in a rotating biological contactor reactor (van Driessel & Christov, 2001). In the Mycopor-process, white-rot fungi were immobilized in polyurethane foam and the system was operated continuously using a trickling filter reactor set-up. Using this process, waste water from the alkaline stage of a sulphate mill resulted in a maximum of 80% decolorizing activity, accompanied by 50% COD reduction and 80% toxicity elimination after one passage through a trickling filter of 1 m length (Jaklin-Farcher et al, 1992). The principle applicability of capillary membrane technology using immobilised white-rot fungi in the bioremediation of industrial waste waters and aromatic pollutants has been demonstrated (Ryan et al, 2000).

**Ligninolytic Enzymes and Their Possible Role in Decolourisation of Waste Waters**

White-rot fungi produce a variety of key ligninolytic enzymes composed of lignin peroxidase (LiP), manganese dependent peroxidase (MnP) and
multiple iso-enzymes of laccase. Confusion was caused when mixtures of peroxidases and/or laccases were studied to demonstrate in vitro depolymerisation of high molecular mass lignin. Depolymerisation was usually accompanied by repolymerisation of the low molecular weight fractions (Lamar, 1992). A mixture of MnPs and laccases from Rigidoporus lignosus could degrade Herea lignin (Lamar, 1992). Mutants of P. chrysosporium lacking the ability to produce MnP and LiP did not show significant decolourisation activity when grown under nitrogen limiting conditions. Direct evidence linking MnP with decolourisation was provided by the in vitro depolymerisation of high molecular weight chlorolignin by MnP in the presence of Mn$^{2+}$ and peroxide (Lamar, 1992). Laccase and MnP were detected in bleach plant effluent treated by C. versicolor (Van Driesssel & Christov, 2001). In vivo studies with purified MnP and LiP in the decolourisation of kraft pulp bleach plant waste water indicated that only MnP had a decolourising activity (Jaspers et al, 1994). Also MnP and not LiP activity was detected when growing P. chrysosporium on the waste water. Many aspects of the mechanisms and functions of the enzymes involved in lignin mineralisation are scarcely known (Manzanares et al, 1995). More studies required to allow construction of an enzyme mixture that prevents the repolymerisation reactions from taking place in a cell-free state (Eriksson, 1993). Therefore, processes based on the whole fungus are still preferable (Ritter et al, 1990).

### Adsorption Studies using Microbial Biomass

Boman & Frostell (1988) reported that chlorinated phenols (40-50%) and adsorbable chloroorganics (10%) were first adsorbed onto the fungal biomass before the actual breakdown started. The initial colour decrease of Kraft pulp mill waste water (up to 30% after 3 days), using P. chrysosporium, could possibly be attributed to adsorption of chromogenic compounds during fungal growth (Feijoo et al, 1995). Jaspers & Penninckx (1996) reported that, depending on the conditions of incubation, pellets of P. chrysosporium strongly adsorbed colour and AOX from kraft bleach plant effluent. Royer et al (1985) regarded the decolourisation process by T. versicolor of bleach waste water to consist of two parts: i) Adsorption (completed after 24 hrs); and ii), Oxidation. About 10% of TOX removal, obtained after treatment of a mixture of first chlorination stage and first alkaline stage waste water with Ganoderma lucidum and C. versicolor, was estimated to be due to adsorption (Wang et al, 1992). The maximum percentage removal of high-molecular weight organochlorine, using municipal waste water sludge as sorbent, was 70%. After 24 hrs, only 8% of the organochlorines desorbed from live biomass, indicating that the process was not readily reversible (Srinivasan & Unwin, 1995). Biosorption of colour from a sulphite mill bleach plant effluent by biomass of Rhizomucor pusillus, a mucoralean fungus, removed up to 90% of the adsorbable colour in the first few hours of treatment (Christov et al, 1999). Cell wall fractions (alkali-resistant, residual and chitosan fractions), extracted from the R. pusillus biomass, appeared to be mainly responsible for the adsorption of chromophores from bleach plant waste water. The residual and chitosan fractions (about 12% of the total dry biomass) removed more colour from the effluent (41%) than did the intact biomass (39%). These fractions, mainly composed of chitin and/or chitosan, seemed to be involved in the mechanism of colour removal from bleach plant effluent by R. pusillus (Van Driesssel & Christov, 2002). Complete regeneration (by alkali treatment) and multiple reuse of this fungus were demonstrated in further decolourisation treatments (Christov et al, 1999). In contrast to R. pusillus, C. versicolor decolourised effluent by adsorption followed by biodegradation (van Driesssel & Christov, 2001). Hernandez et al (1994), who used Streptomyces strains to decolourise paper mill waste water obtained after semi-chemical alkaline pulping of wheat straw, estimated that about 20% of the initial colour was lost due to adsorption by the mycelia. Furthermore, transformation of the major chromophoric groups in effluent occurred only after colour adsorption by microbial biomass. Thus, bio-adsorption and transformation of coloured compounds were linked and decolourisation required initial adsorption of the colour compounds to the mycelia.

### Conclusions

Higher efficiencies of white-rot fungi during biological treatment of bleach plant waste water are required to develop a practical biotreatment process. The high volumes of waste water from the pulp and paper industry pose serious treatment problems. Biological treatments including lignolytic microorganisms, especially white-rot fungi, have potential, but the necessity of including a co-substrate
and the need for aeration would be costly affair. Fungal pretreatment of the bleach plant waste water was shown to be effective in colour reduction of the waste water. Another alternative to the secondary biotreatment of effluents would be their complex utilization in microbial fermentation processes to obtain valuable by-products. Biosorption of chromophores from bleach waste water has several potential advantages. These include the fast rate of the adsorption process and the option of incineration of the spent biomass after repeated cycles of adsorption/desorption and/or use as a supplement in the paper-making process. Because of the complexity of treating paper mill waste water, 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. The eventual implementation of these biotechnologies may lead to considerable reductions of the AOX, chloride levels, toxicity and chemical load of the waste water.

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