

## **EXECUTIVE SUMMARY**

### **BACKGROUND**

Efforts are made by the pulp and paper industry to reduce the chloroorganic/chloride discharges by the substitution of chlorine/chlorine dioxide with other more environmentally friendly bleaching agents. In response to the environmental concerns and stringent emission standards, modifications of the production process at the pulping and bleaching stages have been developed. Physical and chemical methods of removing chloroorganics, although quite effective in decolourisation of pulp and paper mills effluents, are unattractive for industrial applications because of the high costs. However, biological methods of effluent treatment have the advantage of being cost-effective and, in addition to colour, removal they can also reduce both BOD and COD of the effluent.

### **OBJECTIVES**

- Development of a novel for the pulp and paper manufacture technology for bio-sulfite pulping of wood by using white-rot fungi.
- Development of a novel for the pulp and paper manufacture technology for biobleaching of sulfite pulp by using hemicellulolytic enzymes and reduced amounts of bleaching chemicals.
- Improving the quality of the effluents produced as a result of the application of the biopulping and biobleaching technologies and as a result of secondary treatment of the effluents.
- Investigation of the possibilities for the complex utilisation of the effluents as substrates for generating xylanases and/or for production of valuable by-products.

### **RESEARCH APPROACH**

Decolourization and remediation of industrial waste waters from the pulp and paper industry was investigated following microbial pretreatment or bleaching with enzymes. The effluents under study were derived from the pulping and bleaching stages of pulp production. Acid sulfite pulping and elemental chlorine-free bleaching experiments were carried out under standardized conditions. Two industrial pulp types were examined for their bleachability with enzymes: hardwood acid sulfite pulp and hardwood soda-AQ (anthraquinone) pulp. Following pulping and bleaching, pulp properties such as brightness, viscosity and kappa number were determined according to the Standard Methods of the Technical Association of the Pulp and Paper Industry (TAPPI, Atlanta, USA). Most of the waste water analyses such as chemical oxygen demand and colour were carried out as described in the Standard Methods for Examination of Water and

Waste Water (APHA, American Public Health Association, Washington, DC, USA). The microbial pre-treatment of wood chips for biopulping was performed under solid-state fermentation conditions. The submerged fermentation experiments for enzyme production were carried out in shake flasks and evaluation of the cultivation conditions was based on the levels of xylanase activity produced. The efficiency of biosorption and microbial decolourization was evaluated mainly based on their impact on colour, chemical load and chlorinated organic matter.

## RESEARCH

### Biosulfite pulping

- Among a group of ten fungal species tested *Ceriporiopsis subvermispota* SS-3 was found the most promising isolate for future optimization studies of biosulfite pulping of *Eucalyptus grandis* wood chips as judged by results obtained on kappa number, alkali solubility  $S_{10}$  and  $S_{18}$ , brightness and pulp yield. Kappa number is used as a criterion for the oxidative potential and lignin content of pulps and is determined as the volume of 0.1 M potassium permanganate (ml) consumed by 1 g moisture-free pulp (corrected to 50% consumption of the permanganate). Alkali solubilities  $S_{10}$  and  $S_{18}$  provide information on the low molecular weight carbohydrates (degraded cellulose and hemicellulose) in pulp: a 10% sodium hydroxide solution dissolves both degraded cellulose and hemicellulose ( $S_{10}$ ) whereas hemicellulose is soluble in 18% sodium hydroxide solution ( $S_{18}$ ).
- Pretreatment with *C. subvermispota* SS-3 for ten days resulted in a substantial decrease in kappa number (by 29%) and increase in brightness (by 12%). On the other hand 5% of pulp yield was lost, and both viscosity and alkali solubility ( $S_{10}$  and  $S_{18}$ ) were retained at levels comparable to the control.
- When the fungal incubation time was reduced to five days, a 5% reduction of kappa number, 11% increase in brightness and no loss of pulp yield were observed. Alternatively, cooking time or amount of pulping chemicals could possibly be reduced at a given pulp yield and savings in energy or chemicals obtained respectively.
- Beneficial biosulfite pulping could be performed without use of additional carbon source during the fungal pretreatment of chips. However, the use of inoculum levels of as high as 1% on chips (dry weight basis) may preclude the scale-up of the process.
- Fungal pretreatment of wood could improve the selectivity not only of pulping but of the bleaching process as well. As a result, the higher brightness of pulp could be translated in savings of bleaching chemicals which in turn would reduce the chemical load and AOX levels in the bleach plant effluent as well.

### **Biobleaching**

- The impact of enzyme pretreatment of pulp on brightness was dependent on the particular xylanase preparation used. No relation seems to exist between the enzymatic release of reducing sugars from pulp, on one hand, and xylan removal from pulp or bleachability of pulps, on another. However, bleachability of sulfite pulp appears to improve in samples where xylan was enzymatically hydrolyzed and removed to a great extent.
- Brightness gain obtained as result of xylanase pretreatment of pulp seems to decline as the number of chemical bleaching steps increase. The impact of enzyme pretreatment on brightness was dependent on the particular bleaching sequence and regardless of the enzyme dose used.
- Chemical savings of up to 51% (as active chlorine) could be achieved when sulfite pulp was biobleached with *Aureobasidium pullulans* xylanases in sequence X-OD<sub>1</sub>E<sub>0</sub>D<sub>2</sub>P. The dose of chlorine dioxide at D<sub>1</sub> stage could be reduced by up to 80% whereas at D<sub>2</sub> stage it was lowered by up to 50%.
- Recent results indicate that the economics of the biobleaching process could be improved since enzyme charges of as low as 5 U xylanase/g pulp appeared to produce a brightness gain in bleaching of sulfite and soda pulps. Approximately 4.5 tonnes of pulp could be treated with 1 litre of Iogen HS-90 (22 731 U xylanase/ml). The charge per tonne of pulp would be about 0.220 t or 0.250 kg of enzyme (density 1.15 kg/l) and based on a price of US\$12.0/kg of enzyme, the cost to treat a tonne of pulp would be US\$3.0.
- The use of accessory xylan-degrading enzymes such as acetyl xylan esterases did not aid xylanase action on pulp, suggesting that these enzymes are not required in biobleaching. Furthermore, another enzyme,  $\alpha$ -glucuronidase, was found inefficient on sulfite pulp, indicating that this enzyme does not play an important role in xylan degradation.

### **Effluent biotechnology**

- As result of the reduction of the active chlorine charges during biobleaching of dissolving pulp, the chloride and AOX levels of the total bleach pulp effluent were reduced respectively by 31% and 38% whereas COD decreased by 22.5% at D<sub>1</sub>, 7% at E<sub>0</sub> and 20% at D<sub>2</sub>.
- The bleach plant effluent from the alkali extraction stage (E<sub>0</sub>) contained a fraction of coloured compounds (approximately 50% of total) which was adsorbable onto *Rhizomucor pusillus* biomass. The biosorption of colour was a rapid process which could remove approximately 90% of the adsorbable colour in the first few hours of effluent treatment. Treatment with *R.*

*pusillus* decolourised the effluent by 45-55% after 6-8 h and by 43% only after 2 h. On the other hand, treatment of E<sub>0</sub> with the white-rot fungus *Trametes nivosa* 208 required a minimum of 3 days for efficient colour degradation and reduction of about 65-70%. A prolonged treatment for up to 7 days did not decrease further colour of effluent.

- It was demonstrated that the fungal biomass could be completely regenerated and reused on the effluent. This would be beneficial because of the large volumes of wastewater emanating from pulp and paper mills that require remediation.
- Conditions would have to be monitored to prevent microbial contamination, especially when prolonged treatment periods are used at lower temperatures. To avoid this, decontamination of E<sub>0</sub> prior to fungal treatment would be necessary. *Rhizomucor pusillus* could grow and tolerate temperatures of up to 55°C and retain 85% of its decolouring abilities at 55°C as compared to 30°C. The use of elevated temperatures for effluent treatment would reduce the chances of microbial contamination and eliminate the need of cooling the effluent.
- Fungal treatment of E<sub>0</sub> could substantially reduce the COD (20-30%), toxicity (80-130%) and AOX (30%) levels in the effluent. Results from fungal biotreatment of E<sub>0</sub> were sensitive towards variation in the E<sub>0</sub> composition and may vary from batch to batch depending on the initial pH, temperature and colour of E<sub>0</sub>.
- This is the first report of using a mucoralean fungus in decolourisation of pulp and paper mill effluents. Biomass of *R. pusillus* proved to be efficient and compared well with other physico-chemical adsorbents in uptake of colour from the effluent. Under the conditions used in this study, the following descending order of colour removal was established: biomass (51%) > strong anion exchanger (48%) > activated carbon (37%) > chitosan (34%) > chitin (7%) > strong cation exchanger (4%).
- Results from preliminary experiments indicated that the bleach plant effluent could be used as a sole carbon source for xylanase production. In non-optimised conditions the effluent induced the production of cellulase-free enzymes in most of the strains tested. To the best of our knowledge, this is the first report of utilising a bleaching effluent from the pulp and paper industry for enzyme (xylanase) production.

## CONCLUSIONS

- Biopulping of wood chips with selected white-rot fungi as well as biobleaching of sulfite pulp with xylanases are promising in terms of improving the quality of pulp and bleach plant effluent.

- Fungal pretreatment of the bleach plant effluent was shown to be effective in colour reduction of the effluent.
- The eventual implementation of these biotechnologies may lead to savings in chemicals and considerable reductions of the AOX/chloride-levels, toxicity and chemical load of the effluent.
- This work indicates the potential usefulness that some of the effluents have in the production of valuable by-products, in particular xylanases.